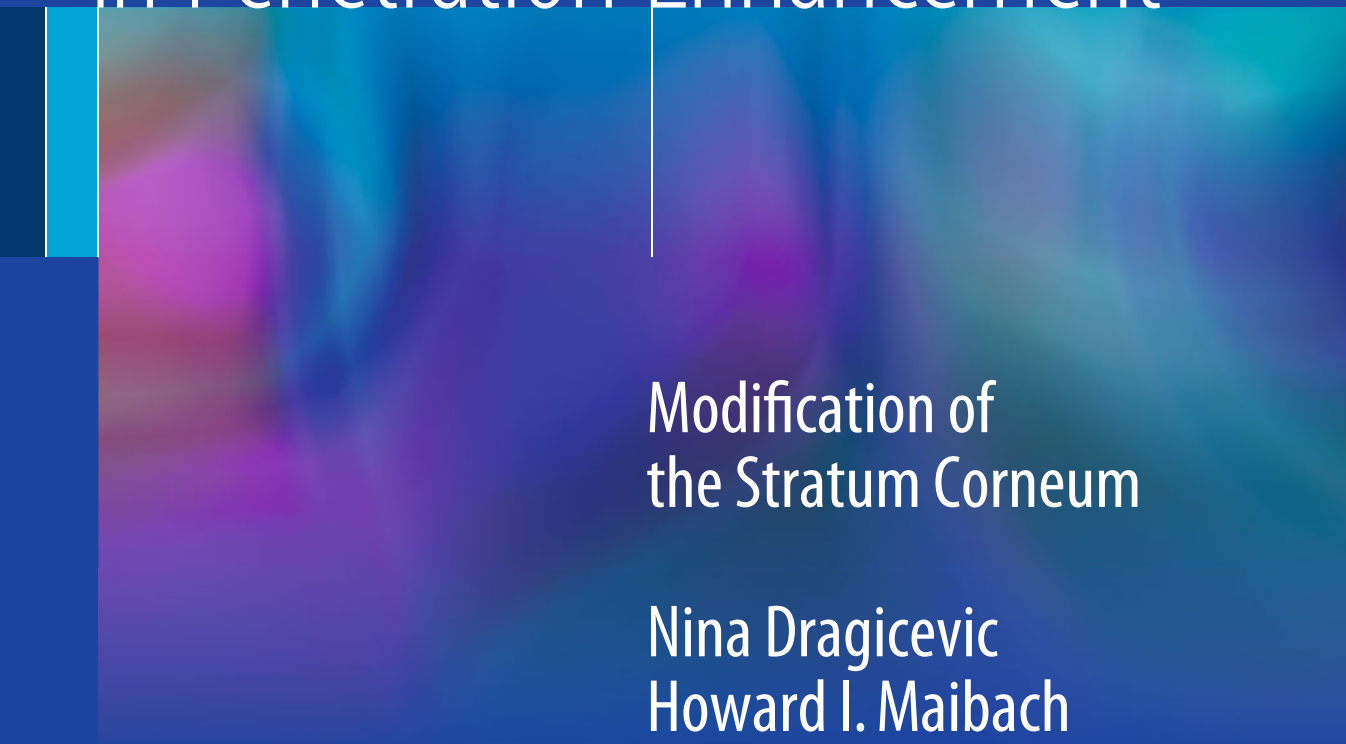
Percutaneous Penetration Enhancers

Chemical Methods in Penetration Enhancement



Modification of the Stratum Corneum

Nina Dragicevic
Howard I. Maibach
Editors

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ISBN 978-3-662-47038-1 ISBN 978-3-662-47039-8 (eBook)
DOI 10.1007/978-3-662-47039-8

Library of Congress Control Number: 2015956039

Springer Berlin, Heidelberg Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer-Verlag GmbH Berlin Heidelberg is part of Springer Science+Business Media (www.springer.com)

Preface

The main function of skin is the protection of the body from the external environment by preventing loss of water and the ingress of exogenous substances. This implies that the skin acts as a barrier for the diffusion of substances into the underlying tissue. Despite this role, the skin has become recognized as an important drug delivery route which can be reached directly. It is an ideal site for the application of drugs for achieving local (topical) and systemic (transdermal) drug effects. Local or topical drug delivery assumes treating various skin diseases, while transdermal delivery aims to achieve systemically active drug levels in order to treat systemic diseases. Drugs have been applied to the skin to achieve also regional drug delivery which involves drug application to the skin to treat or alleviate disease symptoms in deep tissues beneath the skin (such as in musculature, etc.). Topical and transdermal drug delivery offer a number of advantages compared to other conventional routes, and hence they are of great interest to pharmaceutical research, which explains the increasing interest in skin as a site of drug application.

However, skin represents a formidable barrier for percutaneous drug absorption, being of crucial importance for achieving topical and transdermal effects of drugs. Significant efforts have been devoted to developing strategies to overcome the impermeability of intact human skin. There are many ways for circumventing the stratum corneum, which provides the main barrier to drug penetration. These methods can be divided into chemical and physical penetration enhancement methods, i.e. percutaneous penetration enhancers, which are described in this book series *Percutaneous Penetration Enhancers*.

The aim of this book series is to provide to readers working in academia and industry, including young researchers, an up-to-date comprehensive work describing all the important topics required to understand the principles of enhancing transdermal and dermal drug delivery. The book series contains five books.

The book *Chemical Methods in Penetration Enhancement: Drug Manipulation Strategies and Vehicle Effects* begins with a description of the skin, as understanding of its structure, function and especially its penetration pathways is fundamental to understanding how topical and transdermal dosage forms work and how different methods may be employed to enhance percutaneous drug penetration. The first two parts of the book devoted to skin and the stratum corneum, representing its uppermost layer being responsible for its protection, discuss their structure, the importance of the lipid organization in the stratum corneum, the different penetration pathways through the skin

with an emphasis on the increasing importance of the follicular route, as well as the influence of different excipients on the skin. The focus of the book is on the chemical methods used to overcome the impermeability of intact skin, such as different drug manipulation strategies (drug or prodrug selection, chemical potential control, eutectic systems, complexes with cyclodextrines, etc.) and formulation/vehicle effects (influences of: emulsions, nanoemulsions, pickering emulsions, microemulsions, emulsifiers, emollients, liquid crystalline structures, gels, etc.) on the penetration enhancement of drugs.

The book *Chemical Methods in Penetration Enhancement: Nanocarriers* describes similarly to the first book chemical methods used in penetration enhancement of drugs. However, this book is devoted to the application of different kinds of nanocarriers and represents an attempt to familiarize the readers with the importance of nanocarriers used to enhance the percutaneous penetration of drugs as they have numerous advantages in comparison to conventional drug formulations. More recently, different types of nanocarriers have been designed by researchers which allow controlled and targeted drug delivery (dermal or transdermal drug delivery), improved therapeutic effectiveness and reduced side effects of drugs. As carriers they can be classified into lipid-based vesicles (e.g. liposomes, transfersomes, invasomes, etc.), surfactant-based vesicles (e.g. niosomes, novasomes and others), lipid-based particulate carriers (e.g. solid lipid nanoparticles, nanostructured lipid carriers and lipid nanocapsules), polymer-based particulate carriers (e.g. polymeric nano- and microparticles, polymeric nanocapsules, polymeric micelles, dendrimers, dendritic core-multishell nanocarriers, etc.), nanocrystals and others. This book focusing on the different nanocarriers gives a comprehensive review of their use as promising dermal and transdermal drug delivery systems. It also considers the use of nanocarriers for cutaneous immunization offering the important advantage of being painless and having a stronger immune response compared to the intramuscular injection of vaccines. In addition, the book provides insights on the safety of the use of nanoparticles.

The book *Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum* similarly to the aforementioned two books describes the chemical methods used in penetration enhancement of drugs with an emphasis on the enhancing methods used to modify the stratum corneum. It starts with the classification of penetration enhancers, their mode of action and provides insights on the structure–activity relationship of chemical penetration enhancers. The focus of this book is on the most commonly used classes of skin penetration enhancers being investigated in scientific literature and used in commercial topical and transdermal formulations, and their representatives are discussed in more detail, including their mechanism of action, where known. The following penetration enhancers are considered in the book: alcohols (e.g. ethanol, etc.), glycols (e.g. propylene glycol, etc.), amides (e.g. 1-dodecylazacycloheptan-2-one or laurocapram (Azone®), etc.), fatty acids (e.g. oleic acid, etc.), fatty acid esters (e.g. isopropyl myristate, etc.), ether alcohols (e.g. diethylene glycol monoethyl ether (Transcutol®)), pyrrolidones (e.g. N-methyl-2-pyrrolidone, etc.), sulphoxides (e.g. dimethyl sulphoxide, etc.), surfactants (e.g. polysorbates, etc.), terpenes (e.g. L-menthol, etc.), peptides and new classes of enhancers, such as iminosulfuranes,

transcarbams, dimethylamino acid esters and dicarboxylic acid esters. In addition, synergistic effects of different chemical penetration enhancers have been discussed in the book as an important feature of chemical penetration enhancers. Furthermore, the safety profile of chemical penetration enhancers is considered.

The book *Physical Methods in Penetration Enhancement* considers the current status and possible future directions in the emerging area of physical methods being used as potent enhancers for the percutaneous penetration of drugs. It gives a comprehensive overview of the most used methods for enhancing dermal and transdermal drug delivery. It covers sonophoresis, iontophoresis, electroporation, magnetophoresis, microneedles, needle-free jet injectors, ablation methods (electrical, thermal or laser skin ablation) and others. The numerous advantages of these methods have opened new frontiers in the penetration enhancement of drugs for dermal and transdermal drug delivery. Cutaneous vaccination and gene delivery by physical methods have been also discussed in this volume. Consideration was given to new methods, too, such as a novel electrochemical device for penetration enhancement, different waves (e.g. photoacoustic waves, microwaves, etc.), natural submicron injectors, moxibustion and others. Furthermore, the combined use of different physical methods or of physical methods and passive enhancement methods (chemical penetration enhancement methods) are discussed as they provide, due to their synergistic effects, higher percutaneous drug penetration when used together.

The book *Drug Penetration Into/Through the Skin: Methodology and General Considerations* provides fundamental principles of the drug penetration into/through the skin, from covering basic mathematics involved in skin permeation of drugs, influences of drug application conditions and other factors on drug penetration, mechanistic studies of penetration enhancers, influences of the type of skin used (human native or reconstructed skin) to different methods utilized to assess the drug penetration into/through the skin and to determine the amount of permeated drug (such as tape stripping of the stratum corneum, electron spin resonance, Raman spectroscopy, attenuated total reflection, confocal laser scanning microscopy, single and multiphoton microscopy, etc.). Retardation strategies are also discussed as being important for some classes of substances, such as sunscreens. The safety of applied penetration enhancers as well as the research ethics in the investigation of dermal and transdermal drug delivery are addressed in this book. The book ends with the current status and future perspectives of passive/chemical and active/physical penetration enhancement methods as they are gaining extensive interest as promising tools to enable an efficient dermal or transdermal drug delivery.

We are very thankful to all the authors who contributed chapters to the book series *Percutaneous Penetration Enhancers*, as they found time to work on the chapters despite having busy schedules and commitments. All the authors are eminent experts in the scientific field which was the subject of their chapter, and hence their contribution raised the value of the book. We also sincerely thank our collaborators from Springer: Ellen Blasig, Isabella Formento, Sverre Klemp, Srinath Raju, Andre Tournois, Grant Weston and

Portia Formento Wong, for their dedicated work which was necessary to achieve such a high standard of publication. We highly appreciate readers' comments, suggestions and criticisms to improve the next edition of the book series.

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Hyaluronic Acid for Percutaneous Drug Delivery

1

Sarah Hedtrich and Wolfgang Frieß

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1.1 Introduction

Hyaluronic acid (HA), a high-molecular-weight biopolymer, is of special interest as carrier material in drug delivery due to its biocompatibility, biodegradability, special viscoelastic properties, and the ability to create a dense network in which drugs might be incorporated. One major medical application of HA besides for skin care and as vehicle for dermal delivery, specifically of diclofenac sodium, is its intra-articular (IA) injection in the treatment of rheumatoid arthritis. In the course of rheumatoid arthritis, an enhanced HA degradation in the synovial fluid is observed. HA injection into the joints provides visco-supplementation as well as some anti-inflammatory, slight analgesic, and biosynthetic stimulatory effects (Kogan et al. 2007). Another major medical application is in the field of ophthalmology. As a main component of the vitreous body of the eye, HA is applied via intraocular injection to cushion in many surgical procedures, e.g., during cataract removal, corneal transplantation, or repair of a detached retina. Furthermore, HA is used as viscosity enhancer in eye drops proving solutions with remarkable non-Newtonian, shear-thinning properties to lubricate, moisturize, and protect the ocular surface.

HA can be used as a drug carrier, where especially the local treatment of joint diseases could benefit from its use. Lyons et al. incorporated

N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_1

triamcinolone acetonide crystals into an HA gel to provide sustained drug release which is caused by decelerated dissolution of the corticosteroid particles and to reduce the risk of crystal-induced joint inflammation (Lyons et al. 2008). Another patent deals with the incorporation of an antimicrobial agent into an HA gel intended for the treatment or prevention of joint infections upon IA injection of, e.g., levofloxacin, ofloxacin, and lincomycin (Tanaka 2004). Due to its hydrophilic nature, HA-containing gels could specifically provide a highly compatible environment for sensitive protein drugs. These biotech drugs, which currently fill the development pipeline of many pharmaceutical companies, suffer from a lack of available drug delivery vehicles that provide adequate protein stability (Jiskoot et al. 2012). Prisell et al. revealed a significantly slower release of human recombinant insulin-like growth factor-I from HA gels (0.5–2 %) compared to a HA-free formulation both in vitro and in vivo upon subcutaneous injection in rats (Prisell et al. 1992). Meyer et al. found no significant aggregation of recombinant human granulocyte colony-stimulating factor (GCSF) in 2 % HA gels upon storage for 6 weeks at 37 °C, and they found prolonged elevated drug plasma levels of GCSF for up to 4 days (Meyer et al. 1995).

Thus, HA has demonstrated its potential as a drug carrier also outside the area of topical dermatological and cosmetic application. The biopolymer benefits from its special viscoelastic properties and high biocompatibility. But, these pros are at the same time cons as the concentration of HA which can be used is rather limited due to the high viscosity of HA hydrogels, and drug release from HA hydrogels typically occurs rather fast, although this can be modified by the HA concentration, too, namely, higher HA concentration results in slower drug release.

1.2 Physical and Chemical Properties of Hyaluronic Acid

HA is a naturally occurring polyanionic linear, unbranched polysaccharide composed of a repeating disaccharide that consists of D-GLUCURONIC acid-ß(1,3)-N-acetyl-D-GLUCOSAMINE-ß(1,4) (Fig. 1.1).

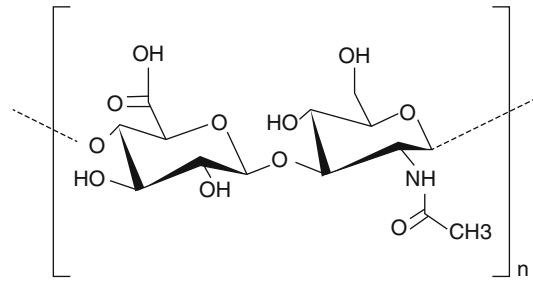

Fig. 1.1 Chemical structure of hyaluronic acid

The acid dissociation constant pKa of approximately 3.0 renders the dissociated salt form under physiological conditions which is sodium hyaluronate (Lapcik et al. 1998). For HA the term hyaluronan and for the sodium salt sodium hyaluronan are used synonymously. For medical applications, almost solely the sodium salt of HA is used. However, most studies do not specify the material; here, HA is used as a general term. Nevertheless, one can presume that the sodium salt was used.

Its molecular weight is typically in the range of a 10^6–10^7 Da. But also lower-molecular-weight HA (LMW-HA) of only 5 or 100 kDa is available. Low-molecular-weight HA is prepared from higher-molecular-weight species by physical, chemical, or enzymatic approaches (Stern et al. 2007; Weindl et al. 2004) and has been shown to be more pro-angiogenic and pro-inflammatory than high-molecular-weight HA (see below). The unique physicochemical properties of HA such as the rheological behavior of aqueous solutions, its polyelectrolytic properties, and the molecular weight characteristics have been described in detail by Milas and Rinaudo (2005). HA acts as a lubricant and shock absorbent in the body. It stabilizes the cartilage matrix and controls the tissue water homeostasis. HA binds to proteins in the extracellular matrix and onto cell surfaces, influences the migration, adhesion, and proliferation of cells, as well as leukocyte functions. In the past, HA for medical and pharmaceutical applications was predominantly isolated from rooster comb. The obtained HA varied in its molecular weight and the three-dimensional

organization of the chains and has to go through extensive purification (Kogan et al. 2007). Today, the isolation of HA from *Streptococcus zooepidemicus* cultures gives access to material of higher purity at a more reasonable price. This bacterial HA exhibits a molecular weight of several hundred kDa and the fermentation conditions still need to be optimized to increase the molecular weight to reach the MDa range of rooster comb-derived HA (Chen et al. 2009; Lai et al. 2012). In mammals, HA is enzymatically degraded by hyaluronidase, ß-D-glucuronidase, and ß-N-acetyl-hexosaminidase (Necas et al. 2008). A variety of chemical modifications have been developed, mostly cross-linking and esterification of HA, to render HA variants with reduced hydrophilicity, less swelling capability, and slower biodegradation (Lapcik et al. 1998).

1.3 Hyaluronic Acid in Cosmetics

HA is widely used in cosmetics, especially in rejuvenative medicine in terms of tissue regeneration, augmentation, and skin hydration. Its popularity increases steadily since 2004. The most important approaches are its usage as a dermal filler in aesthetic dermatology and as a moisturizing agent for the treatment of cutaneous lines and wrinkles. HA is abundant in the human skin and contributes considerably to the maintenance of the extracellular space and the transport of nutrients and ion solutes due to its high water-binding capacity (Weindl et al. 2004). In this course, HA preserves skin hydration and consequently is often used as a moisturizer in cosmetic formulations aiming for a restoration of skin elasticity, thereby achieving an anti-wrinkle effect. Additionally, HA forms a viscous pericellular network restricting the movement of reactive oxygen species or binding them by acting as an iron chelator (Trommer et al. 2003; Moseley et al. 2003). Thus, HA acts as an antioxidant and scavenger of free radicals which is beneficial for cosmetic treatments, too. Furthermore, it exhibits excellent viscoelastic properties and high biocompatibility so that side effects are rare (Brown and Jones 2005).

Currently, there are two ways of HA application: the intradermal injection as dermal filler or the topical application as a cream or a gel.

Injecting HA is considered a real augmentation. After injection, HA retains water and therefore volume at the injection site. Over time, HA is biodegraded by the enzyme hyaluronidase within several months. Degradation seems not to be of clinical relevance until the HA molecular weight and concentration falls below a critical level and the clinical effect diminishes rapidly (Price et al. 2007). Only minor complications occur after HA injections and allergic reactions are not of relevance. Occasionally, a transient erythema and mild swelling is observed at the injection side. Single reports also describe the formation of sarcoidosis or the formation of a sterile abscess. Nevertheless, the application of HA is very safe (Price et al. 2007).

Focusing on the topical application, HA is an attractive agent for the cosmetic industry due to its superior skin hydration effects which helps the skin to retain and maintain skin elasticity, turgor, and moisture. A few small clinical studies have been conducted using HA of different molecular weights showing superior effects with low-molecular-weight species. Consistently, significant improvement of skin hydration and elasticity was found in all test subjects. Following an application of HA fragments (50 and 130 kDa), a significant reduction of wrinkle depth was observed (Pavicic et al. 2011). Additionally, combinations of such fragments with standard antiaging drugs such as retinaldehyde were tested showing decreased wrinkle depth after 3-month application and improved decrease of photoaging (Cordero et al. 2011). However, the significance of these studies often is limited by the lack of adequate control groups and, thus, do not allow final conclusions on the real impact of HA.

The effects of HA are limited by its high molecular weight which can easily reach up to 2 MDa and, thus, is excluded from skin absorption. This drawback is addressed by the cosmetic industry which therefore uses HA fragments, so-called low-molecular-weight (LMW) HA in varying sizes such as 5, 50, 100 kDa, etc. Improved skin absorption of HA fragments is

described; however the measured flux values are in the nanogram range. For example, with 300 kDa HA, the flux was 19.8 ng/cm^{-2}*h^{-1} after 5 h (Farwick et al. 2011). Incorporating LMW-HA in creams, cosmetic companies suggest to upholster the skin from the inside and, thus, to reduce wrinkle depth. However, the size of the HA fragments used in these products are not known and very few studies with mostly insufficient control groups have been conducted. Some studies indicate beneficial effects of fragments in terms of wrinkle depth but further studies are required. Nevertheless, Farwick et al., describes the up- and downregulation of 120 genes upon LMW-HA incubation in reconstructed skin. Especially genes which are important for cell-cell interactions and for the epithelial barrier function of epithelial and endothelial cells such as E-cadherin; occludin; claudin-4, claudin-17, and claudin-1; and other tight junctions proteins were upregulated (Farwick et al. 2011). To our best knowledge, in vivo studies have not been conducted yet to confirm these results.

1.4 Hyaluronic Acid-Based Formulations for Intra-/Transdermal Drug Delivery

The excellent physicochemical properties of HA and its high biocompatibility lead to various clinical applications also in terms of topical drug delivery. The most prominent example is a 2.5 % HA-sodium gel containing 3 % diclofenac sodium (Solaraze®, Almirall Hermal GmbH, Germany) which is approved in the USA and Europe for the treatment of actinic keratosis (AK). AK is a common skin lesion mainly induced by extensive UV exposure which may develop into an invasive squamous cell carcinoma (SCC) being associated with a less favorable prognosis. This progression from AK to SCC forms the basis for the treatment rational of AK and is estimated with 0.025–16 % per year (Brown and Jones 2005). The diclofenac HA gel significantly reduces the number of AK lesions and can produce complete clearance of lesions when applied twice daily for 60 or 90 days. The product is well tolerated and does not induce serious adverse effects (Jarvis and Figgitt 2003). The contribution of HA to this effective treatment was investigated thoroughly. Brown et al. found a significantly enhanced partitioning, retention, and localization of diclofenac in the epidermal layer of human skin when treated with the HA gel in comparison to aqueous solutions, other glycosamines, and conventional pharmaceutical gelling agents clearly indicating penetration-enhancing effects of HA (Brown et al. 1995, 2001; Brown and Jones 2005). The formation of a drug reservoir in the epidermis is the major proposed reason for the superiority of this formulation (Brown et al. 1999; Alam et al. 1995). Facilitated and enhanced drug delivery into the epidermal layer following an application of HA-containing formulations is described also for other drugs such as ibuprofen, clindamycin, and cyclosporine (Brown and Jones 2005). The in vitro studies were further substantiated by the finding that radiolabeled HA itself penetrates into the skin of nude mice and humans (Brown et al. 1999; Birkenfeld et al. 2011). Brown et al. reported the absorption of HA from the skin surface into the skin and various hypotheses are proposed to explain this surprising finding (Brown et al. 1999). Farwick et al. report skin absorption of HA fragments with a size of 50 kDa, although in a very low amount (Farwick et al. 2011). As even the fragments are too big to penetrate via the inter- or transcellular pathway, transappendageal penetration is discussed (Farwick et al. 2011). Results from our group suggest the contrary: We did not find any skin absorption of HA-sodium or HA-sodium fragments (5, 100 kDa) in healthy, tape-stripped skin and even reconstructed skin models (Witting et al. 2015). In our opinion the unquestioned beneficial cosmetic effect of HA is due to the extensive skin hydration resulting in an upholstering of the skin and, thus, a reduction of the wrinkle depth.

As mentioned above, HA-facilitated skin absorption is described in the literature, e.g., for diclofenac, ibuprofen, and cyclosporine. How HA acts as a penetration enhancer is still ambiguous. One plausible explanation is the extensive skin hydration upon HA application which mainly can

influence drug absorption. Depending on the HA concentration and its viscosity, HA hydrogels form an occlusive barrier on the skin, preventing the evaporation of water. Increased hydration loosens the cluster of densely packed corneocytes, intra- and intercellular channels open up and, thus, the drug can penetrate more easily.

HA is also of increasing interest as a (nano-/microparticulate) drug delivery system. The rationale behind this approach is the identification of the CD44 and hyaladherin (RHAMM) as the major cell-surface HA receptors on fibroblasts and keratinocytes. Stimulation of these receptors influences cell migration, differentiation, and proliferation (Bourguignon et al. 2004), and it is believed to facilitate the absorption and distribution of HA in the skin (Fig. 1.2) (Yang et al. 2012). Based on the positive effects of HA on skin absorption, the authors hypothesize that the use of HA nano-/microparticles may further enhance skin absorption. HA particles would act as a receptor-mediated transdermal delivery system. After penetration into the viable epidermis, the particles may initiate further cellular responses (Fig. 1.2) (Yang et al. 2012).

Based on these facts, investigations of HA conjugates aiming for efficient (trans-) dermal drug transport were conducted. Yang et al. described the synthesis of HA-human growth hormone conjugates aiming for efficient transdermal delivery of the protein and found effective transport of the conjugates into the dermal layer of the skin (Yang et al. 2012). The postulated skin absorption mechanism is depicted in Fig. 1.2.

Furthermore, HA-doxorubicin (DOX) conjugates showed increased accumulation in skin tumors as well as significantly stronger impairment of tumor growth compared to DOX alone upon topical treatment of melanoma, based on the fact that melanoma cells are positive for the HA-receptor CD44 and RHAMM. Clearly, the increased efficiency was linked to the conjugation of DOX to HA which increased the antitumor activity (Zhang et al. 2009).

Besides the topical application as hydrogels or particular systems recently, the development of HA-based, dissolving microneedles was described (Liu et al. 2012; Ito et al. 2011). The advantage of these microneedles includes high biocompatibility, mechanical strength, self-disintegration, and fast drug release. Insulin delivery with the microneedles was comparable to the subcutaneous injection. In contrast to other microneedles, HA-based systems ensured a fast drug release ($\leq$1 h) and drug stability (Liu et al. 2012). A similar approach was investigated by Ito et al., studying HA-based dissolving microneedles for the transdermal delivery of the migraine therapeutic sumatriptan. In contrast to dextran-based microneedles, higher plasma concentrations, area under the curve (AUC), and maximum concentration (Cmax) values were detected for the HA-based systems (Ito et al. 2011).

As HA can influence cellular behavior of fibroblasts and keratinocytes by stimulating the CD44 and RHAMM receptors, this polysaccharide is widely discussed for its application in wound healing. Biomaterials such as hydrocolloids, alginates, and HA have the advantage of forming a part of the natural tissue matrix; they are biodegradable and play an active part in normal wound healing and new tissue formation (Boateng et al. 2008). Important for adequate wound healing is the remodeling of the extracellular matrix. Besides its stimulating effects on keratinocyte and fibroblast proliferation, migration, and differentiation via HA receptor activation, HA seems to stimulate the remodeling of the extracellular matrix and collagen deposition (Price et al. 2007). However, the exact mechanism is not understood yet. It was shown that HA mediates physiological functions such as morphogenesis, regeneration, and wound healing via interactions with binding proteins and cell surface receptors via HA receptor stimulation and subsequent rapid tyrosine phosphorylation. Dynamic regulation of cell behavior and signaling was described, too (Hall et al. 1994). The stimulation of fibroblasts' and keratinocytes' proliferation induced by an activation of the CD44 receptor was reported. Similar cellular effects are described for LMW-HA (Barnes et al. 2010; Kaya et al. 2006; Greco et al. 1998; Xie et al. 2011; Mohapatra et al. 1996).

Various studies investigating the effects of HA on acute and chronic wounds are published

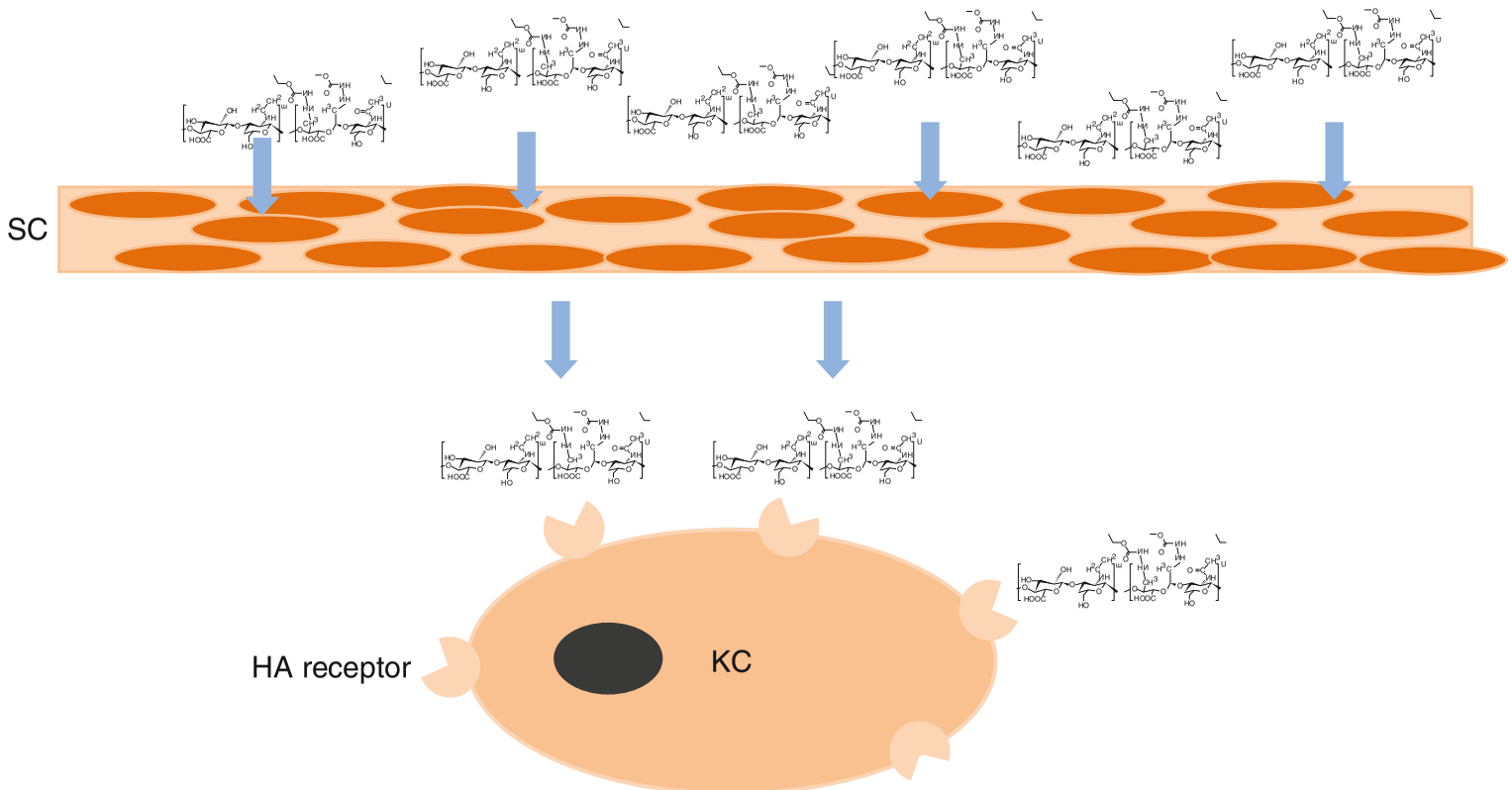

Fig.1.2 Schematic representation of the hypothesis of active HA transport across the stratum corneum (*SC*). *KC* = keratinocyte. HA transport is facilitated by HA receptors on keratinocytes and fibroblasts. Furthermore, it is discussed that after the successful skin absorption HA may bind to the cell surface receptors influencing cell migration, differentiation and proliferation. The figure was adapted and modified from (Yang et al. 2012)

showing improved wound healing and high tolerability of the formulations (Boateng et al. 2008; Voinchet et al. 2006; Vazquez et al. 2003; Colletta et al. 2003). Chronic wounds such as foot ulcers in diabetic patients are still an unsolved problem and ask for new therapeutic approaches. Especially for these conditions, HA-based formulations were studied well. The application of a HA gauze pad for 3 weeks improved the appearance and dimension of a leg ulcer faster compared to a dextranomer paste (Ortonne 2001). Clearly, the addition of HA to wound dressings is beneficial in terms of wound closure and hydration, but the development of a wound dressing solely based on HA failed due to problems with the stability and consistency of the dressings (Rossi et al. 2007).

Beneficial effects of HA formulations have also been reported in burn patients. The application of the gold standard for topical burn therapy sulfadiazine combined with HA resulted in clinically relevant reduced healing time (−4/5 days) and a reduction of the local edema occurring shortly after injury. Reepithelialization process was stimulated compared to a plain sulfadiazine cream (Koller 2004; Costagliola and Agrosi 2005). The twice-daily application of a 0.2 % HA gel in patients undergoing/on radiotherapy resulted in a slight delay of the onset of acute skin reactions such as radioepithelitis and significantly reduced intensities of these conditions and accelerated the healing process (Liguori et al. 1997).

When applying HA fragments on a murine excisional wound model, accelerated wound closure was observed (Gao et al. 2010). Angiogenesis and lymph vessel formation were increased; granulation, collagen deposition, and fibroblast proliferation were accelerated. The authors found significantly upregulated endothelial nitric oxide synthase and procollagen-1 mRNAs as well as a downregulation of the matrix metalloproteinases 9 and 13. As mentioned above, HA also promotes (endothelial cell) proliferation by binding to the cell surface receptor CD44 (Gao et al. 2010).

Zavan et al. described the production of HA-ester-based porous nanoparticles incorporating the platelet-derived growth factor (PDGF) (Zavan et al. 2009). The authors describe a faster wound closure after the PDGF-HA microparticle treatment but the study lacks from valid controls (Zavan et al. 2009). HA is considered to interact intensively with such active substances due to its viscoelasticity and immunocompatibility by potentiating and maximizing tissue responses and by regulating the drug release (Xie et al. 2011). Xie et al. reported significantly enhanced wound healing in a 3D skin model when applying HA-vitronectin-growth hormone complexes. Besides accelerated reepithelialization and wound closure, stimulating effects on the secretion of antimicrobial peptides such as human beta defensin and human cathelicidin were observed (Dusio et al. 2011).

1.5 Safety Considerations

Despite the positive effects found for HA, large-scale, multicenter, and controlled clinical trials are missing to date. Most of the studies described above are limited by the lack of adequate controls and by the heterogeneity of wounds which have been studied, thus not allowing for final recommendations.

Despite the beneficial effects of LMW-HA on wound healing and its broad cosmetic applications, potential pro-inflammatory processes induced by LMW-HA need to be taken into account and require careful evaluation. Very LMW-HA fragments (≤50 kDa/≤250 kDa) trigger the production of pro-inflammatory cytokines such as tumor necrosis factor α (TNFα) and adhesion molecules (Farwick et al. 2011; Rossler and Hinghofer-Szalkay 2003). Studies of Voelcker et al. (2008) and Taylor et al. (2004) describe the activation of Toll-like receptor 2 and 4 by HA fragments, leading to the activation of immune cells such as monocytes, macrophages, and Langerhans cells [for review, see (Stern et al. 2006)], inducing the production of pro-inflammatory cytokines (Voelcker et al. 2008; Taylor et al. 2004). The fragment size is a crucial point and considerably determines the resulting cellular interactions. LMW-HA also seems to promote tumor cell motility, invasion, and angiogenesis, indicating that HA fragments

may facilitate cancer progression [for review, see (Stern et al. 2006)]. These caveats need to be taken into account carefully when using HA fragments also for topical applications. The exact determinations of the fragment size as well as the induced physiological responses need to be elucidated thoroughly.

1.6 Concluding Remarks

Various studies clearly demonstrate the effectiveness of HA-based formulations for skin penetration enhancement. The skin penetration enhancement is most likely due to the loosening of the densely packed corneocytes in the course of skin hydration which then allow for easier drug penetration into the skin. HA gels are excellent vehicles for topical drug application also on wounded skin. A facilitation of reepithelialization is most likely and the hydrophilic environment generated by HA alone is favorable for wounds. To elucidate the real HA effect and impact, large-scale clinical trials are crucial.

Contradictory results on the skin absorption of HA and its fragments themselves are published and final conclusions are currently not possible. However, in our opinion intradermal effects of topically applied HA and its fragments are questionable. Nevertheless, anti-wrinkle effects and increased skin elasticity are well-known effects most likely due to the excellent skin-hydrating effects. The usage of fragments requires careful evaluation of their potential pro-inflammatory effects. To date, HA drug delivery systems for topical application such as micro- and nanoparticles are of minor relevance.

References

Alam CA, Seed MP, Willoughby DA (1995) Angiostasis and vascular regression in chronic granulomatous inflammation induced by diclofenac in combination with hyaluronan in mice. J Pharm Pharmacol 47(5):407–411

Barnes L, Tran C, Sorg O, Hotz R, Grand D, Carraux P et al (2010) Synergistic effect of hyaluronate fragments in retinaldehyde-induced skin hyperplasia which is a Cd44-dependent phenomenon. PLoS One 5(12), e14372

Birkenfeld B, Parafiniuk M, Bielecka-Grzela S, Klimowicz A, Piwowarska-Bilska H, Mikolajczak R et al (2011) The penetration of topically applied ointment containing hyaluronic acid in rabbit tissues. Pol J Vet Sci 14(4):621–627

Boateng JS, Matthews KH, Stevens HN, Eccleston GM (2008) Wound healing dressings and drug delivery systems: a review. J Pharm Sci 97(8):2892–2923

Bourguignon LY, Singleton PA, Diedrich F (2004) Hyaluronan-CD44 interaction with Rac1-dependent protein kinase N-gamma promotes phospholipase Cgamma1 activation, Ca(2+) signaling, and cortactin-cytoskeleton function leading to keratinocyte adhesion and differentiation. J Biol Chem 279(28):29654–29669

Brown MB, Hanpanitcharoen M, Martin GP (2001) An in vitro investigation into the effect of glycosaminoglycans on the skin partitioning and deposition of NSAIDs. Int J Pharm 225(1-2):113–121

Brown MB, Jones SA (2005) Hyaluronic acid: a unique topical vehicle for the localized delivery of drugs to the skin. J Eur Acad Dermatol Venereol 19(3):308–318

Brown MB, Marriott C, Martin GP (1995) The effect of hyaluronan on the in vitro deposition of diclofenac within the skin. Int J Tissue React 17(4):133–140

Brown TJ, Alcorn D, Fraser JR (1999) Absorption of hyaluronan applied to the surface of intact skin. J Invest Dermatol 113(5):740–746

Chen WY, Marcellin E, Hung J, Nielsen LK (2009) Hyaluronan molecular weight is controlled by UDP-N-acetylglucosamine concentration in Streptococcus zooepidemicus. J Biol Chem 284(27):18007–18014

Colletta V, Dioguardi D, Di Lonardo A, Maggio G, Torasso F (2003) A trial to assess the efficacy and tolerability of Hyalofill-F in non-healing venous leg ulcers. J Wound Care 12(9):357–360

Cordero A, Leon-Dorantes G, Pons-Guiraud A, Di Pietro A, Asensi SV, Walkiewicz-Cyraska B et al (2011) Retinaldehyde/hyaluronic acid fragments: a synergistic association for the management of skin aging. J Cosmet Dermatol 10(2):110–117

Costagliola M, Agrosi M (2005) Second-degree burns: a comparative, multicenter, randomized trial of hyaluronic acid plus silver sulfadiazine vs. silver sulfadiazine alone. Curr Med Res Opin 21(8):1235–1240

Dusio GF, Cardani D, Zanobbio L, Mantovani M, Luchini P, Battini L et al (2011) Stimulation of TLRs by LMW-HA induces self-defense mechanisms in vaginal epithelium. Immunol Cell Biol 89(5):630–639

Farwick M, Gauglitz G, Pavicic T, Kohler T, Wegmann M, Schwach-Abdellaoui K et al (2011) Fifty-kDa hyaluronic acid upregulates some epidermal genes without changing TNF-alpha expression in reconstituted epidermis. Skin Pharmacol Physiol 24(4):210–217

Gao F, Liu Y, He Y, Yang C, Wang Y, Shi X et al (2010) Hyaluronan oligosaccharides promote excisional wound healing through enhanced angiogenesis. Matrix Biol 29(2):107–116

Greco RM, Iocono JA, Ehrlich HP (1998) Hyaluronic acid stimulates human fibroblast proliferation within a collagen matrix. J Cell Physiol 177(3):465–473

Hall CL, Wang C, Lange LA, Turley EA (1994) Hyaluronan and the hyaluronan receptor RHAMM promote focal adhesion turnover and transient tyrosine kinase activity. J Cell Biol 126(2):575–588

Ito Y, Kashiwara S, Fukushima K, Takada K (2011) Two-layered dissolving microneedles for percutaneous delivery of sumatriptan in rats. Drug Dev Ind Pharm 37(12):1387–1393

Jarvis B, Figgitt DP (2003) Topical 3% diclofenac in 2.5% hyaluronic acid gel: a review of its use in patients with actinic keratoses. Am J Clin Dermatol 4(3):203–213

Jiskoot W, Randolph TW, Volkin DB, Middaugh CR, Schoneich C, Winter G et al (2012) Protein instability and immunogenicity: roadblocks to clinical application of injectable protein delivery systems for sustained release. J Pharm Sci 101(3):946–954

Kaya G, Tran C, Sorg O, Hotz R, Grand D, Carraux P et al (2006) Hyaluronate fragments reverse skin atrophy by a CD44-dependent mechanism. PLoS Med 3(12), e493

Kogan G, Soltes L, Stern R, Gemeiner P (2007) Hyaluronic acid: a natural biopolymer with a broad range of biomedical and industrial applications. Biotechnol Lett 29(1):17–25

Koller J (2004) Topical treatment of partial thickness burns by silver sulfadiazine plus hyaluronic acid compared to silver sulfadiazine alone: a double-blind, clinical study. Drugs Exp Clin Res 30(5-6): 183–190

Lai ZW, Rahim RA, Ariff AB, Mohamad R (2012) Biosynthesis of high molecular weight hyaluronic acid by Streptococcus zooepidemicus using oxygen vector and optimum impeller tip speed. J Biosci Bioeng 114(3):286–291

Lapcik L Jr, Lapcik L, De Smedt S, Demeester J, Chabrecek P (1998) Hyaluronan: preparation, structure, properties, and applications. Chem Rev 98(8): 2663–2684

Liguori V, Guillemin C, Pesce GF, Mirimanoff RO, Bernier J (1997) Double-blind, randomized clinical study comparing hyaluronic acid cream to placebo in patients treated with radiotherapy. Radiother Oncol 42(2):155–161

Liu S, Jin MN, Quan YS, Kamiyama F, Katsumi H, Sakane T et al (2012) The development and characteristics of novel microneedle arrays fabricated from hyaluronic acid, and their application in the transdermal delivery of insulin. J Control Release 161(3):933–941

Lyons RT, Robinson MR, Trogden JT, Whitcup SM, inventors (2008) Peripherally administered viscous formulations. US 20140356438 A1

Meyer J, Whitcomb L, Treuheit M, Collins D (1995) Sustained in vivo activity of recombinant human granulocyte colony stimulating factor (rHG-CSF) incorporated into hyaluronan. J Control Release 35: 67–72

Milas M, Rinaudo M (2005) Characterization and properties of hyaluronic acid (hyaluronan). In: Dumitriu S (ed) Polysaccharides: structural diversity and functional versatility. Marcel Dekker, New York, pp 535–549

Mohapatra S, Yang X, Wright JA, Turley EA, Greenberg AH (1996) Soluble hyaluronan receptor RHAMM induces mitotic arrest by suppressing Cdc2 and cyclin B1 expression. J Exp Med 183(4):1663–1668

Moseley R, Walker M, Waddington RJ, Chen WY (2003) Comparison of the antioxidant properties of wound dressing materials--carboxymethylcellulose, hyaluronan benzyl ester and hyaluronan, towards polymorphonuclear leukocyte-derived reactive oxygen species. Biomaterials 24(9):1549–1557

Necas J, Bartosikova L, Brauner P, Kolar J (2008) Hyaluronic acid (hyaluronan): a review. Vet Med 53:397–411

Ortonne JP (2001) [Comparative study of the activity of hyaluronic acid and dextranomer in the treatment of leg ulcers of venous origin]. Annales de dermatologie et de venereologie (Suppl):13–16

Pavicic T, Gauglitz GG, Lersch P, Schwach-Abdellaoui K, Malle B, Korting HC et al (2011) Efficacy of cream-based novel formulations of hyaluronic acid of different molecular weights in anti-wrinkle treatment. J Drugs Dermatol 10(9):990–1000

Price RD, Berry MG, Navsaria HA (2007) Hyaluronic acid: the scientific and clinical evidence. J Plast Reconstr Aesthet Surg 60(10):1110–1119

Prisell PT, Camber O, Hiselius J, Norstedt G (1992) Evaluation of hyaluronan as a vehicle for peptide growth factors. Int J Pharm 85:51–60

Rossi S, Marciello M, Sandri G, Ferrari F, Bonferoni MC, Papetti A et al (2007) Wound dressings based on chitosans and hyaluronic acid for the release of chlorhexidine diacetate in skin ulcer therapy. Pharm Dev Technol 12(4):415–422

Rossler A, Hinghofer-Szalkay H (2003) Hyaluronan fragments: an information-carrying system? Horm Metab Res 35(2):67–68

Stern R, Asari AA, Sugahara KN (2006) Hyaluronan fragments: an information-rich system. Eur J Cell Biol 85(8):699–715

Stern R, Kogan G, Jedrzejas MJ, Soltes L (2007) The many ways to cleave hyaluronan. Biotechnol Adv 25(6):537–557

Tanaka K, inventor (2004) Pharmaceutical composition containing viscoelastic substance and medication. US 20040038936 A1

Taylor KR, Trowbridge JM, Rudisill JA, Termeer CC, Simon JC, Gallo RL (2004) Hyaluronan fragments stimulate endothelial recognition of injury through TLR4. J Biol Chem 279(17):17079–17084

Trommer H, Wartewig S, Bottcher R, Poppl A, Hoentsch J, Ozegowski JH et al (2003) The effects of hyaluronan and its fragments on lipid models exposed to UV irradiation. Int J Pharm 254(2): 223–234

Vazquez JR, Short B, Findlow AH, Nixon BP, Boulton AJ, Armstrong DG (2003) Outcomes of hyaluronan therapy in diabetic foot wounds. Diabetes Res Clin Pract 59(2):123–127

Voelcker V, Gebhardt C, Averbeck M, Saalbach A, Wolf V, Weih F et al (2008) Hyaluronan fragments induce cytokine and metalloprotease upregulation in human melanoma cells in part by signalling via TLR4. Exp Dermatol 17(2):100–107

Voinchet V, Vasseur P, Kern J (2006) Efficacy and safety of hyaluronic acid in the management of acute wounds. Am J Clin Dermatol 7(6):353–357

Weindl G, Schaller M, Schafer-Korting M, Korting HC (2004) Hyaluronic acid in the treatment and prevention of skin diseases: molecular biological, pharmaceutical and clinical aspects. Skin Pharmacol Physiol 17(5):207–213

Witting M, Boreham A, Brodwolf R, Vávrová K, Alexiev U, Friess W et al (2015) Interactions of hyaluronic Acid with the skin and implications for the dermal delivery of biomacromolecules. Mol Pharm. 12(5):1391–401

Xie Y, Upton Z, Richards S, Rizzi SC, Leavesley DI (2011) Hyaluronic acid: evaluation as a potential delivery vehicle for vitronectin: growth factor complexes in wound healing applications. J Control Release 153(3):225–232

Yang JA, Kim ES, Kwon JH, Kim H, Shin JH, Yun SH et al (2012) Transdermal delivery of hyaluronic acid – human growth hormone conjugate. Biomaterials 33(25):5947–5954

Zavan B, Vindigni V, Vezzu K, Zorzato G, Luni C, Abatangelo G et al (2009) Hyaluronan based porous nano-particles enriched with growth factors for the treatment of ulcers: a placebo-controlled study. J Mater Sci Mater Med 20(1):235–247

Zhang LS, Greyner HJ, Mummert ME, Petroll WM (2009) Development of a hyaluronan bioconjugate for the topical treatment of melanoma. J Dermatol Sci 55(1):56–59

Chemical Penetration Enhancers: Classification and Mode of Action

2

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Contents

2.1 Introduction

Skin is the largest organ in humans, which covers the whole body and protects it against water loss and against undesired penetration of exogenous substances from the environment into the skin, i.e. body. This barrier property of the skin is the main obstacle for the drug penetration into or through the skin. Various strategies are developed to overcome the impermeability of the stratum corneum (SC), being the outermost layer of the skin which provides the primary barrier to percutaneous drug penetration. Circumventing this barrier is a prerequisite for an efficient dermal/transdermal administration of drugs. One approach for

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N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_2

improving percutaneous drug penetration, which has been extensively investigated, is the use of chemical penetration enhancers (CPE), also called sorption promoters or accelerants.

2.2 Chemical Penetration Enhancers (CPEs)

Chemical penetration enhancers are agents that partition into and interact with the components of the SC, increasing skin permeability in a temporary, reversible manner.

Thus, skin penetration enhancers reversibly reduce the barrier properties, i.e. resistance of the SC to drug penetration, and allow drugs to penetrate more readily into the viable skin tissue and in some cases also into the systemic circulation (CPEs increase drug transport across the skin) (Barry 1991).

Advantages of chemical enhancers over physical enhancers (such as iontophoresis, sonophoresis, electroporation, etc.) are design flexibility, ease of application, the possibility of self-administration and prolonged drug delivery through patches, patient compliance and their incorporation into inexpensive and simple formulations (Karande and Mitragotri 2009). These advantages make chemical penetration enhancers a universally studied subject in dermal and transdermal drug delivery. However, there are a few disadvantages of this penetration enhancement method: a number of chemicals tested as penetration enhancers can cause skin irritation; some of them have low efficacy at used therapeutic levels, and chemical enhancers are not suitable for the delivery of macromolecules.

To be considered as a candidate for a CPE, a chemical compound should meet the following desirable criteria (Williams and Barry 2004):

1. It should not irritate the skin and should be non-toxic and non-allergenic.
2. It should have a rapid but reproducible and predictable effect.
3. It should not have any pharmacological activity.
4. It should work unidirectional, i.e. enhance drug penetration into the skin whilst preventing the loss of endogenous material from the body.
5. Upon removal of the enhancer, a quick and complete recovery of skin properties is expected.
6. It should be colourless and odourless.
7. It should be compatible with drugs and other excipients in the formulation.
8. It should be cosmetically acceptable when applied to the skin.

Most substances fulfil some, but not all of the above criteria. No CPE has yet been developed which possesses all the aforementioned ideal properties. Despite showing some limitations, a consistent group of chemical compounds has been used for years safely and effectively in dermal and transdermal drug delivery systems.

2.3 Classification of Chemical Penetration Enhancers

CPEs form a diverse pool of chemical compounds that can be classified into groups on the basis of how structurally related they are (Table 2.1) (Williams and Barry 2004). An extensive literature review of compounds, cited as skin penetration enhancers, can be found in Osborne and Henke (1997).

Penetration enhancers often work well when used together, i.e. they show synergistic effects in enhancing the penetration of the drug into/through the skin (when used together, they exert higher effects than when used alone). Examples of such synergistic mixtures are Azone® and fatty acids, terpenes and PG, etc. (Williams and Barry 2004).

In addition to the aforementioned classification, Asbill and Michniak (2000) classified chemical penetration enhancers according to their activity into the following groups:

- Penetration enhancers that have both dermal and transdermal activities, i.e. enhance the penetration of the drug into and through the skin, respectively
- Penetration enhancers that have transdermal activity
- Penetration enhancers that have only dermal activity

Table 2.1 Classification of chemical penetration enhancers based on their chemical structure and examples of most used enhancers

Chemical class	Enhancer
Alcohols	*Short-chain alcohols* Ethanol Isopropyl alcohol *Long-chain alcohols* Decanol Octanol *Glycols* Propylene glycol (PG)
Amides	*Cyclic amides* Azone®(1-dodecylazacycloheptan-2-one or laurocapram)
Fatty acids	Lauric acid Oleic acid Linoleic acid
Esters	*Alkyl esters* Ethyl acetate Butyl acetate Methyl acetate *Fatty acid esters* Isopropyl myristate Isopropyl palmitate
Ether alcohols	Transcutol®(diethylene glycol monoethyl ether)
Surfactants	*Anionic surfactants* Sodium lauryl sulphate (SLS) *Cationic surfactants* Benzalkonium chloride Cetylpyridinium chloride Cetyltrimethylammonium bromide; *Nonionic surfactants* Polysorbates (Tween® 20, Tween® 80, etc.) *Zwitterionic surfactants* Dodecyl betaine
Sulphoxides and analogues	Dimethyl sulphoxide (DMSO) Decylmethyl sulphoxide (DCMS)
Essential oils	Eucalyptus Ylang ylang Chenopodium
Terpenes and its derivatives	D-Limonene L-Menthol 1,8-Cineole
Pyrrolidones	N-methyl-1-2-pyrrolidone (NMP) 2-pyrrolidone (2P)
Oxazolidines	4-decyloxazolidin-2-one
Phospholipids	Phosphatidylcholine (PC)
Enzymes	Acid phosphatase, papain, phospholipase C

- Penetration retarders that deliver low concentrations of the drug into the skin and decrease drug flux

2.4 Mechanisms of Action of Chemical Penetration Enhancers

Penetration enhancers can also be classified according to their mechanism of action. Before we review the lipid-protein partitioning (LPP) theory, it would be useful to have a quick reminder of the parameters that determine the diffusion of a molecule through the skin. Factors affecting the drug permeation rate through the SC can be considered using the steady-state flux equation (Eq. 2.1) (Barry 1983):

$$\frac{dm}{dt} = \frac{DC_0K}{h} \tag{2.1}$$

dm/dt is the steady-state flux, representing the cumulative mass of the diffusant, m, passing per unit area of membrane in time t.

C_0 is the constant donor drug concentration.

K is the partition coefficient of a solute between membrane and bathing solution.

D is the diffusion coefficient.

h is the membrane thickness.

With these parameters in mind, effective penetration enhancers can increase transdermal drug delivery by (Williams and Barry 2004):

- Increasing the diffusion coefficient of the drug in the SC (e.g. by disrupting the SC lipid organisation)
- Increasing the drug concentration in the vehicle (e.g. by acting as an anti-solvent)
- Improving the partitioning between the drug formulation and the SC (e.g. by changing the solvent nature of the skin membrane in order to enhance the drug partitioning into the skin)
- Decreasing skin thickness, which is less likely

Barry and co-workers (Barry 1991; Williams and Barry 1991) postulated the lipid-protein partitioning (LPP) concept to help categorise penetration enhancers and to describe how they alter the permeability of the skin. This theory suggests three possible ways in which the permeability of the skin can be adjusted to allow easier drug transport (Fig. 2.1):

1. Disruption of the intercellular lipid bilayer (lipid modification)

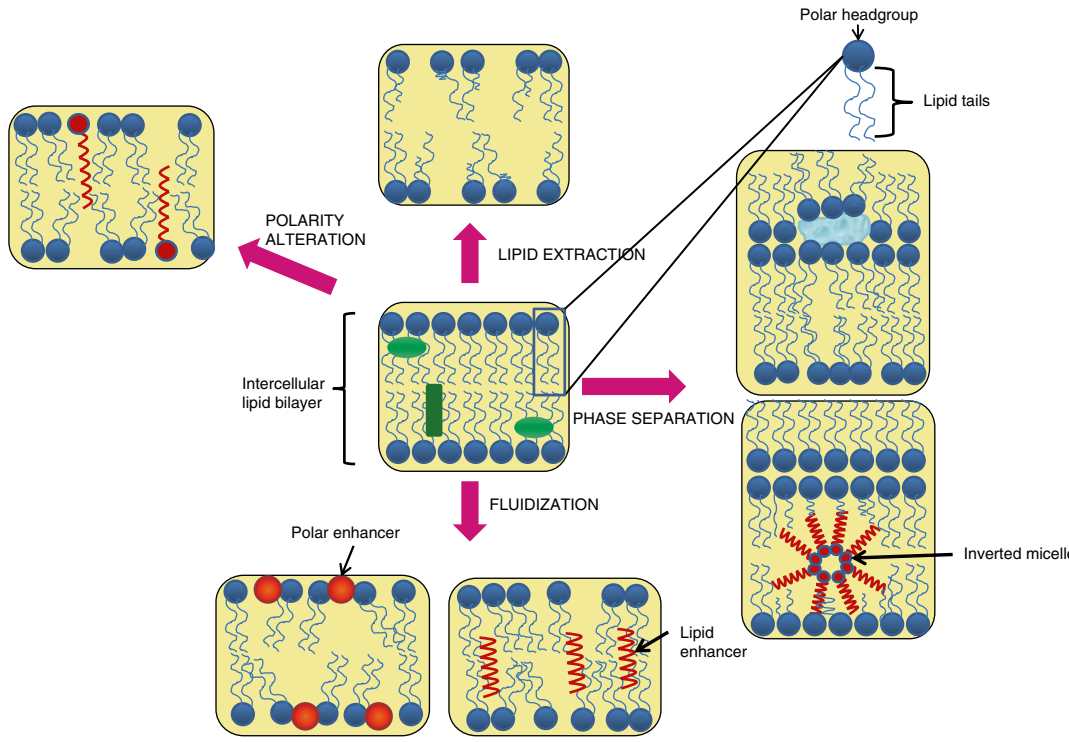

Fig. 2.1 Possible effects of chemical penetration enhancers on the structure of the lipid bilayer (Modified from Barry 2004)

2. Interaction with the intracellular proteins of the SC (protein modification)
3. Increasing partitioning of a drug, co-enhancer or co-solvent into the SC (partitioning promotion)

CPEs act by one or usually by more of the aforementioned modes of action.

2.4.1 Lipid Modification

In this mode of action, the enhancers will change the structural organisation of the lipid bilayers of the SC (Fig. 2.1). As a result, the SC barrier will become more permeable and the diffusion coefficient of the drug would increase (Eq. 2.1). Enhancer molecules will form microcavities within the lipid bilayer and in this way increase the free volume available for drug diffusion. This disturbance of the lipid bilayer can be homogeneous when the enhancer molecules are dispersed evenly throughout the lipids. Azone®, terpenes, fatty acids, alcohols and DMSO favour the homogeneous type of behaviour (Barry 2001; Benson 2005). Penetration enhancers (e.g. oleic acid and terpenes at high concentration) can also concentrate heterogeneously (i.e. they phase separate – the "pooling" phenomenon) within the domains of the lipid bilayers forming permeable "pores" that provide less resistance for penetration of polar molecules (Ongpipattanakul et al. 1991; Cornwell et al. 1996). Solvents such as DMSO, ethanol and chloroform/methanol mixture may extract lipids and form aqueous channels in the SC, which results in a more permeable SC (Menczel 1995; Hatta et al. 2010).

CPEs are believed to interact with lipid bilayers of the SC at three main sites (Lane et al. 2012):

1. Interaction with the polar head groups of the lipids (e.g. Azone®); these enhancers due to forming H bonds and/or by ionic forces disturb the hydration spheres of the lipid bilayers and thereby disrupt their packing order within

the polar plane, which leads to the fluidization of the intercellular lipids as well as to the increase of the water volume between the lipid layers; the consequence is a decreased resistance to drug diffusion.
2. Interaction in the aqueous domain of the lipid bilayers which increases the solubility of this site for the drug (e.g. propylene glycol, ethanol, Transcutol®, N-methyl pyrrolidone, etc.); these solvents change the solubility parameter of the skin in order to match the solubility parameter of the drug/permeant, which leads to an enhanced partitioning of the drug from the vehicle into the SC.
3. Interaction with the lipid alkyl chain – CPEs insert directly between the hydrophobic lipid tails (e.g. long-chained and less polar enhancers, such as oleic acid, isopropyl myristate, etc.); these enhancers disrupt the packing of lipids and thereby increase the fluidization of lipids, which increases drug diffusion through the SC.

The main penetration pathway for drugs is the intercellular route. Consequently penetration enhancers need to modify the intercellular lipids of the SC to be effective. They may interact at the level of polar head groups and/or between the hydrophobic tails of the intercellular lipids arranged in bilayers (Barry 1991). Lipophilic enhancers (i.e. oleic acid and limonene) can directly fluidize ceramide acyl chains, thus increasing the spacing of the lipid packing. In addition, they can modify the acyl chain packing indirectly by decreasing the strength of the intermolecular hydrogen bonds between the polar head groups (Guillard et al. 2009). The same study showed that hydrophilic enhancers (i.e. ethanol and DMSO) have no influence on the acyl chain organisation, but show a complex action on the polar head groups, weakening the hydrogen bonds within the polar head groups of ceramides (Guillard et al. 2009). According to du Plessis et al. (2001), these interactions compete with water-mediated intermolecular hydrogen bonding and ionic forces, thus disturbing the hydrogen spheres of the lipids resulting in alterations in the head group domain. This effect would consequently also alter the packing of the lipid acyl chains, and hence, ethanol has an indirect impact on acyl chains. On the other hand, the action of ethanol on acyl chains would be indirect, and moreover, the main mode of action of this compound is to reduce the barrier function by extraction of the SC lipids.

Moghadam et al. (2013) also showed that all three classes of investigated CPEs (solvents, terpenes and surfactants) with the exception of two solvents, propylene glycol and ethanol, affected SC lipids, i.e. they caused increased disordering of lamellar and lateral packing of lipids. The highest degree of SC lipid disordering was caused by surfactants (especially sodium dodecyl sulphate, didecyldimethylammonium bromide, didecyltrimethylammonium bromide) followed by terpenes, such as nerol.

2.4.2 Protein Modification

Another mode of action of CPEs is by interacting and binding with the keratin filaments. DMSO, ionic surfactants, urea and decylmethyl sulphoxide can interact with the keratin in the corneocytes. This may cause a conformational change in the proteins, disrupting the order in the corneocytes; keratin may be denatured or its conformation may be modified, and vacuoles can be formed. The diffusion of the drug through the SC is then increased (Eq. 2.1), and therefore, the permeation of the drug is increased, too. However, the intracellular penetration pathway is usually not important for drug permeation, and most studies suggest that lipid fluidization is the primary way of modifying the SC, which leads to an enhanced percutaneous drug penetration (Barry 2001). It was shown that solvents used at high concentrations,when enhancing drug permeation, may cause fissuring of the intercellular lipids and splitting of SC squames, by affecting the desmosomes that maintain the cohesion between corneocytes. These phenomena would cause severe skin irritation which is not acceptable, and hence, these enhancers at high concentrations do not present candidates for topical and transdermal formulations (Barry 1991; Benson 2005).

2.4.3 Partitioning Promotion

As seen from Eq. (2.1), partition coefficient (K), diffusion coefficient (D) and drug concentration in the vehicle (C_0) are the parameters that change the rate of drug penetration. Some solvents (ethanol, propylene glycol, Transcutol®, N-methyl pyrrolidone) can penetrate into the SC and change its chemical properties and thereby its solvent properties. This allows for the increase of the partitioning (K in Eq. 2.1) of a drug, co-enhancer and co-solvent into the SC and their increased solubility within the SC (Barry 2001; Benson 2005). An example is the increased penetration of nitroglycerin and estradiol by the use of ethanol as a co-solvent. Further, propylene glycol is used synergistically with Azone®, oleic acid and terpenes in order to enhance their penetration and thereby their concentration in the SC (Barry 2001).

2.4.4 Indirect Penetration Enhancement

Apart from directly affecting the skin by modes of action described above which enhance percutaneous drug permeation, chemical enhancers can modify the drug formulation itself and work indirectly on enhancing drug permeation through the skin. They can act by (Williams and Barry 2004):

1. Changing the thermodynamic activity of the vehicle, i.e. by fast permeation of a solvent from the donor into the skin, the permeant is left in a more thermodynamically active state. This effect is known as the "push effect".
2. The "drag effect", implying that the enhancer-solvent "drags" the drug with it into the skin.
3. Solubilising the permeant in the donor (e.g. surfactants change the barrier properties of the skin directly, but in addition they form micelles which ease solubilisation of the permeant); this is important for permeants with low solubility, where the use of enhancers may decrease depletion effects and extend the drug permeation through the skin (Songkro 2009).

2.5 Different Classes of Chemical Penetration Enhancers

As mentioned in Sect. 2.3, CPEs are typically classified into structurally related chemical compound groups. CPEs are able to use different mechanisms of action, and it is difficult to assign a primary mode of action for many of the chemical enhancers. For these reasons, the classification of CPEs is based on their chemical structure and not on their mechanism of action.

In the following section, some groups of CPEs will be described briefly with their known mechanisms of action. For detailed reviews, please refer to the separate chapters in this volume.

2.5.1 Terpenes

Terpenes are derived from plant essential oils, and both are used as potent penetration enhancers (Herman and Herman 2015). Terpenes are more often used, and they represent not toxic substances, which cause only mild limited irritation of the skin, and they interact reversibly with the SC lipids (Obata et al. 1991; Okabe et al. 1990). They have been classified as being generally regarded as safe (GRAS) substances (Akimoto et al. 2001). Chemical structure of terpenes is based on repeated isoprene units (C_5H_8) (with the exception of lavandulol). Depending on the number of isoprene units, they can be classified into monoterpenes (C_{10}), sesquiterpenes (C_{15}) or diterpenes (C_{20}). In addition to this, terpenes include different chemical groups (hydrocarbons, alcohols, oxides, ketones, esters) (Aqil et al. 2007). Terpenes have been studied as skin penetration enhancers for both hydrophilic (such as propranolol, bupranolol, etc.) and lipophilic drugs (such as ibuprofen, hydrocortisone, etc.) (Zhao and Singh 1999; Babu and Pandit 2005; Brain et al. 2006; Vaddi et al. 2002).

The most extensively studied terpenes are D-limonene, 1.8-cineole and L-menthol, which have been used for penetration enhancement of both hydrophilic and lipophilic drugs (Aqil et al. 2007; Song et al. 2009).

How effective terpenes are as penetration enhancers is determined by their lipophilicity, size and chirality, boiling point and energy of vaporisation and degree of unsaturation (Aqil et al. 2007).

Williams and Barry (1991) evaluated different monoterpenes as enhancers for 5-fluorouracil and found a *structure-activity relationship*. Hori et al. (1991) and Moghimi et al. (1997) confirmed that amongst terpenes, hydrophilic terpenes (alcohols, ketones and oxide terpenes, like fenchone and thymol) are more effective in enhancing the permeation of hydrophilic drugs (propranolol), whereas hydrocarbon terpenes (like limonene and cymene) are more active towards lipophilic drugs (diazepam). Moreover, Tas et al. (2007) observed that anethole, a hydrophobic terpene, enhanced significantly the absorption of the highly lipophilic etodolac, whilst the hydrophilic terpenes, menthol and carvacrol, did not enhance the absorption of etodolac. Hence, high lipophilicity of terpenes is important for enhancing the permeation of lipophilic drugs (Ghafourian et al. 2004).

The *mechanism of drug penetration enhancement* induced by terpenes depends on the applied drug. In the case of hydrophilic drugs (such as 5-fluorouracil), the primary mechanism of action of terpenes is to increase drug diffusivity in the SC by disrupting the lipid structure of the SC (Williams and Barry 1991; Cornwell and Barry 1994). As to partitioning, terpenes do not increase the partitioning of the hydrophilic drug into the human SC, since the drug (e.g. 5-fluorouracil) is less soluble in all terpenes than in water. For more lipophilic drugs (such as estradiol), terpenes increase drug diffusivity, but also increase drug partitioning into the SC (Williams and Barry 1991). The increase in partitioning is proposed to be due to solvent effects, since estradiol is moderately soluble in many terpenes. The permeation of lipophilic drugs increases proportionally to their solubility in the enhancer (Williams and Barry 1991).

The mechanism by which terpenes *increase drug diffusivity* in the SC, being the main mode of terpenes' penetration enhancing action, has been investigated by different techniques. It has been shown by differential scanning calorimetry (DSC) measurements that terpenes reduce lipid phase transition temperatures, indicating that they may increase SC permeability by disrupting the intercellular lipid bilayers (Williams and Barry 1989; Cornwell and Barry 1994), which has been confirmed for 1.8-cineole, menthone and nerolidol (in propylene glycol/water systems), being lipid disruptive, whereas no clear proof was found for the lipid disruptive effect of D-limonene (Yamane et al. 1995; Cornwell et al. 1996). Obtained DSC results revealed that D-Limonene produced a freezing point depression effect on SC lipids, implying little interaction with lipids at skin temperature, whilst its small enhancement effect is believed to be due to phase separation of the oil in SC lipid bilayers (Yamane et al. 1995; Cornwell et al. 1996). In contrast, small angle X-ray diffraction (SAXD) studies have indicated that besides 1,8-cineole, also D-limonene disrupts SC bilayer lipids (i.e. induces reductions in bilayer periodicity), whereas nerolidol (a long-chain sesquiterpene) reinforces the bilayers, possibly by orienting alongside the SC lipids (Cornwell et al. 1996). According to Cornwell and Barry (1994), nerolidol disrupted the organised packing of the SC lipids due to its amphiphilic structure and was able to increase the diffusivity of 5-fluorouracil.

Recent SAXD and wide angle X-ray diffraction (WAXD) measurements have confirmed that D-limonene caused a slight disruption of the organised lipid bilayers' structures (the hexagonal hydrocarbon-chain packing structure was much strongly disrupted than the orthorhombic one), an increase of the repeat distance of the long lamellar structure by incorporating D-limonene molecules and formation of "pools" of D-limonene in the hydrophobic region of the intercellular lipid matrix in the SC (Hatta et al. 2010). As to L-menthol, SAXD and WAXD studies revealed that it increased the repeat distance of the lamellar structure in lipid bilayers and inhibited the formation of hexagonal hydrocarbon chain packing. In addition, DSC studies showed that it decreased the phase transition temperature of ceramide 2/cholesterol mixtures. Thus, L-menthol changed the lamellar structure and the intermolecular interaction between ceramide 2 and

cholesterol, indicating fluidization of lipid structures (ceramide 2/cholesterol mixtures), which promotes the percutaneous drug permeation (Watanabe et al. 2009). Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) study revealed that administration of L-menthol causes disorder of the intercellular lipids in the SC similar to that of heat application (Obata et al. 2010), which was also proposed by Watanabe et al. (2009). DSC and ATR-FTIR studies with 1,8-cineole and L-menthol revealed that both terpenes exerted effects on both lipid acyl tails and polar head groups, as demonstrated by a reduction in the main transition temperature (T_m) and in the non-hydrogen bonded amide I stretching frequency, respectively (Narishetty and Panchagnula 2005). However, at physiological temperature, terpenes mainly act at polar head groups and break the inter- and intralamellar hydrogen bonding network, which decreases integrity in the SC barrier. Terpenes also increased the hydration levels of the lipid system probably by forming new aqueous channels. These results indicated that 1.8-cineole and L-menthol enhanced transdermal permeation of the investigated drug zidovudine by transforming SC lipids from a highly ordered orthorhombic perpendicular subcellular packing to a less ordered hexagonal subcellular packing (Narishetty and Panchagnula 2004).

Jain et al. (2002) showed using FTIR that terpenes with weak self-association or which have the ability of donating or accepting H bonds (like menthol, cineole, menthone) increase the permeation of a water-soluble, polar drug, imipramine hydrochloride, through the breaking of hydrogen bonds between ceramides' polar head groups.

Linalool, carvacrol and terpineol were suggested to interact with SC lipids to enhance the transdermal delivery of haloperidol across human skin (Vaddi et al. 2002).

Terpenes, when applied in high quantities, can increase drug diffusivity also by making "pools" inside the SC. In this case they are phase separated within the SC from undisrupted lipid bilayers, i.e. they exist within separate domains in the SC. In this way permeable "pores" are formed, being important for the permeation of polar drugs (Cornwell and Barry 1994; Cornwell et al. 1996).

Terpenes can also enhance drug diffusivity by extraction of SC lipids. FTIR studies revealed the permeation enhancement of nicorandil by nerolidol and carvone due to partial lipid extraction (Krishnaiah et al. 2006); propranolol hydrochloride by menthone and D-limonene (in combination with ethanol) due to lipid extraction, macroscopic barrier perturbations and increased partitioning of the drug to the SC (Zhao and Singh 1999); and tamoxifen by eugenol and D-limonene (in combination with ethanol) due to lipid extraction and increased partitioning and by menthone only due to lipid extraction (Zhao and Singh 1998). Enhanced permeability of the SC to tamoxifen due to lipid extraction and macroscopic barrier perturbation was also found after application of menthone, eugenol and limonene in combination with propylene glycol. Moreover, the effective diffusion coefficient of tamoxifen through the epidermis was enhanced following treatment with either eugenol or limonene (Zhao and Singh 2000). FTIR and DSC studies revealed that the diterpene forskolin enhanced skin permeation of valsartan by disruption and extraction of lipid bilayers of the SC (Rizwan et al. 2008).

Besides lipid modification, also protein modification can influence the drug diffusivity after application of terpenes, as a proportion of terpenes may distribute into the corneocytes. In the corneocytes, terpenes interact with keratin, opening up the dense protein structure making it more permeable. Despite higher partitioning of terpenes into the lipid domains, their uptake into the protein domains may be significant due to the fact that intracellular protein domains make up 70–95 % of the SC volume (Cornwell et al. 1996). However, the transcellular penetration route has not been recognised as an important route.

As to indirect action of terpenes on the percutaneous drug penetration, it was found that 1,8-cineole increased the permeation of mefenamic acid by the "drag" or "pull" effect (Heard et al. 2006). Terpenes may also increase the drug permeation by increasing its thermodynamic activity in the vehicle (Kanikkannan et al. 2000).

Terpenes have mostly been used together with propylene glycol as they provide in that combination a higher enhancer efficacy, because of their

synergistic effect. The combination of menthol/propylene glycol enhanced the permeation of propofol (Yamato et al. 2009) and imipramine hydrochloride (Shah et al. 2008). DSC and SAXD investigations provided fragmented evidence that terpene/propylene glycol synergy may produce enhanced lipid bilayer disruption (Cornwell et al. 1996). It has also been proposed that propylene glycol increases the partitioning of the terpene into the SC, where it can exert its enhancer effect (Barry 2001). Terpenes have also been used with ethanol. 1,8-cineole and L-menthol applied at 5 % w/v in 66.6 % ethanol as a vehicle significantly enhanced the pseudo-steady-state flux of zidovudine (Narishetty and Panchagnula 2005). Menthone (1, 2, 3 and 5 %) in combination with 50 % ethanol (Zhao et al. 2001) and 5 % w/v of other terpenes (carvone, 1.8-cineole, menthol and thymol) in 50 % ethanol (Gao and Singh 1998) significantly enhanced the flux of tamoxifen compared to the control (50 % ethanol).

2.5.2 Azone

Azone® (1-dodecylazacycloheptan-2-one or laurocapram) was the first synthetic penetration enhancer. It shows very low toxicity and only mild skin irritancy. Structurally it has a polar head group attached to a C12 chain; therefore, it is very lipophilic. It can enhance the penetration of both hydrophilic and lipophilic drugs (Jampilek and Brychtova 2012). Azone® is one of the most studied chemical penetration enhancers, but despite this, it is not available in commercial topical or transdermal products. Azone® is a very effective chemical penetration enhancer at concentrations of 0.1–5 % (Jampilek and Brychtova 2012). Its mechanism of action is still not clear. Different hypotheses were suggested to explain the enhancing activity of Azone®. On the basis of DSC, FTIR and drug permeation studies, it was proposed that Azone® acts on the lipid bilayers of the SC, fluidizing them which leads to an increase of the drug diffusion (Harrison et al. 1996a, b). These authors assumed a homogeneous distribution of Azone® through the SC lipid array.

Due to the structure of Azone®, Hadgraft et al. (1996) suggested that hydrogen bonds are formed between the polar head group of Azone® and ceramides, being important for its penetration-enhancing activity. According to Hadgraft (1999), Azone® due to its long chain easily integrates into the bilayer and then the polar head group oxygen competes for the H bonds in the ceramide head groups, weakening them and thus forming the so-called channels, i.e. areas of increased fluidity in the lamellae that facilitate drug penetration.

Jampilek and Brychtova (2012) suggest that Azone® disrupts the packing of the lipid bilayer of the SC by heterogeneously partitioning into it and spreading across separate domains of the bilayer. This assumption is supported by Azone's angled conformation that forms a shape of a bent spoon which eases its intercalation between ceramides of the SC. Electron diffraction studies provided evidence that Azone® exists or partly exists as a distinct phase (i.e. in separate domains) within the SC lipids (Pilgram et al. 2001).

2.5.3 Fatty Acids

Fatty acids have been approved by the FDA as GRAS and have been used as penetration enhancers in some commercially available products (Ibrahim and Li 2010). Their effectiveness as penetration enhancers depends on their degree of saturation (and site of saturation), chain length and their structure. It was found for saturated fatty acids that a chain length of C10 to C12 would be optimal for an effective penetration enhancer, whilst in unsaturated fatty acids, C18 was optimal (Aungst et al. 1986; Aungst 1989). The higher the degree of unsaturation, the better enhancer the fatty acid would be. In the case of unsaturated fatty acids, the cis conformation allows for a higher disruption of the lipids in the SC than the trans conformation and a higher drug flux was obtained when the distance of the double bond from the carboxylic group was larger (Golden et al. 1987). Oleic acid presents the most investigated fatty acid as it was shown to be a potent penetration enhancer for various drugs

(Choi et al. 2012; Baek et al. 2013; Patel et al. 2014). It has been used in commercial transdermal products (Lane 2013). Oleic acid in its cis conformation was shown to increase lipid fluidity in porcine skin (Golden et al. 1987). From its structure, it is expected that oleic acid interacts with lipid chains in the SC, thus disturbing the lipid packing which would increase the bilayer fluidity. FTIR studies proposed a penetration-enhancing mechanism in which oleic acid exists as a separate phase, i.e. forms a "pool" in the SC lipids (Ongpipattanakul et al. 1991), which was confirmed by freeze fracture electron microscopy studies (Tanojo et al. 1997). Thus, oleic acid enhances drug penetration by forming permeable defects within the SC lipids, which results from the cis double bond of oleic acid favouring it to condense with itself rather than distribute homogeneously in the skin lipids (Lane 2013).

Because of their lipophilic nature, fatty acids are often used with co-solvents (such as propylene glycol, benzyl alcohol, isopropyl myristate). Studies showed that fatty acids and co-solvents act in a synergistic way to enhance the penetration of a drug. The mechanism of action proposed is the increased partitioning of fatty acids into the SC by the application of co-solvents (Barry 2001; Ibrahim and Li 2010).

2.5.4 Alcohols

Transdermal and dermal delivery systems often utilise alcohols to improve penetration enhancement of drugs. Alcohols used can be within the short-chain (ethanol, isopropyl alcohol) or the long-chain group, i.e. fatty alcohols (1-butanol, 1-propanol, 1-octanol, decanol, etc.) (Williams and Barry 2004; Lane 2013). Other alcohols, such as glycols (especially propylene glycol), have also been used in dermal and transdermal products.

Alcohols can exert their permeation-enhancing activity through various mechanisms: extraction of lipids and proteins, fluidization of lipids, increasing drug partitioning and drug solubility and changing the thermodynamic activity of the drug (Lane 2013; Williams and Barry 2004).

Studies with ethanol showed that it was effective in enhancing the flux of levonorgestrel, hydrocortisone, estradiol and 5-fluorouracil through rat skin in vitro (Williams and Barry 2004), estradiol through human skin in vivo (Pershing et al. 1990) and hinokitiol through hairless mouse skin in vitro (Joo et al. 2008). There are reports that suggested that the effect of ethanol on SC is concentration dependent, i.e. with the increase of ethanol concentration, the drug diffusion is enhanced up to an ethanol concentration whereas higher concentrations decrease drug penetration (Berner et al. 1989; Megrab et al. 1995; Thomas and Panchagnula 2003; Watkinson et al. 2009a). Ethanol can cause lipid and protein extraction from the SC when used at high concentrations (75 %v/v), and it will cause dehydration when used as absolute (Goates and Knutson 1994; Megrab et al. 1995). It is believed that skin dehydration which occurs at higher ethanol concentrations reduces percutaneous drug permeation (Williams and Barry 2004).

As to the mechanism of the permeation-enhancing effect of ethanol, ethanol may exert it through various mechanisms.

Being a solvent, ethanol can increase the solubility of the drug in the donor phase, which is important for poorly soluble drugs that may deplete within in the donor. Kadir et al. (1987) suggested that in patch formulations, ethanol can use the "push effect" mechanism to promote a greater driving force for the drug permeation. This effect happens due to the fast permeation of ethanol or ethanol evaporation from the donor phase, which makes the drug concentration higher reaching a supersaturated state, possessing higher driving force for drug permeation. Another mechanism which arises also from the rapid ethanol permeation is the "pull" or "drag" effect, where ethanol carries the permeant with it (Heard et al. 2006). However, that mechanism has been rejected in the case of morphine hydrochloride permeation from formulations where ethanol was used as a solvent (Morimoto et al. 2002).

As to the effect on the skin barrier function by acting on intercellular lipids, many enhancers and also ethanol act mainly in this way. According to Barry (2001), these enhancers may disrupt the SC

lipid organisation by penetrating into the lipid bilayers of the SC, where they can rotate, vibrate and form microcavities, thereby increasing the free volume for drug diffusion. Ethanol as a hydrophilic enhancer exerts a direct effect on the polar head groups of the ceramides in the SC by weakening their H bonds, which results in alterations in the head group domain (Guillard et al. 2009). Due to this effect, ethanol exerts an indirect effect on the organisation of acyl chains in the SC lipid bilayers (du Plessis et al. 2001), i.e. this kind of perturbations leads to fluidization of the lipid domain and increase of the water volume between layers, resulting in decreased resistance to drug diffusion and increased drug permeation (Lane et al. 2012). Hatta et al. (2010) used small- and wide-angle X-ray diffraction to follow the changes in the SC lipids and proteins after applying ethanol. This study showed that ethanol caused a slight disruption of the lipid structure, more strongly at the orthorhombic hydrocarbon chain packaging, and partial disruption of the soft keratin structure in the corneocytes. In addition to this, the authors suggest that ethanol formed "pools" in the hydrophilic region of the intercellular lipid matrix of the SC. The results of this study indicate that ethanol generates routes through which hydrophilic molecules penetrate the skin. When used at high concentrations for a prolonged time, ethanol can extract some of the lipid fraction from within the SC, as aforementioned (Williams and Barry 2004). Thermogravimetric analysis (TGA) and FTIR spectroscopy revealed that the application of ethanol, due to extraction of SC lipids, dehydrated the skin as it decreased the bound water content. This decreased the permeation of the solute, whilst increasing TEWL, and partitioning was predominant for the permeation of the solute (Shah et al. 2008).

As to partitioning promotion, ethanol as a solvent can influence the solubility of the skin tissue which results in improved partitioning of the drug into the skin membrane (Megrab et al. 1995). This effect of ethanol occurs due to its effect on the aqueous domain of the lipid bilayers, which increases the solubility of this site for the drug, i.e. it alters the solubility parameter of the skin by shifting it to a value close to that of the drug (Lane et al. 2012).

Ethanol has been due to its advantages used as a penetration enhancer in various commercial topical and transdermal preparations. For an overview of topical and transdermal preparations containing penetration enhancers available on the market, refer to Lane (2013).

2.5.5 Glycols

Propylene glycol is the most commonly used glycol in dermal and transdermal formulations (for commercial formulations, refer to Lane (2013)). It has been used as a co-solvent and has been recognised for its penetration-enhancing properties. Propylene glycol can be applied alone or as a vehicle for other penetration enhancers (such as oleic acid) and shows synergistic action when used in combination. The exact mechanism of its drug penetration-enhancing ability is not clearly understood; however, it is believed to be similar to that of ethanol. As a co-solvent, propylene glycol permeates rapidly through the skin which may alter the thermodynamic activity of the drug and modify/enhance the driving force for drug diffusion ("push effect") (Williams and Barry 2004). The "pull" or "drag effect" was also proposed for propylene glycol (Hoelgaard and Mollgaard 1985). It has been observed that the permeant penetration depth is highly correlated with the depth of penetration of PG (Pudney et al. 2007; Bonnist et al. 2011). Further, PG affects also the lipid domains in the SC in different ways. One of them is due to the fast partition of propylene glycol into the tissue where it interacts with the aqueous domain of lipid bilayers, changing the solubility properties of this site of skin for the drug, thereby increasing drug solubility and hence drug partitioning into the skin (Lane et al. 2012). The significant permeation enhancement of triprolidine base from PG was explained by this mode of action of PG (Kasting et al. 1993). Watkinson et al. (2009b) also reported that the main penetration-enhancing mechanism of PG in increasing drug (ibuprofen) permeation was by increasing drug solubility and partitioning and not diffusion. Bouwstra et al. (1991) proposed also that PG does not intercalate within the lipid

bilayers but may be incorporated in the polar head group domain of the packed lipids. This is in accordance with a recent study (Brinkmann and Müller-Goymann 2005) which revealed that PG integrates into the hydrophilic regions of the lipid bilayers between the polar head groups in the perpendicular direction to the bilayer, thereby increasing the distance in the lamellar phase. Further, findings from a study suggested that PG caused skin dehydration (Bouwstra et al. 1989). Shah et al. (2008) showed that PG and ethanol decreased the bound water content through protein alterations and extraction of lipids, respectively, causing dehydration and increased partitioning of the drug through dehydrated skin. Several research studies showed a correlation between the PG concentration used and the degree of drug penetration (Trottet et al. 2004; Watkinson et al. 2009b).

Propylene glycol shows synergistic action with terpenes (Cornwell et al. 1996; Yamato et al. 2009; Furuishi et al. 2013), oleic acid (Brinkmann and Müller-Goymann 2005; Choi et al. 2012), Azone® (Brinkmann and Müller-Goymann 2005) and other penetration enhancers.

2.5.6 Surfactants

Many studies that used surfactants in transdermal delivery systems have reported that they are effective as chemical penetration enhancers (Som et al. 2012). The major drawback when using surfactants is their skin irritancy and toxicity, which defines their limited use in vivo (for topical and transdermal penetration enhancement). Surfactants are amphiphatic molecules that have a hydrophobic part (lipophilic alkyl or aryl fatty chain) and a hydrophilic part (polar head group). They can be classified according to the nature of their polar group into: anionic, cationic, zwitterionic and nonionic surfactants. It should be kept in mind when applying surfactants that they are able to form micelles, solubilise the active and lower its thermodynamic activity and ultimately its skin permeation (Lane 2013). Effectiveness as chemical penetration enhancers will depend on the structure, chain length and polarity of the surfactant. Ionic surfactants interact with both keratin in the corneocytes and the lipid matrix of the SC (Som et al. 2012). Anionic surfactants such as sodium lauryl sulphate (SLS) interact with the lipids and keratin in the skin, and after increased exposure to them, they show greater penetration enhancement of the drug but also greater damage to the skin than other surfactants. Cationic surfactants (benzalkonium chloride, cetylpyridinium chloride) are even more potent in penetration enhancement of drugs and unfortunately in damaging the skin (Som et al. 2012). They interact with the SC proteins through polar and hydrophobic interactions. As they are strong skin irritants, they are generally not used as CPE for dermal and transdermal penetration enhancement. Nonionic surfactants (polyoxyethylene sorbitan fatty acid esters (polysorbates) – such as Tween 20, Tween 80, polyoxyethylene alkyl ethers (Brij), etc.) are better tolerated than the surfactants with the charged head group, but are less efficient as CPE (Williams and Barry 2004). The mechanism of action of nonionic surfactants includes the following effects: (1) on the lipid level: increasing the fluidity of the lipid bilayer, solubilisation and extraction of the lipids; (2) on the level of proteins: interactions with the keratin filaments in corneocytes; and (3) on the level of the drug: changing the thermodynamic activity of the drug, allowing more effective penetration into the skin. Zwitterionic surfactants (dodecyl betaine, hexadecyl betaine, hexadecylsulfobetaine, N, N-dimethyl-N-dodecyl amine oxide, dodecyltrimethylammonium bromide) also use fluidization of the lipids as their mechanism of action (Ridout et al. 1991).

2.5.7 Sulphoxides

DMSO has initially been used as a co-solvent in transdermal formulations to help improve drug partitioning into the skin (Karande and Mitragotri 2009). It is colourless, odourless and hygroscopic. Many studies reported its use as a penetration enhancer for both hydrophilic and lipophilic compounds. It has been shown to enhance the percutaneous penetration of antiviral agents, steroids and

antibiotics (Williams and Barry 2004). DMSO can enhance the drug penetration via a number of suggested mechanisms: extraction of skin lipids; interactions with keratin, such as changing the intercellular keratin conformation, from α helical to a β sheet, as well as displacement of bound water from keratin; and interactions with the lipid alkyl chains in the SC (Williams and Barry 2004; Lane 2013). Barry (1987) proposed that DMSO can exert its enhancer activity indirectly forming solvent-filled "pools" where the solubility of the drug is increased and directly by displacing water molecules initially from the proteins and then from the lipid polar head groups. These actions of DMSO would enable the formation of micro-channels between the lipid bilayers. In addition to this, it was proposed that DMSO could interact with the polar head groups of lipids, thus destabilising the regular lipid structure, making it more fluid and less resistant to drug diffusion (Williams and Barry 2004). DMSO may also change the solubility properties of SC for the permeant, by affecting the aqueous domain in the lipid bilayers, and hence facilitate drug partitioning from the vehicle into the SC (Lane et al. 2012; Williams and Barry 2004).

However, for an effective penetration enhancement, high concentrations of DMSO are required (Notman et al. 2008). This in turn can cause skin irritation and the production of a malodourous metabolite in the breath. These factors limit the commercial application of DMSO in transdermal products. Other compounds in the sulphoxide group have been tested for enhancer efficacy, dimethylformamide (DMF), dimethylacetamide (DMAC) and decylmethyl sulphoxide (DCMS), but they too either have adverse effects on the skin (DMF, DMAC) or are less effective enhancers for lipophilic drugs (DCMS) (Williams and Barry 2004).

2.5.8 Esters

The most common ester investigated as a penetration enhancer is isopropyl myristate, which has been used in commercial products (Lane 2013). Different research results were reported regarding the enhancing mechanism of this fatty acid ester. DSC studies indicated that isopropyl myristate integrates itself within the lipid bilayers and causes a more fluid bilayer structure, facilitating the drug flux (Leopold and Lippold 1995). Other data implied that isopropyl myristate pretreatment resulted in a more densely packed lipid bilayer (Brinkmann and Müller-Goymann 2003). In addition, studies from Santos et al. (2012) suggested that isopropyl myristate can increase drug solubility in the SC.

Octyl salicylate (OSAL), commonly used as a chemical sunscreen and regarded as safe (up to concentrations of 5 %), has also been used as a penetration enhancer. It has been used in commercial transdermal products (Lane 2013). Again, various results have been published regarding the mechanism of action for OSAL (Lane 2013). One hypothesis is that OSAL creates solvent "pools", which help to enhance drug diffusion (Santos et al. 2012).

Also other fatty acid esters are used in commercial products, such as sorbitan monooleate, glyceryl monooleate, glyceryl monolaurate, etc. (Lane 2013).

2.5.9 Transcutol® (Diethylene Glycol Monoethyl Ether)

Transcutol® represents a potent, non-toxic, biodegradable solubiliser able to significantly enhance percutaneous penetration of different drugs, such as lidocaine (Cázares-Delgadillo et al. 2005), clonazepam (Mura et al. 2000), etc. Thus, Transcutol® has been used in a number of dermal and transdermal commercial products (Lane 2013). It has been usually used in combination with a co-solvent, such as propylene glycol (Mura et al. 2000). It has also been used together with Azone® as it shows in that combination a higher enhancing effect than when used alone (Escobar-Chávez et al. 2005).

According to Harrison et al. (1996a), Transcutol® enhanced the permeation of the model permeant 4-cyanophenol by increasing its solubility in the SC. However, further studies are needed to explain its mechanism of enhancing the percutaneous drug penetration.

2.5.10 Water

Water is the most commonly used compound in transdermal formulations and the most natural penetration enhancer. In the SC, 15–20 % of the tissue dry weight is made of water that either is bound to some structural elements of the SC (25–35 % of water in the SC) or is in a free form, able to act as a solvent for polar molecules (Williams and Barry 2004).

Mechanism of action of water in penetration is not clear. It is believed that increased hydration generally will enhance drug flux across the SC for a range of drugs. However, some authors found that occlusion does not always increase drug permeation (Bucks and Maibach 1999). For hydrophilic drugs, the proposed mode of action of water is that water increases the solubility of the polar drug and then its partitioning into the SC (Williams and Barry 2004). Results about water's ability to modify the lipid membrane are contradictory. Bouwstra et al. (2003) showed that water does not affect the packing of lipids in the SC. Van Hal et al. (1996) showed the existence of water "pools" and occasionally vesicle-like structures within the intercellular lipid bilayers, but without significant disruption of the lipid domains. The existence of water "pools" suggests that a lipid-water phase separation occurred. Elias et al. (2002) proposed the existence of an "aqueous pore pathway" in the SC. In this model, lacunar domains that are heterogeneously distributed in the lipid bilayer under physiological conditions would under special conditions (extensive hydration, occlusion, etc.) interconnect to form a continuous "aqueous pore pathway" that would allow enhanced drug permeation.

Conclusion

Despite the extensive research carried out in the field of chemical penetration enhancers for dermal and transdermal drug delivery, very few of these compounds have found their application in commercial dermal and transdermal products. Limitations of their use stem from low efficacy in enhancing dermal/transdermal drug delivery when used at low concentrations and skin irritation (Karande and Mitragotri 2009). In addition to this, the experimental design used in studies with CPEs represents a problem, i.e. a number of studies have been carried out, but mostly in vitro and in non-human skin. Further, infinite doses are mostly used instead of finite doses which simulate the in vivo application of creams, gels, lotions and sprays in patients. Experiments that simulate clinical situations would improve the current knowledge about the possible use of CPEs and contribute to a better understanding of their mode of action and their therapeutic effectiveness. The potential of chemical enhancers lies in their combined use with other enhancers – due to their possible synergistic penetration-enhancing effect – either with other chemicals (which enables higher effectiveness concomitant with a reduction of their applied concentration and thus lower skin irritation) or with physical methods for dermal and transdermal drug delivery enhancement.

References

Akimoto T, Kawahara K, Nagase Y, Aoyagi T (2001) Polymeric transdermal drug penetration enhancer. The enhancing effect of oligodimethylsiloxane containing a glucopyranosyl end group. J Control Release 77: 49–57

Aqil M, Ahad A, Sultana Y, Ali A (2007) Status of terpenes as skin penetration enhancers. Drug Discov Today 12(23–24):1061–1067

Asbill CS, Michniak BB (2000) Percutaneous penetration enhancers: local versus transdermal activity. Pharm Sci Technol Today 3(1):36–41

Aungst BJ (1989) Structure/effect studies of fatty acid isomers as skin penetration enhancers and skin irritants. Pharm Res 6(3):244–247

Aungst BJ, Rogers N, Shefter E (1986) Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactants, sulfoxides and amides. Int J Pharm 33(1–3):225–234

Babu RJ, Pandit JK (2005) Effect of penetration enhancers on the transdermal delivery of bupranolol through rat skin. Drug Deliv 12:165–169

Baek JS, Lim JH, Kang JS, Shin SC, Jung SH, Cho CW (2013) Enhanced transdermal drug delivery of zaltoprofen using a novel formulation. Int J Pharm 453(2):358–362

Barry BW (1983) Dermatological formulations: percutaneous absorption. Marcel Dekker, New York

Barry BW (1987) Mode of action of penetration enhancers in human skin. J Control Release 6(1):85–97

Barry BW (1991) Lipid–protein-partitioning theory of skin penetration enhancement. J Control Release 15: 237–248

Barry BW (2001) Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci 2:101–114

Barry BW (2004) Breaching the skin's barrier to drugs. Nat Biotechnol 22(2):165–167

Benson HA (2005) Transdermal drug delivery: penetration enhancement techniques. Curr Drug Deliv 2(1): 23–33

Berner B, Mazzenga GC, Otte JH, Steffens RJ, Juang R-H, Ebert CD (1989) Ethanol: water mutually enhanced transdermal therapeutic system: II. Skin permeation of ethanol and nitroglycerin. J Pharm Sci 78:402–407

Bonnist EY, Gorce JP, Mackay C, Pendlington RU, Pudney PD (2011) Measuring the penetration of a skin sensitizer and its delivery vehicles simultaneously with confocal Raman spectroscopy. Skin Pharmacol Physiol 24(5):274–283

Bouwstra JA, Peschier LJC, Brussee J, Boddé HE (1989) Effect of N-alkyl-azocycloheptan-2-ones including azone on the thermal behaviour of human stratum corneum. Int J Pharm 52:47–54

Bouwstra JA, de Vries MA, Gooris GS, Bras W, Brussee J, Ponec M (1991) Thermodynamic and structural aspects of skin barrier. J Control Release 1:209–220

Bouwstra JA, de Graaff A, Gooris GS, Nijsse J, Wiechers JW, van Aelst AC (2003) Water distribution and related morphology in human stratum corneum at different hydration levels. J Invest Dermatol 120(5):750–758

Brain KR, Green DM, Dykes PJ, Marks R, Bola TS (2006) The role of menthol in skin penetration from topical formulations of ibuprofen 5% in vivo. Skin Pharmacol Physiol 19:17–21

Brinkmann I, Müller-Goymann CC (2003) Role of isopropyl myristate, isopropyl alcohol and a combination of both in hydrocortisone permeation across the human stratum corneum. Skin Pharmacol Appl Skin Physiol 16:393–404

Brinkmann I, Müller-Goymann CC (2005) An attempt to clarify the influence of glycerol, propylene glycol, isopropyl myristate and a combination of propylene glycol and isopropyl myristate on human stratum corneum. Pharmazie 60(3):215–220

Bucks D, and Maibach HI (1999) Occlusion Does Not Uniformly Enhance Penetration *In Vivo*. p. 81–105 in Percutaneous Absorption, 3rd ed., Bronaugh RL, and Maibach HI, eds. New York: Marcel Dekker, Inc.

Cázares-Delgadillo J, Naik A, Kalia YN, Quintanar-Guerrero D, Ganem-Quintanar A (2005) Skin permeation enhancement by sucrose esters: a pH-dependent phenomenon. Int J Pharm 297(1–2):204–212

Choi J, Choi MK, Chong S, Chung SJ, Shim CK, Kim DD (2012) Effect of fatty acids on the transdermal delivery of donepezil: in vitro and in vivo evaluation. Int J Pharm 422(1–2):83–90

Cornwell PA, Barry BW (1994) Sesquiterpene components of volatile oils as skin penetration enhancers for the hydrophilic permeant 5-fluorouracil. J Pharm Pharmacol 46(4):261–269

Cornwell PA, Barry BW, Bouwstra JA, Gooris GS (1996) Modes of action of terpene penetration enhancers in human skin; differential scanning calorimetry, small-angle X-ray diffraction and enhancer uptake studies. Int J Pharm 127:9–26

Du Plessis J, Pugh WJ, Judefeind A, Hadgraft J (2001) The effect of hydrogen bonding on diffusion across model membranes: consideration of the number of H-bonding groups. Eur J Pharm Sci 13:135–141

Elias PM, Tsai J, Menon GK, Holleran WM, Feingold KR (2002) The potential of metabolic interventions to enhance transdermal drug delivery. J Investig Dermatol Symp Proc 7(1):79–85

Escobar-Chávez JJ, Quintanar-Guerrero D, Ganem-Quintanar A (2005) In vivo skin permeation of sodium naproxen formulated in pluronic F-127 gels: effect of Azone and Transcutol. Drug Dev Ind Pharm 31(4–5):447–454

Furuishi T, Kato Y, Fukami T, Suzuki T, Endo T, Nagase H, Ueda H, Tomono K (2013) Effect of terpenes on the skin permeation of lomerizine dihydrochloride. J Pharm Pharm Sci 16(4):551–563

Gao S, Singh J (1998) In vitro percutaneous absorption enhancement of a lipophilic drug tamoxifen by terpenes. J Control Release 51(2–3):193–199

Ghafourian T, Zandasrar P, Hamishekar H, Nokhodchi A (2004) The effect of penetration enhancers on drug delivery through skin: a QSAR study. J Control Release 99:113–125

Goates CY, Knutson K (1994) Enhanced permeation of polar compounds through human epidermis. I. Permeability and membrane structural changes in the presence of short chain alcohol. Biochim Biophys Acta 1195:169–179

Golden GM, McKie JE, Potts RO (1987) Role of stratum corneum lipid fluidity in transdermal drug flux. J Pharm Sci 98:4085–4099

Guillard EC, Laugel C, Baillet-Guffroy A (2009) Molecular interactions of penetration enhancers within ceramides organization: a FTIR approach. Eur J Pharm Sci 36:192–199

Hadgraft J (1999) Passive enhancement strategies in topical and transdermal drug delivery. Int J Pharm 184(1):1–6

Hadgraft J, Peck J, Williams D, Pugh J, Allan G (1996) Mechanisms of action of skin penetration enhancers/retarders: azone and analogues. Int J Pharm 141(1–2): 17–25

Harrison JE, Watkinson AC, Green DM, Hadgraft J, Brain K (1996a) The relative effect of azone and transcutol on permeant diffusivity and solubility in human stratum corneum. Pharm Res 13(4):542–546

Harrison JE, Groundwater PW, Brain K, Hadgraft J (1996b) Azone® induced fluidity in human stratum corneum. A fourier transform infrared spectroscopy investigation using the perdeuterated analogue. J Control Release 41(3):283–290

Hatta I, Nakazawa H, Obata Y, Ohta N, Inoue K, Yagi N (2010) Novel method to observe subtle structural modulation of stratum corneum on applying chemical agents. Chem Phys Lipids 163:381–389

Heard CM, Kung D, Thomas CP (2006) Skin penetration enhancement of mefenamic acid by ethanol and 1,8-cineole can be explained by the 'pull' effect. Int J Pharm 321:167–170

Herman A, Herman AP (2015) Essential oils and their constituents as skin penetration enhancer for transdermal drug delivery: a review. J Pharm Pharmacol 67(4):473–485

Hoelgaard A, Mollgaard B (1985) Dermal drug delivery – improvement by choice of vehicle or drug derivative. J Control Release 2:111–120

Hori M, Satoh S, Maibach HI, Guy RH (1991) Enhancement of propranolol hydrochloride and diazepam skin absorption in vitro: effect of enhancer lipophilicity. J Pharm Sci 80:32–35

Ibrahim SA, Li SK (2010) Efficiency of fatty acids as chemical penetration enhancers: mechanisms and structure enhancement relationship. Pharm Res 27(1):115–125

Jain AK, Thomas NS, Panchagnula R (2002) Transdermal drug delivery of imipramine hydrochloride: I. Effect of terpenes. J Control Release 79:93–101

Jampilek J, Brychtova K (2012) Azone analogues: classification, design, and transdermal penetration principles. Med Res Rev 32(5):907–947

Joo HH, Kim JC, Lee HY (2008) In vitro permeation study of hinokitiol: effects of vehicles and enhancers. Drug Deliv 15:19–22

Kadir R, Stempler D, Liron Z, Cohen S (1987) Delivery of theophylline into excised human skin from alkanoic acid solutions: a 'push– pull' mechanism. J Pharm Sci 76:774–779

Kanikkannan N, Kandimalla K, Lamba SS, Singh M (2000) Structure-activity relationship of chemical penetration enhancers in transdermal drug delivery. Curr Med Chem 7:593–608

Karande P, Mitragotri S (2009) Enhancement of transdermal drug delivery via synergistic action of chemicals. Biochim Biophys Acta 1788(11):2362–2373

Kasting GB, Francis WR, Roberts GE (1993) Skin penetration enhancement of triprolidine base by propylene glycol. J Pharm Sci 82(5):551–552

Krishnaiah YS, Al-Saidan SM, Chandrasekha DV, Rama B (2006) Effect of nerodilol and carvone on in vitro permeation of nicorandil across rat epidermal membrane. Drug Dev Ind Pharm 32:423–435

Lane ME (2013) Skin penetration enhancers. Int J Pharm 447:12–21

Lane ME, Santos P, Watkinson AC, Hadgraft J (2012) Passive skin permeation enhancement. In: Benson HE, Watkinson AC (eds) Topical and transdermal drug delivery. Wiley, Hoboken, pp 23–42

Leopold CS, Lippold BC (1995) An attempt to clarify the mechanism of the penetration enhancing effects of lipophilic vehicles with differential scanning calorimetry (DSC). J Pharm Pharmacol 47(4):276–281

Megrab NA, Williams AC, Barry BW (1995) Oestradiol permeation across human skin, silastic and snake skin membranes: the effects of ethanol/water co-solvent system. Int J Pharm 116:101–112

Menczel EM (1995) Delipidization of the cutaneous permeability barrier and percutaneous penetration. In: Smith EW, Maibach HI (eds) Percutaneous penetration enhancers. CRC Press, Boca Raton, pp 383–392

Moghadam SH, Saliaj E, Wettig SD, Dong C, Ivanova MV, Huzil JT, Foldvari M (2013) Effect of chemical permeation enhancers on stratum corneum barrier lipid organizational structure and interferon alpha permeability. Mol Pharm 10(6):2248–2260

Moghimi HR, Williams AC, Barry BW (1997) A lamellar matrix model for stratum corneum intercellular lipids. V. Effect of terpene penetration enhancers on the structure and thermal behaviour of the matrix. Int J Pharm 146:41–54

Morimoto H, Wada Y, Seki T, Sugibayashi K (2002) In vitro skin permeation of morphine hydrochloride during the finite application of penetration-enhancing system containing water, ethanol and L-menthol. Biol Pharm Bull 25:134–136

Mura P, Faucci MT, Bramanti G, Corti P (2000) Evaluation of transcutol as a clonazepam transdermal permeation enhancer from hydrophilic gel formulations. Eur J Pharm Sci 9(4):365–372

Narishetty ST, Panchagnula R (2004) Transdermal delivery of zidovudine: effect of terpenes and their mechanism of action. J Control Release 95(3):367–379

Narishetty ST, Panchagnula R (2005) Effect of L-menthol and 1,8-cineole on phase behavior and molecular organization of SC lipids and skin permeation of zidovudine. J Control Release 102(1):59–70

Notman R, Anwar J, Briels WJ, Noro MG, den Otter WK (2008) Simulations of skin barrier function: free energies of hydrophobic and hydrophilic transmembrane pores in ceramide bilayers. Biophys J. 95(10): 4763–71.

Obata Y, Takayama K, Machida Y, Nagai T (1991) Combined effect of cyclic monoterpenes and ethanol on percutaneous absorption of diclofenac sodium. Drug Des Discov 8:137–144

Obata Y, Utsumi S, Watanabe H, Suda M, Tokudome Y, Otsuka M, Takayama K (2010) Infrared spectroscopic study of lipid interaction in stratum corneum treated with transdermal absorption enhancers. Int J Pharm 389:18–23

Okabe H, Obata Y, Takayama K, Nagai T (1990) Percutaneous absorption enhancing effect and skin irritation of monocyclic monoterpenes. Drug Des Deliv 6:229–238

Ongpipattanakul B, Burnette RR, Potts RO, Francoeur ML (1991) Evidence that oleic acid exists in a separate phase within stratum corneum lipids. Pharm Res 7:350–354

Osborne DW, Henke JJ (1997) Skin penetration enhancers cited in the technical literature. Pharm Technol 21:58–66

Patel RP, Gaiakwad DR, Patel NA (2014) Formulation, optimization, and evaluation of a transdermal patch of heparin sodium. Drug Discov Ther 8(4):185–193

Pershing LK, Lambert LD, Knutson K (1990) Mechanism of ethanol-enhanced estradiol permeation across human skin in vivo. Pharm Res 7(2):170–175

Pilgram GS, van der Meulen J, Gooris GS, Koerten HK, Bouwstra JA (2001) The influence of two azones and sebaceous lipids on the lateral organization of lipids isolated from human stratum corneum. Biochim Biophys Acta 1511(2):244–254

Pudney PD, Mélot M, Caspers PJ, Van Der Pol A, Puppels GJ (2007) An in vivo confocal Raman study of the delivery of trans retinol to the skin. Appl Spectrosc 61(8):804–811

Ridout G, Hinz RS, Hostynek JJ, Reddy AK, Wiersema RJ, Hodson CD, Lorence CR, Guy RH (1991) The effects of zwitterionic surfactants on skin barrier function. Fundam Appl Toxicol 16(1):41–50

Rizwan M, Aqil M, Ahad A, Sultana Y, Ali MM (2008) Transdermal delivery of valsartan: I. Effect of various terpenes. Drug Dev Ind Pharm 34:618–626

Santos P, Watkinson AC, Hadgraft J, Lane ME (2012) Influence of penetration enhancer on drug permeation from volatile formulations. Int J Pharm 439(1–2): 260–268

Shah DK, Khandavilli S, Panchagnula R (2008) Alteration of skin hydration and its barrier function by vehicle and permeation enhancers: a study using TGA, FTIR, TEWL and drug permeation as markers. Methods Find Exp Clin Pharmacol 30:499–512

Som I, Bhatia K, Yasir M (2012) Status of surfactants as penetration enhancers in transdermal drug delivery. J Pharm Bioallied Sci 4(1):2–9

Song YH, Gwak HS, Chun IK (2009) The effects of terpenes on the permeation of lidocaine and ofloxacin from moisture-activated patches. Drug Deliv 16:75–81

Songkro S (2009) An overview of skin penetration enhancers: penetration enhancing activity, skin irritation potential and mechanism of action. Songklanakarin J Sci Technol 31(3):299–321

Tanojo H, Bosvangeest A, Bouwstra JA, Junginger HE, Bodde HE (1997) In-vitro human skin barrier perturbation by oleic-acid – thermal-analysis and freeze-fracture electron-microscopy studies. Thermochim Acta 293(1–2):77–85

Tas C, Ozkan Y, Okyar A, Savaser A (2007) In vitro and ex vivo permeation studies of etodolac from hydrophilic gels and effect of terpenes as enhancers. Drug Deliv 14:453–459

Thomas NS, Panchagnula R (2003) Transdermal delivery of zidovudine: effect of vehicles on permeation across rat skin and their mechanism of action. Eur J Pharm Sci 18:71–79

Trottet L, Merly C, Mirza M, Hadgraft J, Davis AF (2004) Effect of finite doses of propylene glycol on enhancement of in vitro percutaneous permeation of loperamide hydrochloride. Int J Pharm 274(1–2):213–219

Vaddi HK, Ho PC, Chan YW, Chan SY (2002) Terpenes in propylene glycol as skin-penetration enhancers: permeation and partition of haloperidol, fourier transform infrared spectroscopy, and differential scanning calorimetry. J Pharm Sci 91:1639–1651

Van Hal DA, Jeremiasse E, Junginger HE, Spies F, Bouwstra JA (1996) Structure of fully hydrated human stratum corneum: a freeze fracture electron microscopy study. J Invest Dermatol 106:89–95

Watanabe H, Obata Y, Ishida K, Takayama K (2009) Effect of l-menthol on the thermotropic behavior of ceramide 2/cholesterol mixtures as a model for the intercellular lipids in stratum corneum. Colloids Surf B Biointerfaces 73:116–121

Watkinson RM, Herkenne C, Guy RH, Hadgraft J, Oliveira G, Lane ME (2009a) Influence of ethanol on the solubility, ionization and permeation characteristics of ibuprofen in silicone and human skin. Skin Pharmacol Physiol 22:15–21

Watkinson RM, Guy RH, Hadgraft J, Lane ME (2009b) Optimisation of cosolvent concentration for topical drug delivery – II: influence of propylene glycol on ibuprofen permeation. Skin Pharmacol Physiol 22: 225–230

Williams AC, Barry BW (1989) Permeation, FTIR and DSC investigations of terpene penetration enhancers in human skin. J Pharm Pharmacol 41:12P

Williams AC, Barry BW (1991) The enhancement index concept applied to terpene penetration enhancers for human skin and model lipophilic (oestradiol) and hydrophilic (5-fluorouracil) drugs. Int J Pharm 74: 157–168

Williams AC, Barry BW (2004) Penetration enhancers. Adv Drug Deliv Rev 56(5):603–618

Yamane MA, Williams AC, Barry BW (1995) Terpene penetration enhancers in propylene glycol/water cosolvent systems: effectiveness and mechanism of action. J Pharm Pharmacol 47:978–989

Yamato K, Takahashi Y, Akiyama H, Tsuji K, Onishi H, Machida Y (2009) Effect of penetration enhancers on transdermal delivery of propofol. Biol Pharm Bull 32:267–274

Zhao K, Singh J (1998) Mechanisms of percutaneous absorption of tamoxifen by terpenes: eugenol, D-limonene and menthone. J Control Release 55(2–3): 253–260

Zhao K, Singh J (1999) In vitro percutaneous absorption enhancement of propranolol hydrochloride through porcine epidermis by terpenes/ethanol. J Control Release 62:359–366

Zhao K, Singh J (2000) Mechanism(s) of in vitro percutaneous absorption enhancement of tamoxifen by enhancers. J Pharm Sci 89(6):771–780

Zhao K, Singh S, Singh J (2001) Effect of menthone on the in vitro percutaneous absorption of tamoxifen and skin reversibility. Int J Pharm 219:177–181

Penetration Enhancers and Their Mechanism Studied on a Molecular Level

3

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3.1 Introduction

The application of topical formulations often purposes to maintain and strengthen the skin penetration barrier against perturbing outer influences, especially in case of diseased skin such as atopic dermatitis or psoriasis. Nevertheless, for some cases of skin treatment, a temporary barrier impairment can be desirable, e.g., for improving the dermal or transdermal effect of active components, which need to penetrate into the deeper skin layers or traverse the skin to reach the blood circulation. There are several ways in order to overcome the skin barrier, e.g., by removing the uppermost and main barrier, the stratum corneum (SC). But this would lead to an increased transepidermal water loss and an irreversible damage of the skin, which is not desirable for long-lasting medications.

It has been established that the lipid matrix is the main route through which molecules cross through the skin (Bodde et al. 1991; Talreja et al. 2001). Consequently, the manipulation of the SC lipid matrix is another possible method to achieve an increased amount of penetrated or permeated drug, by way of using so-called *penetration enhancers* of either hydrophilic or lipophilic nature. So far, there exist two main theories in which way penetration modulators are thought to influence the intercellular arrangement of the SC lipids (see Fig. 3.1). The small hydrophilic

N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_3

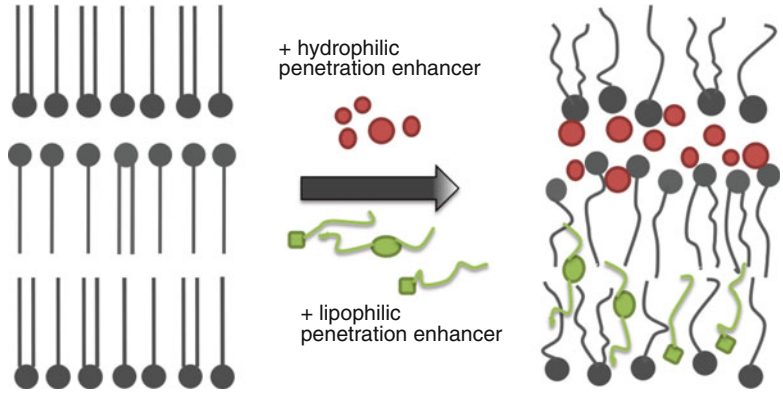

Fig. 3.1 Schematic representation of the penetration routes though the SC intercellular lipid matrix and possible ways of interaction with penetration enhancers (Modified according to (Barry 1991))

penetration enhancers are assumed to interact with the polar head groups of the SC lipids, thereby loosening the tight hydrogen bonds. Thus, the intermembrane region between the adjacent head groups increases and becomes more susceptible to passing molecules. On the other hand, the activity of lipophilic penetration accelerator is presumably based on a disordering or fluidizing effect on the lipid chains (Barry and Bennett 1987) or even causing lipid phase separation (Ongpipattanakul et al. 1991a, b) (see Fig. 3.1).

Nevertheless, the exact mechanisms on a molecular level are an almost unexplored field and remain to be proven.

3.2 Hydrophilic-Enhancing Substances and Their Influence on the Molecular Scale

3.2.1 Influence of Water

The most abundant hydrophilic compound, which is known to facilitate the passing of both hydrophilic and lipophilic drugs through the skin's most efficient barrier, the SC, is water. As it is a native component of the SC, with approximately 15–20 % of the tissue dry weight (Williams and Barry 2004), this natural occurrence was used to receive higher pharmaceutical drug contents at the desired site of action, e.g., by occlusion as stated by Williams and coworkers (Williams and Barry 2004). Such an approach leads to an enormous increase in the water content in the SC. This massive hydration can cause interactions between water and the polar head groups of the SC lipids, which provokes grave changes in the lipid phase such as lipid membrane disruption, as reported in differential scanning calorimetry (DSC) studies (Barry 1987). However, as the absorption of water and its binding within the SC is a highly complex process, even the extensive research carried out in order to understand the mechanisms could not clarify the penetration enhancement effect due to water.

3.2.2 Influence of DMSO

Many other substances are known to also manipulate the lipid ordering of the SC lipid matrix. The high polarity of the solvent dimethyl sulfoxide (DMSO), as an example for a nonnatural penetration enhancer, is reasoned by the S-O bond and results in a potent cosolvent for both hydrophilic and even lipophilic components (Trommer and Neubert 2006).

To explain the interacting mode of DMSO with the SC components, different investigations with varying analyzing methods were carried out. Oertel and coworkers could prove by Fourier transform (FT) infrared spectroscopic studies a change in the keratin conformations (Oertel 1977). In another study using FT Raman spectroscopy, Anigbogu et al. discovered an influence of DMSO on the order of the intercellular lipid lamellae expressed by an increase in the lipid chain disorder and the denaturation of the SC

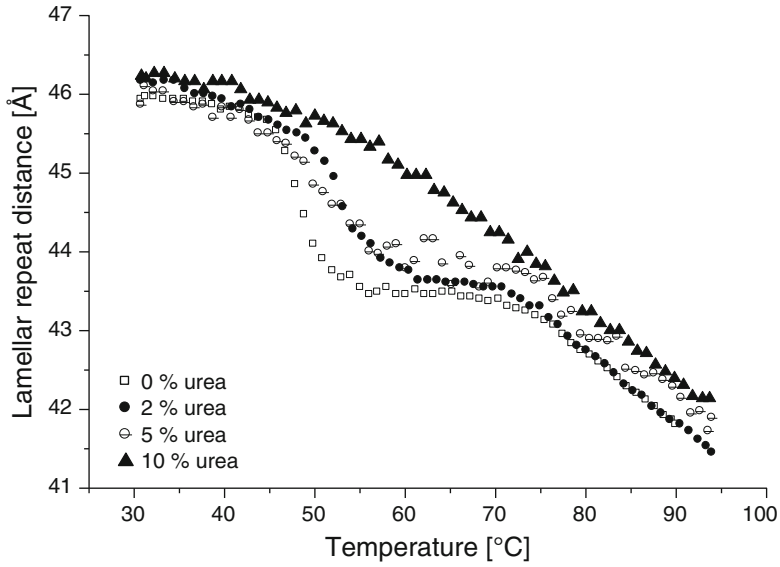

Fig. 3.2 Temperature dependence of the lamellar repeat distance of the SC lipid model membrane containing varying urea concentration (Reprinted from (Zbytovska et al. 2009) with permission from Elsevier)

proteins, resulting in a change of the keratin conformation (Anigbogu et al. 1995). Furthermore, it is known for DMSO to extract lipids from the SC, which loosens the tight multilamellar arrangement of the SC lipid matrix, making it more permeable for drugs (Menczel 1995). According to the work of Friend and coworkers, DMSO also extensively increases the water flux through the skin by a factor of 10^3 investigated in hairless mouse skin (Friend and Heller 1993).

3.2.3 Influence of Urea

Next to several alkanols (Kai et al. 1990; Liu et al. 1991; Pershing et al. 1990; Thomas and Panchagnula 2003), urea is one the hydrophilic penetration modulators, which has been studied extensively (Beastall et al. 1986).

Urea as a very hydrophilic and well water-soluble compound is suspected to be mainly concentrated in the water phase of the skin. The modest penetration-enhancing effect is probably attributed to the increase of the water content in the SC (Williams and Barry 2004), whereby water then acts as the actual enhancer as described above. Furthermore, it is thought that urea facilitates the pathway through the skin by influencing the solubility of the substances followed by a drug distribution into the SC lipids (Williams and Barry 2004). So as to increase the enhancing activity of urea, derivatives of urea were created such as the cyclic urea analogue, which was found to be very potent as penetration enhancer (Wong et al. 1988).

In order to evaluate the interaction of urea with the SC lipids on a molecular scale, Zbytovska and coworkers investigated multilamellar SC lipid vesicles based on ceramide [AP] (CER[AP]) with temperature-dependent small angle X-ray diffraction (Zbytovska et al. 2009). From the position of the diffraction peaks, no influence of urea on the membrane thickness at 32 °C was detected, evidencing that the thickness of the water layer between adjacent lipid head groups is not influenced by urea. On the other hand, a concentration-dependent shift in the phase transition temperature was detected for all urea concentrations, with transference to higher temperature for the first phase transition in the presence of 2–5 % urea. When the amount of urea was further increased, both phase transitions unify into a very broad phase transition (see Fig. 3.2). It was concluded that high amounts of urea induce an increase in the miscibility of the SC lipids by displacing hydrogen-bonded water molecules.

Such an approach using SC lipid model systems can help to identify and to understand the

interaction between urea and the different lipid subclasses of the SC. Consequently more investigation with lipid model differing in their composition need to be carried out in order to fully grasp the functionality of urea as a penetration accelerator.

3.3 Lipophilic Penetration Modulators and Their Influence on the Molecular Scale

In addition to the hydrophilic enhancers, there are also the lipophilic ones, which are employed to increase the penetrating activities of drug substances. Next to the natural compounds in the SC lipid membranes, many studies with similar ingredients were made.

3.3.1 Influence of Oleic Acid

Cooper and coworkers were the first ones who proved the positive effects of the monounsaturated fatty acid specie oleic acid (OA). Already in 1985 they recognized the high potential OA as a penetration promoter for the antiviral compound acyclovir (Cooper et al. 1985), while a little later, Niazy proved similar results for dihydroergotamine (Niazy 1991).

In order to identify possible structural impact of this *cis*-unsaturated C18 fatty acid, Ongpipattanakul and coworkers applied FT infrared spectroscopy and reported a phase separation effect due to the application of OA (Ongpipattanakul et al. 1991a, b). They argued that OA forms a separate liquid phase within the liquid-crystalline ordering of the other SC lipids, forming thereby permeable defects at the fluid-solid interface. Naik and coworkers applied OA and the deuterated derivative onto the human forearm in vivo and performed attenuated total reflectance infrared spectroscopic (ATR-IR) studies. They also found a disordering effect caused by an interrupting of the lamellar structure but only in the superficial layers and reasoned that the enhancing effect is due to a lipid fluidization as well as phase separation (Naik et al. 1995). In another study carried out by Golden and coworkers, the effect of different free fatty acids was studied, especially with respect to the difference between the conformation of this unsaturated fatty acid (Golden et al. 1987). They found using spectroscopic calorimetry and flux techniques that after treatment with the *cis-derivative* of OA, the SC lipids displayed an increased fluidity, and an enhanced flux of salicylic acid through porcine skin was observed. In contrast, the investigation demonstrated that the *trans*-olefinic bond did not alter the phase transition temperature and consequently had no effect on the SC lipids. They argued that the more bulky structure of the *cis*-olefinic bond requires more space and thus disrupts the ordered lipid packing of SC lipids (Golden et al. 1987). To gain more information about the mode of action of OA on a molecular scale, Tanojo et al. analyzed in vitro models of the skin by differential thermal analysis and freeze-fracture electron microscopy. They detected significant changes in the thermal profiles of the SC caused by OA. The final speculation resulted into a new type of lipid domains formed by OA molecules and the SC lipids causing a lower capacity of the cutaneous barrier function (Tanojo et al. 1997). In line with this, Rowat et al. applied solid-state 2H nuclear magnetic resonance (NMR) spectroscopy to monitor SC lipid model membranes based on bovine brain ceramide (CER) in dependence on the fatty acid concentration as well as the temperature (Rowat et al. 2006). They could demonstrate that below the phase transition temperature of 40 °C, the unsaturated fatty acid OA is not incorporated in the lamellar phase of the other lipids, although above 40 °C an interaction occurs. Moreover, they found that OA is able to extract a certain amount of the other lipids, further promoting the separation of the lipids, whereby the extent of the crystalline domains decreases and the fluid more permeable to OA-rich domains expands. To further gain insights into the mode of action and to identify the possible interaction of this fatty acid with the SC lipids, Zbytovska and coworkers applied temperature-dependent X-ray diffraction on SC lipid model systems in the presence of OA

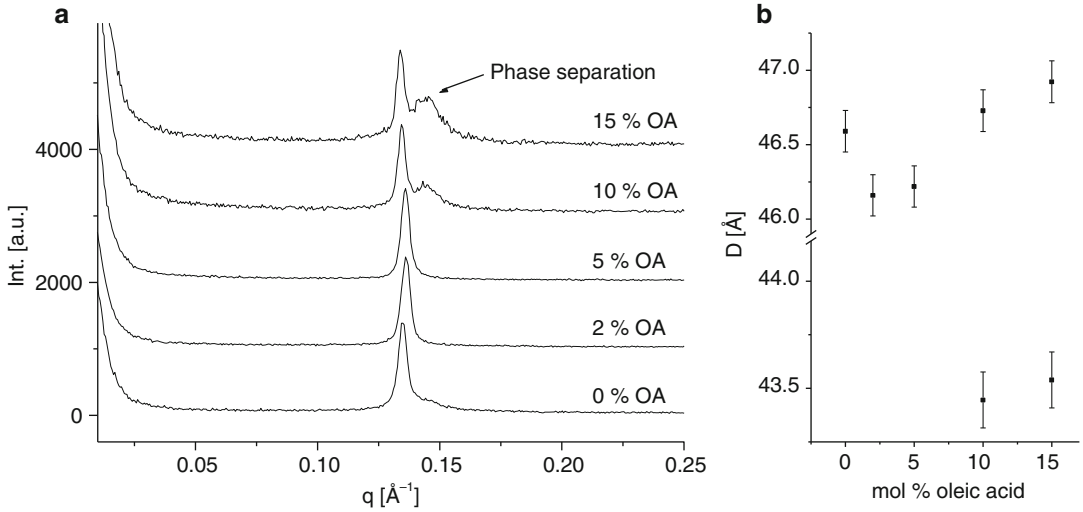

Fig. 3.3 *Left*: X-ray diffraction patterns of the SC lipid MLVs in dependence on the OA concentration measured at 32 °C. *Right*: Display of the corresponding membrane repeat distance (Reprinted from (Zbytovska et al. 2009) with permission from Elsevier)

(Zbytovska et al. 2009). They investigated the concentration-dependent influence of OA on multilamellar vesicle (MLV) mixtures composed of ceramide [AP] (CER[AP]), palmitic acid (PA), cholesterol (CHOL), and its derivative cholesterol sulfate (ChS). As described for other studies above, they also detected a phase separation effect caused by high amounts of OA already at the skin's temperature of 32 °C, whereas no influence on the membrane thickness and on the phase transition temperature could be observed (see Fig. 3.3).

To receive more detailed insights of the impact of OA on the bilayer architecture of SC lipids, Engelbrecht and coworkers applied neutron diffraction on oriented lamellar SC lipid model membranes based on CER[AP], CHOL, PA, and ChS (Engelbrecht et al. 2011). To localize the penetration accelerator molecules within the SC lipid membrane bilayer, the specifically deuterium-labeled OA-d_2 was applied and compared to the sample containing the protonated variety. In contrast to the above-described investigations, a phase separation due the presence of 10 % m/m OA was not detected in the investigated model system, which the authors of this study attributed to the differences in the investigated model systems and the applied techniques. They found that the unsaturated fatty acid is completely embedded in this model membrane, which was further corroborated by the application of the deuterium-labeled OA-d_2 (see Fig. 3.4). Nevertheless, the neutron diffraction data revealed a substantial loss of lamellar order within the membrane, which was accredited to the perturbation of the proper alkyl chain arrangement caused by OA. Again, the fluidizing effect of *cis*-double bond of OA was argued to be responsible for the observed lamellar disorder and, therefore, for the penetration-enhancing effect of OA.

Although the influence of unsaturated fatty acids as penetration accelerators is indisputable, their application, especially of the double unsaturated linoleic acid, is limited by their potential skin irritation when used at higher concentrations (Boelsma et al. 1996; Stillman et al. 1975; Tanojo et al. 1999).

3.3.2 Influence of Isopropyl Myristate

Several experiments with esters of fatty acids revealed their enormous penetration enhancement potential. Especially the pharmaceutically relevant fluid wax isopropyl myristate (IPM)

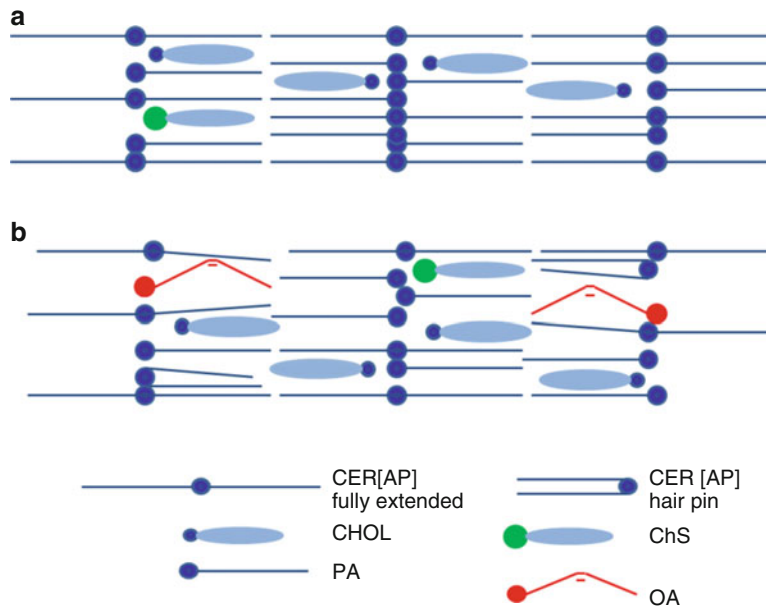

Fig. 3.4 Sketch of the lamellar SC lipid arrangement of the SC lipid model membranes based on CER[AP]. (**a**) The basic model containing CER[AP]/CHOL/PA/ChS. (**b**) Sample basic OA is also composed of CER[AP]/CHOL/PA/ChS, but additionally contains oleic acid (*OA*) (Reprinted from (Engelbrecht et al. 2011) with permission from Elsevier)

constituted the main point of interest. Next to studies focusing on the penetration acceleration of the drugs piroxicam (Santoyo et al. 1995) and nicorandil (Sato et al. 1988) in formulations containing IPM, this wax has also proven to be an effective permeation promoter for the transport of estradiol through the skin (Goldberg-Cettina et al. 1995). As the potential of IPM to increase the transport of drugs through the skin became apparent, the research was focused on the identification of the exact mode of action on a molecular level. Consequently, Engelbrecht and coworkers applied neutron diffraction on SC lipid model membranes based on CER[AP], CHOL, PA, and ChS containing either IPM or its specifically deuterated derivative in order to define the interaction of IPM with the SC lipids (Engelbrecht et al. 2012). The specifically labeled IPM specie was used to localize the IPM molecules within the lipid lamellae. The authors of this study indicated a bilayer perturbation and disordering effect caused by the wax. The neutron diffraction data revealed that the addition of IPM to this SC lipid model membrane resulted in the appearance of another lamellar phase; consequently IPM led to a phase separation of the lipids investigated. Both lamellar phases exhibit a membrane thickness in the range of the system without IPM, whereby one phase (labeled as phase B) has a slightly decrease spacing which indicates an increased state of lipid fluidity and alkyl chain disorder. Nevertheless, both phases of the IPM-containing system show a higher degree of disorder, when compared to the IPM-free SC lipid model membrane. Next to the localization of IPM inside both phase-separated lipid lamellae, the labeled IPM derivative revealed that both phases exhibit distinct differences. While for one phase (referred to as phase B) only one maximum for the deuterium label was detected, the other lipid lamellae (denoted as phase A) featured three maxima (see Fig. 3.5).

Thus, Engelbrecht et al. argued that IPM or its deuterated specie is arranged differently in both phases. In phase B the enhancer molecules are positioned with their labeled CH_3 group directed toward the center of the membrane causing the maximum in the deuterium distribution curve as depicted in Fig. 3.5 (right side), whereby in phase A two different positions for IPM are possible: a fraction of IPM points its myristoyl side chain toward the bilayer center as found for phase B, whereby the deuterium atoms cause the maximum observed in center of the membrane. In the other possibility, a portion of IPM is arranged in the way that the terminal methyl group points

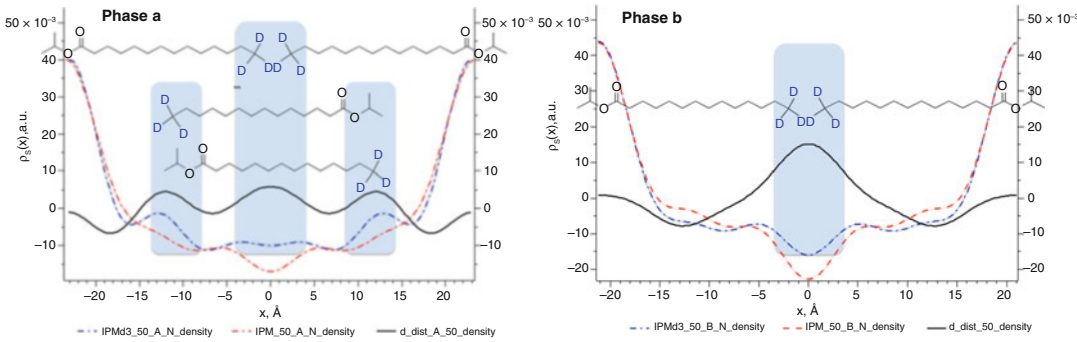

Fig. 3.5 Comparison of neutron scattering length density profiles of the two lamellar phases (phases **a** and **b**), respectively in the presence of either IPM (*blue*) or its deuterated derivative IPM-d_3 (*red*). The difference profile, the deuterium distribution is displayed in a *solid black line*. For a better understanding the possible arrangement of the IPM molecules is included (Modified according to (Engelbrecht et al. 2012))

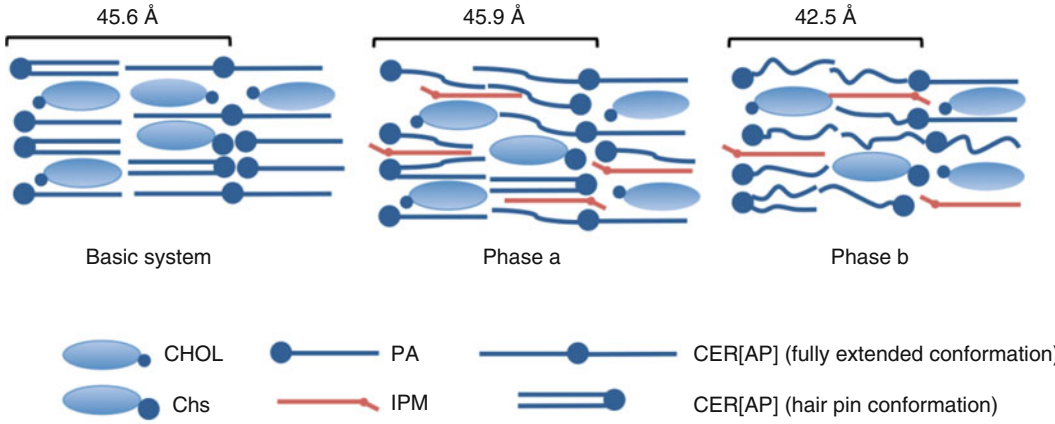

Fig. 3.6 Schematical presentation of the assumed lamellar structure in the presence of 10 wt.% deuterated IPM. *Left:* Lamellar assembly of the SC lipid components CER[AP], cholesterol (*CHOL*), palmitic acid (*PA*), and cholesterol sulfate (*ChS*) in the short periodicity phase as reported previously (Kiselev et al. 2005) (denoted as reference system). *Middle:* Assumed bilayer structure of phase A, whereby IPM is either anchored with its ester group in the hydrophilic head group region or completely inserted into the hydrophobic membrane region. *Right:* The membrane structure and assembly of IPM in phase B. Note the decreased lamellar spacing, indicating an increased state of lipid fluidity and alkyl chain disorder (Reprinted with permission from Elsevier from (Engelbrecht et al. 2012))

toward the region of polar head groups as depicted in Fig. 3.6. It was argued that this rather surprising twisted arrangement of IPM in the unit cell of the SC lipids is possible due to its comparatively high lipophilicity and its lack of the typical hydrophilic head-hydrophobic tail structure.

Additional investigation has to be carried out in order to establish the mode of action of IPM; consequently different simplistic but also more complex SC lipid model membranes have to be studied accordingly.

3.4 Summary and Final Remarks

The functionality of the assortment of enhancing substances is still a wide unknown field with only a few insights into the mechanisms, which were identified in the last decades. The numerous analytical methods complete each other in several ways, and so it will be only a question of time until further findings will complete the means of the functioning of penetration accelerators in their interactions with the SC lipids. Nevertheless,

the knowledge of the exact mode of action of penetration enhancers within the SC lipid matrix can contribute in the development of specifically designed drug delivery formulations, which are more effective for the treatment of different skin diseases. Consequently, there is a high demand to identify the exact mode of action of these penetration modulators on a nanostructural scale.

References

Anigbogu ANC, Williams AC, Barry BW, Edwards HGM (1995) Fourier transform raman spectroscopy of interactions between the penetration enhancer dimethyl sulfoxide and human stratum corneum. Int J Pharm 125:265–282

Barry BW (1987) Mode of action of penetration enhancers in human skin. J Control Release 6:97

Barry BW (1991) Lipid-protein-partitioning theory of skin penetration enhancement. J Control Release 15:237–248

Barry BW, Bennett SL (1987) Effect of penetration enhancers on the permeation of mannitol, hydrocortisone and progesterone through human skin. J Pharm Pharmacol 39:535–546

Beastall J, Guy RH, Hadgraft J, Wilding I (1986) The influence of urea on percutaneous-absorption. Pharm Res 3:294–297

Bodde HE, Vandenbrink I, Koerten HK, Dehaan FHN (1991) Visualization of in vitro percutaneous penetration of mercuric-chloride – transport through intercellular space versus cellular uptake through desmosomes. J Control Release 15:227–236

Boelsma E, Tanojo H, Boddé HE, Ponec M (1996) Assessment of the potential irritancy of oleic acid on human skin: evaluation in vitro and in vivo. Toxicol In Vitro 10:742

Cooper ER, Merritt EW, Smith RL (1985) Effect of fatty acids and alcohols on the penetration of acyclovir across human skin in vitro. J Pharm Sci 74:688–689

Engelbrecht TN, Schroeter A, Hauss T, Neubert RH (2011) Lipophilic penetration enhancers and their impact to the bilayer structure of stratum corneum lipid model membranes: neutron diffraction studies based on the example oleic acid. Biochim Biophys Acta 1808:2798–2806

Engelbrecht TN, Deme B, Dobner B, Neubert RHH (2012) Study of the influence of the penetration enhancer isopropyl myristate on the nanostructure of stratum corneum lipid model membranes using neutron diffraction and deuterium labelling. Skin Pharmacol Physiol 25:200–207

Friend DR, Heller J (1993) Simple alkyl esters as skin penetration enhancers. In: Walters KA, Hadgraft J (eds) Pharmaceutical skin penetration enhancement, vol 59. Marcel Dekker, New York, pp 31–56

Goldberg-Cettina M, Liu P, Nightingale J, Kurihara-Bergstrom T (1995) Enhanced transdermal delivery of estradiol in vitro using binary vehicles of isopropyl myristate and short-chain alkanols. Int J Pharm 114:237–245

Golden GM, McKie JE, Potts RO (1987) Role of stratum corneum lipid fluidity in transdermal drug flux. J Pharm Sci 76:25–28

Kai T, Mak VHW, Potts RO, Guy RH (1990) Mechanism of percutaneous penetration enhancement: effect of n-alkanols on the permeability barrier of hairless mouse skin. J Control Release 12:103–112

Kiselev MA, Ryabova NY, Balagurov AM, Dante S, Hauss T, Zbytovska J, Wartewig S, Neubert RH (2005) New insights into the structure and hydration of a stratum corneum lipid model membrane by neutron diffraction. Eur Biophys J 34:1030–1040

Liu P, Kurihara-Bergstrom T, Good WR (1991) Cotransport of estradiol and ethanol through human skin in vitro: understanding the permeant/enhancer flux relationship. Pharm Res 8:938–944

Menczel E (1995) Delipidization of the cutaneous permeability barrier and percutaneous penetration. In: Smith EW, Maibach HI (eds) Percutaneous penetration enhancers. CRC Press Inc, Boca Raton, pp 383–392

Naik A, Pechtold LARM, Potts RO, Guy RH (1995) Mechanism of oleic acid-induced skin penetration enhancement in vivo in humans. J Control Release 37:299–306

Niazy EM (1991) Influence of oleic acid and other permeation promoters on transdermal delivery of dihydroergotamine through rabbit skin. Int J Pharm 67:97–100

Oertel RP (1977) Protein conformational changes induced in human stratum corneum by organic sulfoxides: an infrared spectroscopic investigation. Biopolymers 16: 2329–2345

Ongpipattanakul B, Burnette RR, Potts RO, Francoeur ML (1991a) Evidence that oleic acid exists in a separate phase within stratum corneum lipids. Pharm Res 8:350–354

Ongpipattanakul B, Francoeur ML, Burnette RR, Potts RO (1991b) Phase-separation of oleic-acid in the stratum-corneum lipids. J Invest Dermatol 96:619

Pershing LK, Lambert LD, Knutson K (1990) Mechanism of ethanol-enhanced estradiol permeation across human skin in vivo. Pharm Res 7:170–175

Rowat AC, Kitson N, Thewalt JL (2006) Interactions of oleic acid and model stratum corneum membranes as seen by 2H NMR. Int J Pharm 307:225–231

Santoyo S, Arellano A, Ygartua P, Martin C (1995) Penetration enhancer effects on the in vitro percutaneous absorption of piroxicam through rat skin. Int J Pharm 117:219–224

Sato K, Sugibayashi K, Morimoto Y (1988) Effect and mode of action of aliphatic esters on the in vitro skin permeation of nicorandil. Int J Pharm 43:31–40

Stillman MA, Maibach HI, Shallita AR (1975) Relative irritancy of free fatty acids of different chain length. Contact Dermatitis 1:65–69

Talreja P, Kleene NK, Pickens WL, Wang TF, Kasting GB (2001) Visualization of the lipid barrier and

measurement of lipid path length in human stratum corneum. AAPS PharmSci 3, E13

Tanojo H, Bos-van Geest A, Bouwstra JA, Junginger HE, Boodé HE (1997) In vitro human skin barrier perturbation by oleic acid: thermal analysis and freeze fracture electron microscopy studies. Thermochim Acta 293:77–85

Tanojo H, Boelsma E, Junginger HE, Ponec M, Bodde HE (1999) In vivo human skin permeability enhancement by oleic acid: a laser Doppler velocimetry study. J Control Release 58:97–104

Thomas NS, Panchagnula R (2003) Transdermal delivery of zidovudine: effect of vehicles on permeation across rat skin and their mechanism of action. Eur J Pharm Sci 18:71–79

Trommer H, Neubert RH (2006) Overcoming the stratum corneum: the modulation of skin penetration. A review. Skin Pharmacol Physiol 19:106–121

Williams AC, Barry BW (2004) Penetration enhancers. Adv Drug Deliv Rev 56:618

Wong O, Huntington J, Konishi R, Rytting JH, Higuchi T (1988) Unsaturated cyclic ureas as new nontoxic biodegradable transdermal penetration enhancers I: synthesis. J Pharm Sci 77:967–971

Zbytovska J, Vavrova K, Kiselev MA, Lessieur P, Wartewig S, Neubert RHH (2009) The effects of transdermal permeation enhancers on thermotropic phase behaviour of a stratum corneum lipid model. Colloid Surf A 351:30–37

Structure-Activity Relationship of Chemical Penetration Enhancers

4

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4.1 Introduction

Transdermal drug delivery offers several advantages over the conventional routes of administration. Elimination of hepatic first-pass effects, reduced side effects through optimization of the blood concentration profile, and extended duration of activity are some of the benefits of transdermal delivery. However, the highly organized structure of the stratum corneum forms an effective barrier to the permeation of a diverse range of agents, which must be modified if poorly penetrating drugs are to be administered. The stratum corneum consists of dead, anucleate, keratinized cells embedded in a lipid matrix.

The use of chemical penetration enhancers would significantly increase the number of candidates suitable for transdermal delivery. According to the lipid-protein partitioning (LPP) theory (Williams and Barry 1991a), chemical penetration enhancers would act by one or more of three major mechanisms: (a) disruption of the stratum corneum lipid matrix, (b) interaction with intracellular protein, and (c) improvement in partitioning of a drug or solvent into the stratum corneum. The LPP theory was extended to recognize (d) disruption of the corneocyte envelope by compounds such as phenol and hydrocarbons; (e) effects on protein junctions, such as desmosomes; and (f) alteration of the partitioning between stratum corneum components (proteinaceous) and the

N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_4

lipid in the diffusion pathway (Menon et al. 1998; Magnusson et al. 2001).

Compounds with a wide variety of chemical structures have been evaluated as skin penetration enhancers. These compounds include fatty acids, fatty alcohols, terpenes, pyrrolidones, surfactants, amides, Azone and its derivatives, urea and its derivatives, sulfoxides, alkanes, and esters. The differences in the structure and physicochemical properties among each class of the enhancers were accounted for their penetration enhancement potencies. Structure-activity relationship (SAR) represents an attempt to correlate the structure or physicochemical property of a compound with its enhancement activity. The physicochemical descriptors include molecular shape, size, lipophilicity, hydrophilicity, molecular geometry, electronic, and steric effects that have strong influence in the biological activity of the compounds. SAR is currently being applied in many disciplines pertaining to drug design, proteomics, and environmental risk assessment. In this chapter, the relationship between the chemical structure and skin permeation enhancement effect of some of the extensively studied penetration enhancers such as fatty acids, fatty alcohols, terpenes, pyrrolidones, and surfactants has been discussed.

4.2 Fatty Acids

Saturated and unsaturated fatty acids have been established as effective enhancers for transdermal permeation of drugs (Aungst et al. 1986; Tanojo et al. 1997a; Thomas and Panchagnula 2003). The chemical formulae of some of the commonly used fatty acids are presented in Table 4.1. The SAR of fatty acids is discussed in detail in this section.

Table 4.1 Chemical formulae of commonly used fatty acids

Name	Formula
Saturated fatty acids	
Caprylic acid	$CH3(CH2)_6COOH$
Capric acid	$CH3(CH2)_8COOH$
Lauric acid	$CH3(CH2)_{10}COOH$
Myristic acid	$CH3(CH2)_{12}COOH$
Palmitic acid	$CH3(CH2)_{14}COOH$
Stearic acid	$CH3(CH2)_{16}COOH$
Unsaturated fatty acids	
Oleic acid	$CH3(CH2)_7CH=CH(CH2)_7COOH$
Linoleic acid	$CH3(CH2)_4CH=CHCH2CH=CH(CH2)_7COOH$
Linolenic acid	$CH3CH2CH=CHCH2CH=CHCH2CH=CH(CH2)_7COOH$

4.2.1 Effect of Carbon Chain Length

There are many reports on the effect of carbon chain length of fatty acids on the percutaneous permeation enhancement of drugs. Aungst et al. (1986) studied the effect of carbon chain length of saturated fatty acids (C7–C18) on the penetration of naloxone through human skin. As the carbon chain length increased from C7 to C12, there was an increase in the permeation of naloxone. An increase in the carbon chain length beyond C12 decreased the flux of naloxone. Maximum permeation was observed with C9–C12.

Ogiso and Shintani (1990) examined the effect of a series of saturated fatty acids on the permeation of propranolol through rabbit skin using gel formulations. Lauric acid and myristic acid were the most effective agents among the fatty acids used in increasing the penetration of propranolol, and the enhancement was significantly larger than those in short and long-chain fatty acids. Lee et al. (1993) investigated the effect of a series of saturated fatty acids (C6–C18) and unsaturated fatty acids (oleic and linoleic acid) on the permeation of the 5-fluorouracil prodrug Tegafur across hairless mouse skin. The fatty acids enhanced the skin permeation of Tegafur in the ethanol/panasate 800 (tricaprylin) (40:60) binary vehicle in the following order: oleic acid>C12>linoleic acid>C10>C8>C6>no fatty acid>C14>C16>C18. All fatty acids increased the skin permeation of Tegafur in the ethanol/water (60:40) binary vehicle. The skin permeation of Tegafur decreased in the following order: C12>C10>linoleic acid>oleic acid>C8>C6>no fatty acid. The skin permeation of Tegafur in the presence of fatty acids was significantly higher with ethanol/panasate 800 (40:60) when compared with

ethanol: water (60:40). These results suggest that the vehicle plays an important role in the skin permeation enhancement effect of fatty acids.

The skin permeation enhancement of a number of fatty acids, namely, straight chain saturated, monounsaturated, and polyunsaturated acids, was evaluated using human stratum corneum (Tanojo et al. 1997a). Saturated fatty acids with 6–12 carbons showed a parabolic correlation between enhancement effect and chain length, with a maximum at nonanoic-decanoic acids (with 9 and 10 carbons). A parabolic relationship between carbon chain length of fatty acids and skin permeation enhancement was also observed with thiamine disulfide (Komata et al. 1992), testosterone (Yu et al. 1991), and indomethacin (Chien et al. 1988).

Kandimalla et al. (1999) studied the effect of saturated fatty acids (C9–C14) on the permeation of melatonin across excised rat skin. A sharp increase in the permeation of melatonin was observed, as the fatty acid chain length increased from 9 to 10 carbons. A further increase in the permeation of melatonin was observed when the chain length was increased to 11. However, the permeation of melatonin decreased when the chain length was increased beyond 11 carbons. It can be observed that the permeation of melatonin has a parabolic relationship with the chain length of the saturated fatty acids. In general, medium chain fatty acids have showed greater permeation enhancement effect compared to short or long-chain fatty acids.

It has been proposed that fatty acids with a certain chain length, that is, around 12 carbons, possess an optimal balance between partition coefficient or solubility parameter and affinity to skin (Ogiso and Shintani 1990). Shorter chain fatty acids would have insufficient lipophilicity for skin permeation, whereas longer chain fatty acids would have much higher affinity to lipids in stratum corneum and thereby retard their own permeation and that of other permeants. The parallel effect with the permeation enhancement suggests that the mode of action of saturated fatty acids as enhancers is dependent on their own permeation across the stratum corneum (Tanojo et al. 1997a).

The mechanism by which fatty acids increase skin permeability appears to involve disruption of the lipids that fill the extracellular spaces of the stratum corneum (Aungst 1989; Barry 2001). Treatment of rabbit stratum corneum with various unsaturated fatty acids resulted in a shift to higher frequency for the C-H asymmetric stretch peak near 2920 cm^{-1} on Fourier transform infrared spectroscopy (FTIR) spectra, which primarily results from the acyl chains of intercellular lipid in the stratum corneum (Morimoto et al. 1996).

4.2.2 Saturated and Unsaturated Fatty Acids

The effect of saturated long-chain fatty acids [stearic acid (C18), myristic acid (C14), and lauric acid (C12)] on the percutaneous transport of thiamine disulfide from propylene glycol was studied through excised rat skin (Komata et al. 1992). The permeation of thiamine disulfide was enhanced 31 times by C12 and 1.4 times by C14 and suppressed to 80 % of its original value by C18. However, with unsaturated fatty acids, the permeation of indomethacin was enhanced in the following order: C20 > C22 > C18 = C16 > C14, and the flux values were correlated well with the uptake of these compounds into the stratum corneum (Morimoto et al. 1996). Oleic acid (C18, unsaturated) has been shown in several studies to be an effective skin permeation enhancer, whereas stearic acid (C18, saturated) is not a good skin permeation enhancer. Chi et al. (1995) reported an increase of 6.5-fold to 17.5-fold in the permeation rate of flurbiprofen by unsaturated fatty acids, while no significant increase was observed with saturated fatty acids. Thus, saturated and unsaturated fatty acids behave differently on the skin permeation enhancement.

4.2.3 Branched Versus Unbranched Fatty Acids

Aungst (1989) reported that maximum flux of naloxone was observed with C9–C12 branched and unbranched fatty acids through human skin.

The branched and unbranched isomers of C5–C14 fatty acids showed similar effects. However, isostearic acid [$(CH_3)_2CH(CH_2)_{14}COOH$] was a more effective permeation enhancer than stearic acid. The higher permeation enhancement effect of isostearic acid than stearic acid was attributed to its lower melting point and greater solubility in propylene glycol (Aungst 1995).

4.2.4 Position of Double Bond

Tanojo et al. (1997a) studied the effect of position of double bond on the percutaneous absorption of para amino benzoic acid in human stratum corneum using cis-octadecenoic acid with a double bond at 6th, 9th, 11th, or 13th position counted from the carboxyl head group. There was no significant difference in the effect of these acids on the permeation of para amino benzoic acid. Morimoto et al. (1996) studied the effect of double bond positions of unsaturated fatty acids (C18) on the permeation of indomethacin through rat skin. The permeation of indomethacin with oleic acid (cis-9), asclepic acid (cis-11), and petroselinic acid (cis-6) was not affected by the position of the double bonds.

4.2.5 Geometric Isomers

The effect of geometric isomers of unsaturated fatty acids on the permeation of indomethacin through rat skin was studied (Morimoto et al. 1996). The indomethacin flux with elaidic acid (trans-9-octadecenoic acid) was significantly lower than that of oleic acid (cis-9-octadecenoic acid). The flux of salicylic acid enhanced by trans isomers of 9-octadecenoic acid was lower than that of their cis-isomers (Golden et al. 1987). However, there was no significant difference between cis and trans unsaturated C16–C18 fatty acid isomers in their effects on naloxone flux across human skin (Aungst 1989). The discrepancy in these results may be due to the difference in the properties of drugs employed and the variation in the skin species used for the studies.

4.2.6 Number of Double Bonds

A significant increase in the flux of naloxone was observed as the number of double bonds in the C18 fatty acid increased from one (oleic acid) to two (linoleic acid) (Aungst et al. 1986). An increase in the number of double bonds to three (linolenic acid), however, did not increase the flux further. Tanojo et al. (1997a) investigated the effect of number of double bonds (in cis-conformation) in straight chain polyunsaturated acids on the permeation of para amino benzoic acid in human stratum corneum. Polyunsaturated fatty acids such as linoleic, linolenic, and arachidonic acid with, respectively, two, three, and four double bonds produced a significantly higher permeation of para amino benzoic acid than the monounsaturated fatty acid. However, there was no significant difference in the permeation enhancement effects among the polyunsaturated fatty acids. Carelli et al. (1992) also reported that the enhancement of flux of alprazolam by linoleic acid was greater than that of oleic acid through hairless mouse skin. However, the flux of indomethacin was not affected by the number of double bonds (Morimoto et al. 1996).

Kandimalla et al. (1999) investigated the effect of oleic acid, linoleic, and linolenic acid on the permeation of melatonin across excised rat skin. As the number of double bonds increased, there was a slight increase in the permeation of melatonin. The flux of melatonin with linolenic acid was significantly higher than that of oleic acid ($P<0.05$). However, there was no significant difference in the flux values of linoleic acid and linolenic acid ($P>0.05$). Fang et al. (2003) studied the effect of oleic acid, linoleic acid, and linolenic acid on the permeation of flurbiprofen through the mouse skin. The permeation of flurbiprofen increased with an increase in the number of double bonds in the fatty acid.

Oleic acid has been reported to be an effective skin penetration enhancer for polar and nonpolar drugs (Narishetty and Panchagnula 2004; Goodman and Barry 1988). Cis-unsaturated fatty acids (e.g., oleic acid, linoleic acid, and linolenic acid) have been reported to form separate domains within the stratum corneum lipids that effectively

decrease the diffusional path length or the resistance (Ongpipattanakul et al. 1991; Tanojo et al. 1997b). The formation of separate domains would provide permeability defects within the bilayer lipids and facilitate the permeation of hydrophilic permeants. The presence of double bonds in the structure has been proposed to cause the formation of kinks in the lipid matrix to allow water permeation across the skin (Potts and Francoeur 1990). An increase in the number of double bonds increases the flux of drugs, possibly by causing more kinks in the lipid structure of the skin.

Recently, Ibrahim and Li (2010) evaluated the effects of fatty acids (e.g., oleic acid, lauric acid, decanoic acid, and undecanoic acid) commonly present in cosmetic and topical formulations on permeation enhancement across human epidermal membrane (HEM) lipoidal pathway when the fatty acids saturated the SC lipid domain without cosolvents (E_{max}). E_{max} of fatty acids was shown to increase with their octanol solubilities and decrease with their lipophilicities. Solid fatty acids (saturated fatty acids) had lower enhancement efficiency when compared to the liquid fatty acids (unsaturated fatty acids). E_{max} of solid fatty acids was shown to depend on their melting points, an important parameter to the effectiveness of the enhancers. The estradiol uptake results suggested that enhancer-induced permeation enhancement across HEM was related to enhanced partitioning into the SC lipid domain.

4.3 Fatty Alcohols

The chemical formulae of some of the commonly used fatty alcohols are presented in Table 4.2. The effect of saturated alcohols (C8-OH to C18-OH) on the flux of naloxone from propylene glycol was investigated through human skin (Aungst et al. 1986). A parabolic effect of alkyl chain length was observed with C10-OH and C12-OH being most effective. The effect of a series of straight chain alkanols on the transdermal delivery of levonorgestrel through excised rat and human cadaver skin was investigated by Friend and coworkers (1988). The flux of levonorgestrel increased as the alkyl chain increased from C2 to C4, but decreased as the chain length increased above 1-butanol.

Table 4.2 Chemical formulae of commonly used fatty alcohols

Name	Formula
Saturated fatty alcohols	
Octanol	$CH3(CH2)_7OH$
Capric alcohol	$CH3(CH2)_9OH$
Lauryl alcohol	$CH3(CH2)_{11}OH$
Myristyl alcohol	$CH3(CH2)_{13}OH$
Cetyl alcohol	$CH3(CH2)_{15}OH$
Unsaturated fatty alcohols	
Oleyl alcohol	$CH3(CH2)_7CH=CH(CH2)_8OH$
Linoleyl alcohol	$CH3(CH2)_4CH=CHCH2CH=CH(CH2)_8OH$
Linolenyl alcohol	$CH3CH2CH=CHCH2CH=CHCH2CH=CH(CH2)_8OH$

Lee et al. (1993) examined the effect of a series of fatty alcohols in ethanol/panasate 800 and ethanol/water on the permeation of Tegafur across hairless mouse skin. All fatty alcohols, except the C18-OH, increased the skin permeation of Tegafur in the ethanol/panasate 800 (60:40) binary vehicle. The degree of permeation percentage of Tegafur obtained was same at 12 h (64.1–67.9 % of dose) in all cases, and no significant difference between them was observed. However, all fatty alcohols significantly enhanced the skin permeation of Tegafur with ethanol/water (60:40) binary vehicle. The flux of Tegafur increased with an increase in alkyl chain length, reached a maximum permeation in C12-OH, and then decreased as the alkyl chain length increased further. The skin permeability of Tegafur was in the following order: C12-OH > C10-OH > C9-OH > C8-OH > C14-OH > C16-OH > C18-OH > no fatty alcohol. Fatty alcohols with 9, 10, and 12 carbon atoms provided the greatest permeation enhancement of Tegafur at 12 h in the ethanol/water (60:40) binary vehicle. These results suggest that the vehicle plays an important role in the permeation enhancement effect of fatty alcohols.

The effect of n-alkanols on the permeation of a polar, nonelectrolyte penetrant, nicotinamide through hairless mouse skin was investigated by Kai et al. (1990). The enhancement versus alkanol chain length profile was parabolic,

C6-OH being the maximum. The alkanol flux after a 6-h contact period, versus carbon number, was also a parabolic function. Alkanol uptake on the other hand increased with increasing chain length. The authors suggested that the primary mechanism by which alkanols increase percutaneous absorption is extraction of stratum corneum intercellular lipids. Sloan et al. (1998) studied the fluxes of theophylline through hairless mouse skin from suspensions in straight alkyl chain alkanols. The flux of theophylline was the lowest from methanol (C1-OH), increased by almost 100-fold from pentanol (C5-OH), hexanol (C6-OH), heptanol (C7-OH), octanol (C8-OH), and nonanol (C9-OH), and then decreased tenfold from undecanol (C11-OH).

The effect of saturated fatty alcohols (C8-OH to C14-OH) on the permeation of melatonin across excised hairless rat skin was investigated (Kanikkannan and Singh 2002). All saturated fatty alcohols increased the permeation of melatonin through hairless rat skin, and the permeation of melatonin was found to be related to the carbon chain length of the fatty alcohols. An increase in the flux of melatonin was observed when the fatty alcohol chain length increased from 8 to 10 carbons. However, the flux of melatonin decreased when the chain length was increased beyond ten carbons. The maximum permeation of melatonin was observed with decanol. The parabolic relationship between carbon chain length of fatty alcohol and skin permeation enhancement was also observed for testosterone (Yu et al. 1991) and indomethacin (Chien et al. 1988).

The effect of number of double bonds in the C18 fatty alcohol on the permeation of naloxone across human skin was studied (Aungst et al. 1986). The permeation of naloxone was increased with an increase in the number of double bonds. Like fatty acids, fatty alcohols also act by disrupting the stratum corneum lipid matrix (Barry 2001). The influence of hydrocarbon chain branching on the effectiveness of alkanol skin permeation enhancers was investigated using corticosterone as a model drug across hairless mouse skin (Chantasart et al. 2004). The branched-chain alkanols showed lower enhancer potency than the 1-alkanols of the same molecular formula; the potency decreases as the hydroxyl group moves from the end of the chain toward the center of the enhancer alkyl chain. The authors also reported that the intrinsic potencies of the 1-alkyl enhancers (1-alkanols, 1-alkyl-2-pyrrolidones, and 1-alkyl-2-azacycloheptanones) are essentially the same and independent of their alkyl chain length at their isoenhancement concentrations (Warner et al. 2001; Warner et al. 2003; Chantasart et al. 2004).

Ding et al. (2006) investigated the relationship between the skin permeation enhancement of alkanols and their physicochemical parameters including octanol-phosphate buffered saline (PBS, pH 7.4). The authors established the correlation equations between enhancement potencies and the physicochemical parameters relevant to lipophilicity and position of hydroxyl group for 16 alkanols (e.g., 1-hexanol, 1-heptanol, 1-octanol, 1-nonanol, 2-nonanol, 3-nonanol, 4-nonanol, and 5-nonanol) using stepwise multiple linear regression analysis. The enhancement potency of alkanols increased as their lipophilicity increased but decreased as the hydroxyl group moves from the end of the alkyl chain toward the center.

It has been reported that the most effective chain lengths (C10–C12) correspond to the length of the steroid nucleus of cholesterol, suggesting that these may act by disrupting ceramide-cholesterol or cholesterol-cholesterol interaction (Brain and Walters 1993). Ackermann et al. (1987) studied the permeation of a series of alkanols (C1-OH to C8-OH) across the nude mouse skin. The permeability coefficients of alkanols increased linearly as the chain length increases. Further, the permeability coefficients of n-alkanols correlated well with their ether-water partition coefficients. These results could be used to explain the permeation enhancement effect of different alkanols. The increase in the enhancement effect of lower alkanols with increase in the alkyl chain length may be attributed to the increased permeation of alkanols through the skin.

4.3.1 Fatty Acids Versus Fatty Alcohols

Fatty acids have a higher melting point than their corresponding fatty alcohols, but lower solubility

parameters. If the enhancement by these fatty acids and alcohols was solely due to solubility effects, then it would be expected that the alcohols would be more effective than the acids, whereas the reverse is true for alkyl chains up to C18. This suggests that more specific interactions must occur (Brain and Walters 1993). Introduction of double bonds into long alkyl chains modifies the effect significantly, and, for the C18 compounds, there was little difference between the corresponding fatty acids and alcohols. There was a greater concentration dependence of permeation enhancement for lauric acid than lauryl alcohol (Aungst et al. 1986).

4.4 Terpenes

Terpenes are naturally occurring compounds, which consists of isoprene (C_5H_8) units. Terpenes are classified according to the number of isoprene units they contain: monoterpenes (C_{10}) have two isoprene units, sesquiterpenes (C_{15}) have three, and diterpenes (C_{20}) have four. The structural formulae of different types of terpenes (hydrocarbon, ketone, alcohol, oxide, and cyclic ether terpenes) evaluated as skin penetration enhancers are shown in Fig. 4.1.

Terpenes have been widely studied as skin penetration enhancers for various drugs (Williams and Barry 1991a; Okabe et al. 1989; Gao and Singh 1998; Godwin and Michniak 1999). Okabe et al. (1989) studied ten cyclic monoterpenes as penetration enhancers for lipophilic drug indomethacin in rats. The absorption of indomethacin from gel ointment was substantially enhanced by hydrocarbon terpenes such as d-limonene. However, the oxygen-containing terpenes did not affect the permeation of indomethacin. Only those cyclic monoterpenes with a lipophilic index (log k') of greater than 0 exhibited absorption promoting effects, suggesting that the lipophilicity of these compounds plays an important role in drug transport across the skin. But the alcohol and ketone terpenes were less effective for lipophilic drugs such as diazepam (Hori et al. 1991) and estradiol (Williams and Barry 1991b).

Williams and Barry (1991b) evaluated a series of terpenes as skin penetration enhancers for the hydrophilic drug 5-fluorouracil in human skin. Cyclic terpenes were chosen from the chemical classes of hydrocarbons, alcohols, ketones, and oxides. Of the terpenes studied, hydrocarbons were poor enhancers and alcohols and ketones were more effective. The epoxides showed mild enhancing activity, whereas the cyclic ethers were very effective; ascaridole, 7-oxabicyclo[2.2.1] heptane, and 1,8-cineole all induce a near 90-fold increase in the permeability coefficient of 5-fluorouracil. The five-membered cyclopentene oxide showed higher enhancing activity than the six-membered cyclohexene oxide.

The effect of 12 sesquiterpenes on the permeation of 5-flurouracil was studied across human skin (Cornwell and Barry 1994). Pretreatment of epidermal membranes with sesquiterpene oils or using solid sesquiterpenes saturated in dimethyl isosorbide enhanced the absorption of 5-fluorouracil. Enhancers containing polar functional groups were generally more effective than pure hydrocarbons, and enhancers with the least "bunched" structures were the most active.

Obata et al. (1990) reported that percutaneous absorption of hydrophilic diclofenac sodium was substantially enhanced in the presence of l-menthol and dl-menthone, while it was little enhanced by d-limonene and p-menthane. Overall, the skin permeation-enhancing effect of terpenes depends on the physicochemical properties of the drugs. In general hydrocarbon terpenes are effective for lipophilic drugs and oxygen-containing terpenes are effective for hydrophilic drugs. Okamoto and coworkers (1987, 1988) evaluated the compounds containing azacyclo ring and acyclic terpene hydrocarbon chains as enhancers for a variety of drugs. These studies demonstrated that azocyclo ring size (C5–C6) has little effect on the potency of the enhancers, whereas the length of hydrophobic terpene chain has a significant effect; a chain length of 12 carbons provided maximum effect.

El-Kattan et al. (2000) investigated the effect of terpene lipophilicity (log P 1.06–5.36) (terpene-4-ol, verbenone, fenchone, carvone, menthone, alpha-terpineol, cineole, geraniol, thymol, cymene, d-limonene, and nerolidol) on the percutaneous absorption of hydrocortisone from hydroxypropyl methyl cellulose gel formulations using hairless

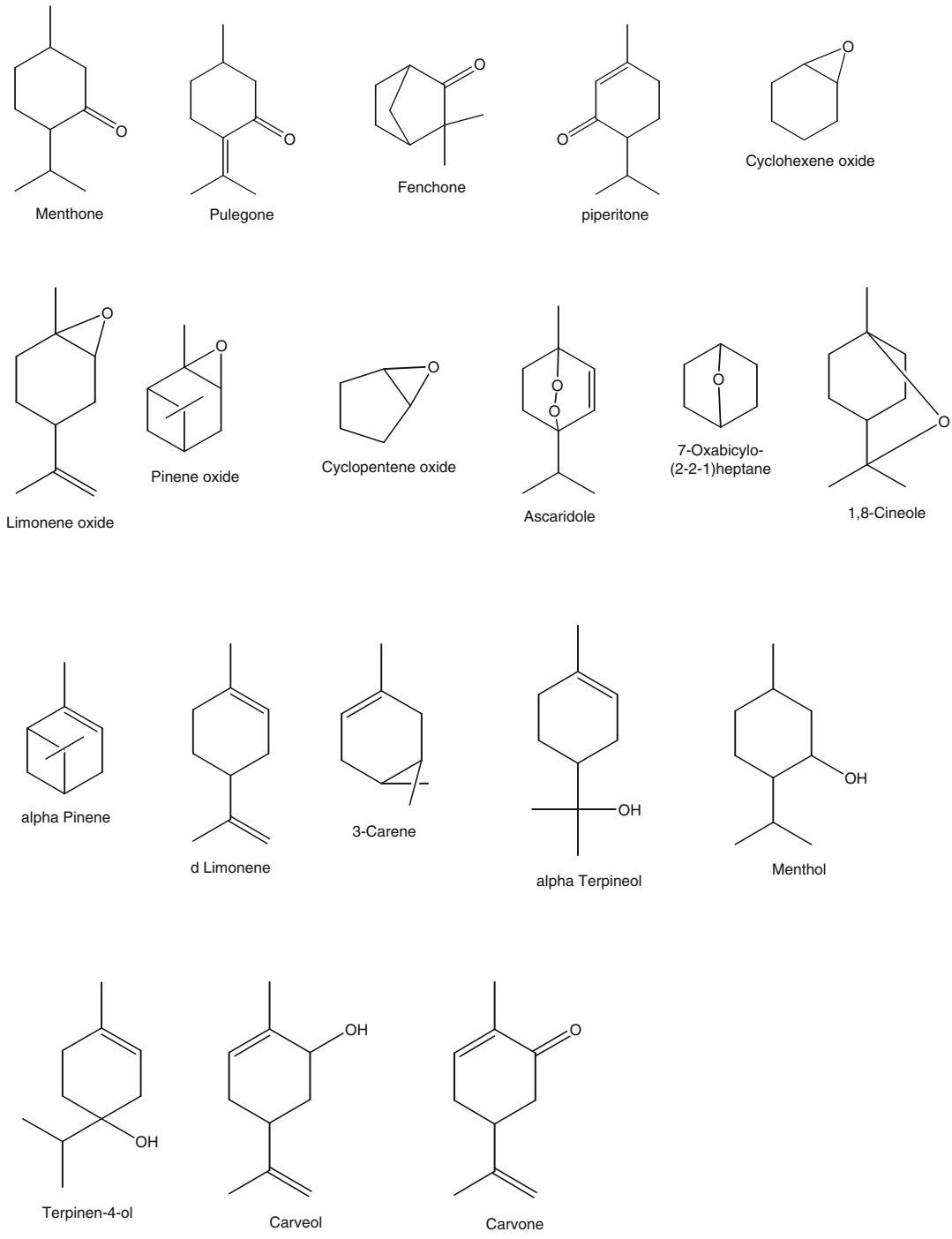

Fig. 4.1 Structural formulae of various types of terpenes (hydrocarbon, ketone, alcohol, oxide, and cyclic ether terpenes) evaluated as skin penetration enhancers

mouse skin in vitro. A linear relationship was found between the log P of terpene and the cumulative amount of hydrocortisone in the receptor compartment after 24 h. An increase in terpene lipophilicity was associated with an increase in the cumulative amount of hydrocortisone transported.

The effects of terpene enhancers (fenchone, thymol, d-limonene, and nerolidol) on the percutaneous absorption of drugs with different lipophilicities (nicardipine hydrochloride, hydrocortisone, carbamazepine, and tamoxifen) were studied (El-Kattan et al. 2001). Nerolidol (highest lipophilicity) provided the highest increase in the flux of the model drugs. The lowest increase in the flux was observed with fenchone (lowest lipophilicity). The results indicated that these four enhancers were more effective at enhancing the penetration of hydrophilic drugs rather than lipophilic drugs. The terpenes act mainly by disrupting the lipid matrix of the stratum corneum (Williams and Barry 1991b). Spectroscopic studies have also suggested that terpenes could exist within separate domains in stratum corneum lipids (Cornwell et al. 1996).

Kang et al. (2007) investigated the effects of physicochemical properties of terpenes on the permeation of a model drug (haloperidol) through the human stratum corneum using 49 terpenes. A full spectrum of terpenes was selected to include monoterpene, sesquiterpene, diterpene, triterpene, and tetraterpene with various functional groups such as hydrocarbons, alcohols, aldehydes, esters, ketones, and oxides. Liquid terpenes produced better enhancing effects than solid terpenes. Triterpenes and tetraterpenes showed poor penetration-enhancing effects compared to other types of terpenes. Terpenes with aldehyde and ester functional groups tend to increase $\text{Log}K_p$, while those with acid functional groups tend to decrease $\text{Log}K_p$. In general, terpenes with higher LogP values were found to be more effective enhancers than those with lower LogP.

Recently, Chantasart et al. (2009) investigated the effects of oxygen-containing terpenes as skin permeation enhancers on the lipoidal pathways of HEM. The enhancement (E(HEM)) effects of menthol, thymol, carvacrol, menthone, and cineole on the transport of a model drug, corticosterone, across HEM were determined. It was found that the enhancer potencies of menthol, thymol, carvacrol, and menthone were essentially the same and higher than that of cineole based on their aqueous concentration in the diffusion cell chamber at E(HEM) = 4. Thymol and carvacrol also had the same E(HEM) = 10 concentration further supporting that they had the same enhancer potency based on the aqueous concentration. The amount of terpene absorbed into the HEM stratum corneum (SC) intercellular lipid under the same conditions indicated that the intrinsic potencies of the studied terpenes are the same based on their concentration in the SC.

4.5 Pyrrolidones

Pyrrolidones and their derivatives have been investigated as potential skin penetration enhancers (Williams and Barry 2004). 2-Pyrrolidone and N-methyl-2-pyrrolidone (NMP) have been evaluated as penetration enhancers for a variety of drugs (Hoelgaard et al. 1988; Bhatia and Singh 1997). Figure 4.2 presents the chemical structures of some pyrrolidones, which have been evaluated as skin penetration enhancers. Aoyagi et al. (1991) synthesized a new group of 2-pyrrolidone enhancers containing a short alkyl group, such as methyl, ethyl, propyl, or butyl group, at the 1-position and a dodecyl group at the 3-position of a 2-pyrrolidone ring. The enhancing effect of these compounds was evaluated using indomethacin as a model drug. The length of the short alkyl group at the 1-position greatly impacted the enhancing activity of the 2-pyrrolidone derivatives. 1-Propyl and 1-butyl-3-dodecyl-2-pyrrolidone provided the greatest permeation enhancement effects for indomethacin through the skin.

The skin permeation enhancement activity of a series of alkyl substituted pyrrolidones was evaluated using phenol red as a model drug across rat skin in vitro and in vivo (Sasaki et al. 1988, 1990a, b, 1991). A correlation between the flux of phenol red and partition coefficient of the pyrrolidones was observed. The percutaneous penetration enhancement of 6-mercaptopurine by nine azacycloalkanone derivatives with an alkyl or terpene chain was studied using excised guinea pig skin (Okamoto et al. 1988). The number of carbonyl groups in the chain influenced the enhancing activity more effectively than the ring size.

It has been reported that pyrrolidone derivatives alter the liposomal membrane made with stratum corneum lipid (Kim et al. 1993). Yoneto

et al. (1995) studied the effects of 1-ethyl, 1-butyl, 1-hexyl, and 1-octyl-2-pyrrolidones on the transport of beta-estradiol, hydrocortisone, and corticosterone across hairless mouse skin. The results showed a 3.5-fold increase in enhancement potency per methylene group introduced at the 1-N position. The authors reported that the 1-alkyl-2-pyrrolidones may act via the intercalation of the alkyl group of the enhancer into the highly ordered interfacial region of the lipid bilayers, inducing significant disorder and enhancing microenvironmental fluidity. The authors studied the fluidizing effects of alkyl pyrrolidones upon the stratum corneum lipid liposome bilayer using steady-state anisotropy and fluorescence lifetime studies (Yoneto et al. 1996). The results suggested that the alkyl pyrrolidones might induce a general fluidizing effect upon the lipid bilayer. As a continuing effort to understand the mechanism of action, the authors studied the influence of the alkyl pyrrolidones on permeant partitioning into hairless mouse stratum corneum under the isoenhancement concentration conditions using beta-estradiol as the model drug (Yoneta et al. 1998). The results suggested that inducing a higher partitioning tendency for beta-estradiol into the lipoidal pathway of hairless mouse stratum corneum is a principal mechanism of action of the alkyl pyrrolidones in enhancing percutaneous absorption.

The transdermal permeation-enhancing effects of 16 pyrrolidinone derivatives toward

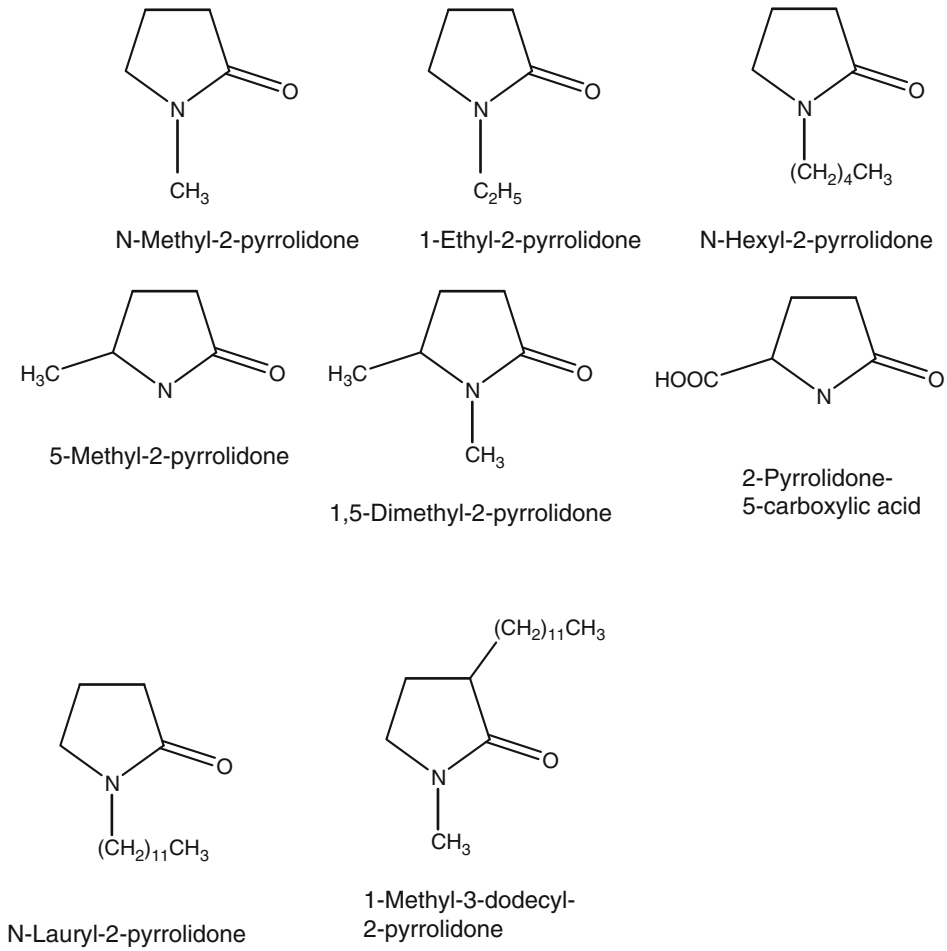

Fig. 4.2 Structural formulae of pyrrolidone enhancers

hydrocortisone have been measured using hairless mouse skin in vitro (Ghafourian et al. 2004). Enhancement ratios were calculated for the permeability coefficient [ER(kp)] and the 24-h receptor concentration ER(Q24, μM). The relationships of log ER(kp) and log ER(Q24) surface area squared (SA^2) indicate that larger pyrrolidinone derivatives are better enhancers for hydrocortisone penetration. Surface area is often correlated with hydrophobicity of molecules, and with this pyrrolidinone series, the correlation between SA^2 and log P had an r^2 value of 0.809. The correlation of log ER (kp) with log P was found to be a weak positive correlation.

4.6 Surfactants

Surfactants generally consist of a lipophilic alkyl or aryl chain with a hydrophilic head group. Surfactants may be classified according to the nature of the head group as anionic, cationic, nonionic, or zwitterionic. Surfactants have been used as skin permeation enhancers in several studies (Lopez et al. 2000; Park et al. 2000; Nokhodchi et al. 2003). In general, the penetration enhancement of drugs by surfactants is in the following order: cationic surfactants > anionic surfactants > nonionic surfactants. Ashton et al. (1992) compared the effects of dodecyl trimethyl ammonium bromide (DTAB), sodium lauryl sulfate (SLS), and polyoxyethylene fatty ether (Brij 36T™, Croda, USA) on the flux of methyl nicotinamide across excised human skin. The permeation enhancement of methyl nicotinamide was in the following order: DTAB > SLS > Brij 36 T ™. However, Brij 36 T ™ exhibited a smaller, but more immediate effect on the permeation of methyl nicotinate, resulting in the highest degree of flux enhancement over the first 24-h period. Walters et al. (1988) investigated the influence of several polyethoxylated nonionic surfactants on the transport of methyl nicotinate across hairless mouse skin in vitro. The surfactants having a linear alkyl chain greater than C8 and an ethylene oxide chain length (E) of less than E14 caused significant increases in the flux of methyl nicotinate. Surfactants having branched or aromatic moieties in the hydrophobic portion were ineffective. Maximum enhancement of flux was obtained using polyoxyethylene (10) lauryl ether (Brij 36T™). The authors proposed two possible modes of surfactant action. The surfactant may first penetrate into the intercellular regions of the stratum corneum, enhance fluidity, and solubilize and extract lipid components. Later, penetration of the surfactant into the intracellular matrix followed by interaction and binding with the keratin filaments may result in a disruption of order within the corneocyte. The structural aspect required for the latter mechanism may explain, to some extent, the maximum activity seen with the C12 surfactant.

The effect of 17 polyoxyethylene (POE) alkyl ethers on the transport of ibuprofen across rat skin was studied (Park et al. 2000). The transdermal flux through excised rat skin was found in the decreasing order of POE(5)cetyl/oleyl ether (110.24 μg/cm²/h) > POE(2) lauryl ether (99.91 μg/cm²/h) > POE(2)oleyl ether (67.46 μg/cm²/h) > POE(10)stearyl ether (66.19 μg/cm²/h). The enhancers containing the EO chain length of 2–5, hydrophilic lipophilic balance (HLB) value of 7–9, and an alkyl chain length of C16–C18 were effective promoters of ibuprofen flux.

The effects of various cationic surfactants (alkyl trimethyl ammonium halides, alkyl dimethylbenzylammonium halides, and alkyl pyridinium halides) on the permeation of radiolabeled water and lidocaine through excised human epidermis have been studied (Kushla and Zatz 1991). All surfactants increased the flux of water and lidocaine by two- to fourfold compared to the initial control period. However, there was no significant difference in the enhancing effects of these three hexadecyl derivatives. The maximum flux enhancement was observed from those derivatives with an alkyl chain length of 12–14 carbons. Cooper and Berner (1984) reported that the optimal chain length for skin barrier impairment might be attributed to the factors such as solubility of the surfactant in the donor vehicle, the critical micellar concentration, the stratum corneum-vehicle partition coefficient, and the binding affinity of the surfactant for epidermal keratin. An optimum chain length of 12–14

carbons may represent compromise between water solubility and lipophilic character. Furthermore, stratum corneum keratin may bind preferentially with carbon chains of specific length.

Cappel and Kreuter (1991) compared the enhancement potential of polysorbates 20, 21, 80, and 81. The results of these studies showed that polysorbates had a lesser effect on the transdermal permeation of methanol. Maximum permeation enhancement was achieved in the presence of polysorbates 21 and 81, which enhanced the permeation of methanol of two- to threefold, indicating that the more lipophilic polysorbates alter the barrier properties of the skin to a greater extent than their hydrophilic analogs.

Lopez et al. (2000) investigated the influence of the polar functional group on the skin permeation enhancement effects of nonionic surfactants. Their results indicated that the nature of the enhancer head group greatly influences cutaneous barrier impairment. Sorbitan monolaurate (Span®20, Croda, USA) showed greater permeation enhancement of all compounds compared to polysorbate 20 (Tween®20, Croda, USA). Ionic surfactants interact well with keratin filaments in the corneocytes and make them more permeable and increase the diffusion coefficient of the drug (Barry 2001). Surfactants may also modify peptide or protein material in the bilayer domain of the stratum corneum (Williams and Barry 1991a).

Kitagawa et al. (2001) studied the effects of the double-chained cationic surfactants dimethyldialkylammoniums (CH3)2 N+ (CnH(2n + 1))2 on the permeation of benzoic acid across excised guinea pig skin. Out of five dimethyldialkylammoniums tested ($n = 10$–18), dimethyldidecylammonium ($n = 10$) showed dose-dependent enhancement effects at concentrations of more than 20 μM. Compared with the significant enhancement effects of dimethyldialkylammoniums with relatively shorter alkyl chains, those of long-chain dimethyldialkylammoniums ($n = 16$, 18) were much less. The results suggest that dimethyldialkylammoniums with relatively shorter alkyl chains, which form either vesicles with looser molecular packing or micelles and appear to be present as surfactant monomers in higher ratios than those with longer alkyl chains, favor the interaction with the skin.

4.7 Recent Studies

Ibrahim and Li (2009) investigated the enhancement effects of a wide variety of compounds from different classes (e.g., oleic acid, octanol, oleyl alcohol, isopropyl myristate, iso-menthone, 1-octyl-2-pyrrolidone, and laurocapram) on the transdermal permeation of corticosterone across HEM. The potencies of these chemical enhancers-maximum enhancement, E_{max}, were compared at their highest thermodynamic activity in equilibrium with HEM. A relationship between the maximum intrinsic enhancement factor (E_{max}) and enhancer lipophilicity (K_{oct}) was observed with the enhancers, in which the enhancer potency decreased with increasing enhancer lipophilicity. The E_{max} versus K_{oct} relationship suggests that the potency of an enhancer is relatively independent of specific interactions between the enhancer and SC lipids. These results also suggest that the solubility of the enhancer in SC is an important factor for transdermal permeation enhancement. E_{max} of the nonalkyl chain enhancers was found to be lower than that of the alkyl chain enhancers when compared at the same lipophilicity. This study also proposed the possibility of using enhancer solubility in silicone as a predictive tool for determining the potency of an enhancer.

Iyer et al. (2007) constructed quantitative structure-activity relationship (QSAR) models for four different skin penetration enhancer data sets of 61, 44, 42, and 17 compounds using classic QSAR descriptors and 4D fingerprints. Three data sets involved skin penetration enhancement of hydrocortisone and hydrocortisone acetate, and the fourth data set involved skin penetration enhancement of fluorouracil. Significant QSAR models could be built using multidimensional linear regression fitting and genetic function model optimization for all four data sets when both classic and 4D fingerprint descriptors were used in the trial descriptor pool. Overall, the

QSAR models for the penetration enhancer systems appear meaningfully different from one another, suggesting that there were distinct mechanisms of skin penetration enhancement that depend on the chemistry of both the enhancer and the penetrant.

Golla et al. (2012) used a combination of genetic algorithms (GA) and quantitative structure-property relationship (QSPR) techniques to develop the computer-aided molecular design (CAMD) algorithm for virtual design of chemical penetration enhancers for transdermal drug delivery. The target properties of chemical penetration enhancers were identified by literature survey and analysis of their molecular properties. Using a database of 272 chemical penetration enhancers cited in the literature as seed molecules, new molecules were generated using genetic operators such as crossover, mutation, and functional group addition. QSPR models developed using artificial neural networks (ANNs) were used to predict the target physicochemical properties including skin penetration coefficient, octanol/water partition coefficient, melting point, skin sensitization, and skin irritation of the newly generated molecules. To validate the design methodology results, identified potential chemical penetration enhancers were tested experimentally for toxicity and skin permeation. Four molecules were found to be effective in enhancing insulin permeation through the skin with minimal or no toxic effects. This approach appears to have a few major drawbacks. Some of the virtually designed chemical penetration enhancers were not effective in transporting insulin through the skin, and the authors attributed this to lack of accurate knowledge of chemical penetration enhancer-drug interaction in the predesign stage. Another major impediment to experimental validation of the newly generated chemicals is the lack of commercial availability of the chemicals.

Conclusions

A large number of chemical compounds have been evaluated as skin penetration enhancers. The list of potential drugs that can be effectively delivered via transdermal route continues to increase. Structure-permeation enhancement relationship studies have significantly increased our understanding of the effect of penetration enhancers for different types of drugs. In general, a parabolic relationship between the carbon chain length of fatty acids and fatty alcohols and skin permeation enhancement has been observed with several drugs. Solid fatty acids (saturated fatty acids) had lower enhancement efficiency when compared to the liquid fatty acids (unsaturated fatty acids). Terpenes with higher LogP values were found to be more effective enhancers than those with lower LogP. With pyrrolidones, the number of carbonyl groups in the chain influenced the enhancing activity more effectively than the ring size. In general, ionic surfactants produced a greater flux of drugs than nonionic surfactants. Unfortunately, many of the penetration enhancers that showed good permeation enhancement effect also cause skin irritation (Kanikkannan and Singh 2002). The practical use of chemical penetration enhancers requires careful balancing of their benefits and risks, i.e., penetration rates and irritation. Further studies are needed in the areas of evaluation of skin permeation enhancement vis-a-vis skin irritation in order to choose penetration enhancers, which possess optimum enhancement effect with no skin irritation.

References

Ackermann C, Flynn GL, Smith WM (1987) Ether-water partitioning and permeability through nude mouse skin in vitro. II. Hydrocortisone 21-n-alkyl esters, alkanols and hydrophilic compounds. Int J Pharm 36:67–71

Aoyagi T, Yamamura M, Suzuki N, Matsui K, Nagase Y (1991) Preparation of substituted pyrrolidone derivatives and their evaluation as transdermal penetration enhancers. Drug Des Discov 8:37–46

Ashton P, Walters KA, Brain KR, Hadgraft J (1992) Surfactant effects in percutaneous absorption. I. Effects on the transdermal flux of methyl nicotinate. Int J Pharm 87:261–264

Aungst BJ (1989) Structure/effect studies of fatty acid isomers as skin penetration enhancers and skin irritants. Pharm Res 6:244–247

Aungst BJ (1995) Fatty acids as skin permeation enhancers. In: Smith EW, Maibach HI (eds) Percutaneous penetration enhancers. CRC Press, New York, pp 277–287

Aungst BJ, Rogers NJ, Shefter E (1986) Enhancement of naloxone penetration through human skin in-vitro using fatty acids, fatty alcohols, surfactants, sulfoxides and amides. Int J Pharm 33:225–234

Barry BW (2001) Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci 14:101–114

Bhatia KS, Singh J (1997) Percutaneous absorption of LHRH through porcine skin: effect of N-methyl 2-pyrrolidone and isopropyl myristate. Drug Dev Ind Pharm 23:1111–1114

Brain KR, Walters KA (1993) Molecular modeling of skin permeation enhancement by chemical agents. In: Walter KA, Hadgraft J (eds) Pharmaceutical skin penetration enhancement. Marcel Dekker, New York, pp 389–416

Cappel MJ, Kreuter J (1991) Effect of nonionic surfactants on transdermal drug delivery. I. Polysorbates. Int J Pharm 69:143–153

Carelli V, Di Colo G, Nannipieri E, Serafini MF (1992) Enhancement effects in the permeation of alprazolam through hairless mouse skin. Int J Pharm 88:89–97

Chantasart D, Li K, He N, Warner KS, Prakongpan S, Higuchi WI (2004) Mechanistic studies of branched-chain alkanols as skin permeation enhancers. J Pharm Sci 93:762–779

Chantasart D, Pongjanyakul T, Higuchi WI, Li SK (2009) Effects of oxygen-containing terpenes as skin permeation enhancers on the lipoidal pathways of human epidermal membrane. J Pharm Sci 98:3617–3632

Chi SC, Park ES, Kim H (1995) Effect of penetration enhancers on flurbiprofen permeation through rat skin. Int J Pharm 126:267–274

Chien YW, Xu H, Chiang CC, Huang YC (1988) Transdermal controlled administration of indomethacin. I. Enhancement of skin permeability. Pharm Res 5:103–106

Cooper ER, Berner B (1984) Interactions of surfactants with epidermal tissues- physicochemical aspects, in Surfactants In Cosmetics. Rieger MM. Marcel Dekker, New York. pp 195–210

Cornwell PA, Barry BW (1994) Sesquiterpene components of volatile oils as skin penetration enhancers for the hydrophilic permeant 5-fluorouracil. J Pharm Pharmacol 46:261–269

Cornwell PA, Barry BW, Bouwstra JA, Gooris GS (1996) Modes of action of terpene penetration enhancers in human skin; differential scanning calorimetry, small-angle X-ray diffraction and enhancer uptake studies. Int J Pharm 127:9–26

Ding BY, Fu XC, Liang WQ (2006) Branched-chain alkanols as skin permeation enhancers: quantitative structure-activity relationship. Pharmazie 61:298

El-Kattan A, Asbill CS, Michniak BB (2000) The effect of terpene enhancer lipophilicity on the percutaneous permeation of hydrocortisone formulated in HPMC gel systems. Int J Pharm 198:179–189

El-Kattan A, Asbill CS, Kim N, Michniak BB (2001) The effects of terpene enhancers on the percutaneous permeation of drugs with different lipophilicities. Int J Pharm 215:229–240

Fang JY, Hwang TL, Leu YL (2003) Effect of enhancers and retarders on percutaneous absorption of flurbiprofen from hydrogels. Int J Pharm 50:313–325

Friend D, Catz P, Heller J, Reid J, Baker R (1988) Transdermal delivery of levonorgestrel I: alkanols as permeation enhancers in vitro. J Control Release 7: 243–250

Gao S, Singh J (1998) In vitro percutaneous absorption enhancement of a lipophilic drug tamoxifen by terpenes. J Control Release 51:193–199

Ghafourian T, Zandasrar P, Hamishekar H, Nokhodchi A (2004) The effect of penetration enhancers on drug delivery through skin: a QSAR study. J Control Release 99:113–125

Godwin DA, Michniak BB (1999) Influence of drug lipophilicity on terpenes as penetration enhancers. Drug Dev Ind Pharm 25:905–915

Golden GM, McKie JE, Potts RO (1987) Role of SC lipid fluidity in transdermal drug flux. J Pharm Sci 76:25–28

Golla S, Neely BJ, Whitebay E, Madihally S, Robinson RL Jr, Gasem KA (2012) Virtual design of chemical penetration enhancers for transdermal delivery. Chem Biol Drug Des 79:478–487

Goodman M, Barry BW (1988) Action of penetration enhancers on human skin as assessed by the permeation of model drugs 5-fluorouracil and estradiol. I. Infinite dose technique. J Invest Dermatol 91:323–327

Hoelgaard A, Mollgaard B, Baker E (1988) Vehicle effect on topical drug delivery. IV. Effect of N-methylpyrrolidone and polar lipids on percutaneous drug transport. Int J Pharm 43:233–240

Hori M, Satoh S, Maibach HI, Guy RH (1991) Enhancement of propranolol hydrochloride and diazepam skin absorption in vitro: effect of enhancer lipophilicity. J Pharm Sci 80:32–35

Ibrahim SA, Li SK (2009) Effects of chemical enhancers on human epidermal membrane: structure-enhancement relationship based on maximum enhancement (E_{max}). J Pharm Sci 98:926–944

Ibrahim SA, Li SK (2010) Efficiency of fatty acids as chemical enhancers: mechanisms and structure enhancement relationship. Pharm Res 27:115–125

Iyer M, Zheng T, Hopfinger AJ, Tseng YJ (2007) QSAR analyses of skin penetration enhancers. J Chem Inf Model 47:1130–1149

Kai T, Mak VHW, Potts RO, Guy RH (1990) Mechanism of percutaneous penetration enhancement: effect of n-alkanols on the permeability barrier of hairless mouse skin. J Control Release 12:103–112

Kandimalla K, Kanikkannan N, Andega S, Singh M (1999) Effect of fatty acids on the permeation of melatonin across rat and pig skin in vitro and on the transepidermal water loss in vivo. J Pharm Pharmacol 51:783–790

Kang L, Yap CW, Lim PF, Chen YZ, Ho PC, Chan YW, Wong GP, Chan SY (2007) Formulation development

of transdermal dosage forms: quantitative structure-activity relationship model for predicting activities of terpenes that enhance drug penetration through human skin. J Control Release 120:211–219

Kanikkannan N, Singh M (2002) Skin permeation enhancement effect and skin irritation of saturated fatty alcohols. Int J Pharm 248:219–228

Kim CK, Hong MS, Kim YB, Han SK (1993) Effect of penetration enhancers (pyrrolidone derivatives) on multilamellar liposomes of SC lipid: a study by UV spectroscopy and differential scanning calorimetry. Int J Pharm 95:43–50

Kitagawa S, Kasamaki M, Hiyama F (2001) Effects of double-chained cationic surfactants n-dimethyldialkylammoniums on skin permeation of benzoic acid through excised guinea pig dorsal skin: comparison of their enhancement effects with hemolytic effects on erythrocytes. Chem Pharm Bull 49:1155–1158

Komata Y, Inaoka M, Kaneko A, Fujie T (1992) In vitro percutaneous absorption of thiamine disulfide from a mixture of propylene glycol and fatty acid. J Pharm Sci 81:744–746

Kushla GP, Zatz JL (1991) Correlation of water and lidocaine flux enhancement by cationic surfactants in vitro. J Pharm Sci 80:1079–1083

Lee CK, Uchida T, Noguchi E, Kim NS, Goto S (1993) Skin permeation enhancement of tegafur by ethanol/panasate 800 or ethanol/water binary vehicle and combined effects of fatty acids and fatty alcohols. J Pharm Sci 82:1155–1159

Lopez A, Llinares F, Cortell C, Herraez M (2000) Comparative enhancer effects of Span®20 with Tween®20 and Azone® on the in vitro percutaneous penetration of compounds with different lipophilicities. Int J Pharm 202:133–140

Magnusson BM, Walters KA, Roberts MS (2001) Veterinary drug delivery: potential for skin penetration enhancement. Adv Drug Deliv Rev 50:205–227

Menon GK, Lee SH, Roberts M (1998) Ultrastructural effects of some solvents and vehicles on the stratum corneum and other skin components: evidence for an 'extended mosaic partitioning model of the skin barrier'. In: Roberts MS, Walters KA (eds) Dermal absorption and toxicity assessment. Marcel Dekker, New York, pp 727–751

Morimoto K, Tojima H, Haruta T, Suzuki M, Kakemi M (1996) Enhancement effects of unsaturated fatty acids with various structures on the permeation of indomethacin through rat skin. J Pharm Pharmacol 48:1133–1137

Narishetty ST, Panchagnula R (2004) Transdermal delivery system for zidovudine: in vitro, ex vivo and in vivo evaluation. Biopharm Drug Dispos 25:9–20

Nokhodchi A, Shokri J, Dashbolaghi A, Hassan-Zadeh D, Ghafourian T, Barzegar-Jalali M (2003) The enhancement effect of surfactants on the penetration of lorazepam through rat skin. Int J Pharm 250:359–369

Obata Y, Takayama K, Okabe H, Nagai T (1990) Effect of cyclic monoterpenes on percutaneous absorption in the case of a water-soluble drug (diclofenac sodium). Drug Des Deliv 6:319–328

Ogiso T, Shintani M (1990) Mechanism for the enhancement effect of fatty acids on the percutaneous absorption of propranolol. J Pharm Sci 79:1065–1071

Okabe H, Takayama K, Ogura A, Nagai T (1989) Effect of limonene and related compounds on the percutaneous absorption of indomethacin. Drug Des Deliv 4:313–321

Okamoto H, Ohyabu M, Hashida M, Sezaki H (1987) Enhanced penetration of mitomycin C through hairless mouse and rat skin by enhancers with terpene moieties. J Pharm Pharmacol 39:531–534

Okamoto H, Hashida M, Sezaki H (1988) Structure activity relationship of 1-alkyl-or 1-alkenylazacycloalkanone derivatives as percutaneous penetration enhancers. J Pharm Sci 77:418–424

Ongpipattanakul B, Burnette R, Potts RO (1991) Evidence that oleic acid exists as a separate phase within stratum corneum. Pharm Res 8:350–354

Park ES, Chang SY, Hahn M, Chi SC (2000) Enhancing effect of polyoxyethylene alkyl ethers on the skin permeation of ibuprofen. Int J Pharm 209:109–119

Potts RO, Francoeur ML (1990) Lipid biophysics of water loss through the skin. Proc Natl Acad Sci U S A 87:3871–3873

Sasaki H, Kojima M, Mori Y, Nakamura J, Shibasaki J (1988) Enhancing effect of pyrrolidone derivatives on transdermal drug delivery. Int J Pharm 44:15–24

Sasaki H, Kojima M, Nakamura J, Shibasaki J (1990a) Enhancing effect of pyrrolidone derivatives on transdermal penetration of phenolsulfonphthalein and indomethacin from aqueous vehicle. Chem Pharm Bull (Tokyo) 38:797–799

Sasaki H, Kojima M, Nakamura J, Shibasaki J (1990b) Enhancing effect of pyrrolidone derivatives on transdermal penetration of sulfaguanidine, aminopyrine, Sudan III. J Pharmacobiodyn 13:200–205

Sasaki H, Kojima M, Mori Y, Nakamura J, Shibasaki J (1991) Enhancing effect of pyrrolidone derivatives on transdermal penetration of 5-flurouracil, triamcinolone acetate, indomethacin, and flurbiprofen. J Pharm Sci 80:533–538

Sloan KB, Beal HD, Taylor HE, Getz JJ, Villaneuva R, Nipper R, Smith K (1998) Transdermal delivery of theophylline from alcohol vehicles. Int J Pharm 171: 185–193

Tanojo H, Geest AB, Bouwstra JA, Junginger HE, Bodde HE (1997a) In-vitro Human skin barrier perturbation by oleic acid: thermal analysis and freeze fracture electron microscopy studies. Thermochim Acta 293:77

Tanojo H, Bouwstra JA, Junginger HE, Bodde HA (1997b) In-vitro Human skin barrier modulation by fatty acids: skin permeation and thermal analysis studies. Pharm Res 14:42–49

Thomas NS, Panchagnula R (2003) Combination strategies to enhance transdermal permeation of zidovudine (AZT). Pharmazie 58:895–898

Walters KA, Walker M, Olejnik O (1988) Non-ionic surfactant effects on hairless mouse skin permeability characteristics. J Pharm Pharmacol 40:525–529

Warner KS, Li SK, Higuchi WI (2001) Influences of alkyl group chain length and polar head group on chemical

skin permeation enhancement. J Pharm Sci 90: 1143–1153

Warner KS, Li SK, He N, Suhonen TM, Chantasart D, Bolikal D, Higuchi WI (2003) Structure-activity relationship for chemical skin permeation enhancers: probing the chemical microenvironment of the site of action. J Pharm Sci 92:1305–1322

Williams AC, Barry BW (1991a) Terpenes and the lipid-protein partitioning theory of skin penetration enhancement. Pharm Res 8:17–24

Williams AC, Barry BT (1991b) enhancement index concept applied to penetration enhancers for human skin and model lipophilic (estradiol) and hydrophilic (5-flurouracil) drugs. Int J Pharm 74:157–168

Williams AC, Barry B (2004) Penetration enhancers. Adv Drug Deliv Rev 56:603–618

Yoneto K, Ghanem AH, Higuchi WI, Peck KD, Li SK (1995) Mechanistic studies of the 1-alkyl-2-pyrrolidones as skin permeation enhancers. J Pharm Sci 84:312–317

Yoneto K, Li K, Higuchi WI, Jiskoot W, Herron JN (1996) Fluorescent probe studies of the interactions of 1-alkyl-2-pyrrolidones with stratum corneum lipid liposomes. J Pharm Sci 85:511–517

Yoneto K, Li K, Higuchi WI, Shimabayashi S (1998) Influence of the permeation enhancers 1-alkyl-2-pyrrolidones on permeant partitioning into the stratum corneum. J Pharm Sci 87:209–214

Yu J, Chien T, Chien Y (1991) Transdermal dual-controlled delivery of testosterone and estradiol: (II) enhanced skin permeability and membrane moderated delivery. Drug Dev Ind Pharm 17:1905–1930

5 Quantitative Structure–Enhancement Relationship and the Microenvironment of the Enhancer Site of Action

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5.1 Introduction

Over the past 40 years, numerous studies on the influence of chemical permeation enhancers upon drug permeation across skin have been performed. The literature is abundant with hundreds of articles, patents, and reviews on this topic (e.g., reviewed in Lee et al. 1991; Williams and Barry 1992; Smith and Maibach 1995; Walters and Hadgraft 1993; Potts and Guy 1997). It is generally believed that the mechanisms of action for most transdermal enhancers are through physical enhancer–membrane interactions (e.g., intercalation and perturbation) and by fluidizing the lipids in the stratum corneum (SC) (e.g., Barry 1987; Yoneto et al. 1996). Some enhancers can also act by SC lipid solubilization and lipid extraction (e.g., Goates and Knutson 1994; Ogiso et al. 1995). Despite continuing advances in the knowledge of transdermal absorption and enhancer mechanisms, the effects of enhancers upon drug permeation have been quite unpredictable, and the mechanism of action of permeation enhancers and their site of action in the SC are not fully understood. A clear quantitative structure–enhancement relationship (relationship between the enhancer molecular structures and their enhancing potencies) for predicting the effectiveness of enhancers is not available. Arbitrary screening remains a common approach in industrial practice to identify effective permeation

N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_5

enhancers for improving percutaneous absorption as opposed to a rational design approach. A screening approach generally is not effective, and therefore, obtaining a quantitative structure–enhancement relationship for permeation enhancers based on a clear understanding of the mechanism of action of enhancers and the nature of the microenvironment of the enhancer site of action is important. If one does not understand the structure–enhancement relationship, the rational design of an effective enhancer and the prediction of the enhancer effects will remain difficult tasks.

There are more than dozens of papers on the subjects of the relationships between the chemical structures of permeation enhancers and their effects on drug permeation across SC. For example, Vavrova et al. (2005) recently reviewed the structure–enhancement relationships of amphiphilic permeation enhancers such as the effects of the hydrophobic chain length as well as polar head group characteristics of the enhancers. Other studies investigated the effects of enhancer chemical structures such as acyl chain length of structurally simplified amphiphiles of carboxylic acid esters and short-chain ceramide analogues upon their abilities to perturb the skin barrier (Novotny et al. 2009a, b). The effects of acyl chain branching of permeation enhancers have also been investigated (Klimentová et al. 2006). A complete review of all important studies involving skin chemical permeation enhancers was not the intent of the authors of this chapter.

This chapter reviews our recent findings and addresses the following questions. How does the nature of the enhancer polar head group and the hydrocarbon group contribute to the potency of a chemical permeation enhancer? What is the relationship between the physicochemical properties of an enhancer such as lipophilicity (e.g., octanol–water partition coefficient) and its potency? What is the nature of the microenvironment of the enhancer site of action? In this chapter, we will establish a quantitative structure–enhancement relationship of the studied enhancers for the lipoidal pathway of the SC. Such a structure–enhancement relationship would provide basic insights into the mechanism of action of chemical permeation enhancers. These insights can aid pharmaceutical scientists in employing physical–chemical principles rather than trial-and-error screening methods in the search for effective enhancers. The structure–enhancement relationship, moreover, will provide direct information regarding the nature of the microenvironment of the enhancer site of action in the SC.

5.2 Methods

A review of the percutaneous absorption literature has indicated that most transdermal absorption studies for the purpose of enhancer evaluation have been conducted in an asymmetric enhancer configuration: the test enhancer solution with the drug is applied on the SC, while the dermis side remains in contact with the receiver solution that is usually an aqueous buffer solution (Warner et al. 2003). Although this experimental setup is adequate in assessing the effects of permeation enhancers upon the delivery of a particular drug and mimics the practical situation of a transdermal delivery system, there are potential fundamental problems. For example, the asymmetric setup can result in an enhancer concentration gradient across the skin membrane. This leads to a complex situation in which the local permeation enhancement varies with the position within the membrane, making mechanistic analysis of the data difficult (Liu et al. 1992). Another shortcoming in some past studies is that the importance of different pathways in the SC (i.e., parallel lipoidal and pore pathways) has not been recognized even though it is a general view that permeation enhancers can affect either or both the SC lipoidal and pore pathways to enhance the permeation of lipophilic and polar compounds. A parallel lipoidal and pore pathway SC transport model should be utilized to delineate the enhancement effects upon the lipoidal pathway induced by the enhancers (e.g., Warner et al. 2001). Other studies have not corrected for the changes in permeant thermodynamic activities in the presence of enhancers and cosolvents in the donor chamber in data analysis. From a mechanistic point of view, the effects of the enhancers and cosolvents upon permeant activities that affect permeant transport should be properly taken into account in assessing the potencies of the enhancers.

Studies in our laboratory have involved the use of a different experimental approach to establish a quantitative structure–enhancement relationship for the lipoidal pathway of the SC and to address the question of the nature of the microenvironment of the enhancer site of action (Kim et al. 1992; Yoneto et al. 1995; Warner et al. 2003; He et al. 2004). In these studies, permeation experiments were conducted under symmetric and equilibrium conditions (i.e., aqueous enhancer solution in both the donor and receiver chambers in equilibrium with the SC). The permeability enhancement factor, E, the ratio of the permeant flux with the enhancer solution to that with the control phosphate-buffered saline solution (PBS), was determined in these experiments with a model lipophilic permeant. The enhancement factor was corrected for any changes in the chemical potential of the permeant in the enhancer solution with respect to that in PBS; this allowed the comparison of enhancement factors at the same permeant thermodynamic activity. Corticosterone (CS) was selected as the model permeant because the lipoidal pathway of the SC is the main transport rate-determining pathway for this permeant. Transport experiments were also conducted with a polar permeant to correct for possible effects of the enhancers upon the pore pathway. The equilibrium concentration of the enhancer in the SC intercellular lipid domain was determined in separate enhancer uptake experiments with SC and delipidized SC. In these experiments, a sample of SC or delipidized SC was weighed and soaked in the enhancer solution until the membrane was in equilibrium with the enhancer solution. If depletion of the enhancer in the enhancer solution was observed, the solution was replaced to maintain a constant enhancer concentration. After equilibration, the SC was removed from the solution and weighed. Then, the enhancer in the SC was extracted with 100 % ethanol and assayed. The details of the experimental procedure for both the transport and uptake experiments can be found in previous studies (Yoneto et al. 1995; Warner et al. 2003; Chantasart et al. 2004; He et al. 2004).

Figure 5.1 shows representative plots of enhancement factor vs. aqueous enhancer concentration for CS permeation across the skin lipoidal pathway with 1-butyl-, 1-hexyl-, and 1-octyl-2-pyrrolidones as the enhancers. From plots such as those in Fig. 5.1, the corresponding enhancer aqueous concentrations for $E=10$ (which are in thermodynamic equilibrium with the skin) were determined for the enhancers studied. These concentrations are defined as the aqueous isoenhancement concentrations of $E=10$ and will be used as a means to evaluate the potencies of the enhancers. Similarly, the aqueous isoenhancement concentration of $E=4$ can be obtained. The relative potencies of the enhancers were assessed and compared based on (a) their aqueous isoenhancement concentrations and (b) their SC intercellular lipid concentrations.

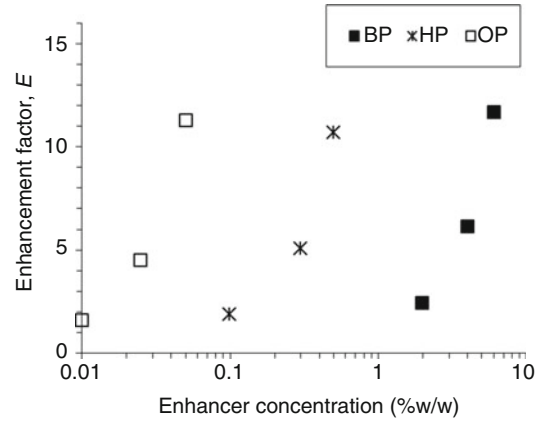
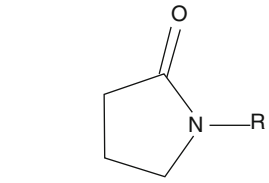


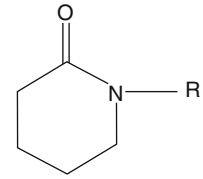
Fig. 5.1 Representative plots of permeability enhancement factor across the SC lipoidal pathway (E) vs. aqueous enhancer concentration for corticosterone with enhancers 1-butyl-2-pyrrolidone (BP), 1-hexyl-2-pyrrolidone (HP), and 1-octyl-2-pyrrolidone (OP)

5.3 Quantitative Structure–Enhancement Relationships Based on Aqueous Concentrations of the Enhancer

Figure 5.2 shows the enhancers studied in our laboratory using the isoenhancement concentration approach. They include homologous series of



1-alkyl-2-pyrrolidones (AP)



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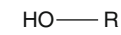
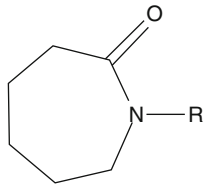
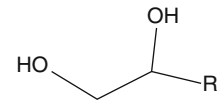


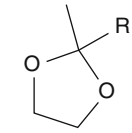
1-alkanols (AL)



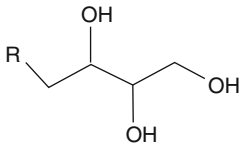
1-alkyl-2-azacycloheptanones (AZ)



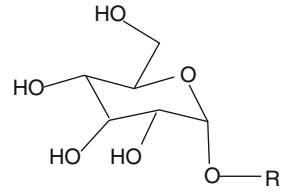
1,2-alkanediols (AD)



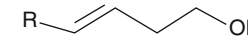
2-(1-alkyl)-2-methyl-
1,3-dioxolanes (MD)



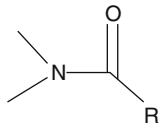
1,2,3-alkanetriols (AT)



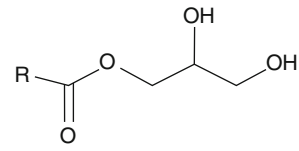
n-alkyl-β-D-glucopyranosides (AG)



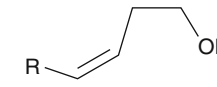
trans-3-alken-1-ols (TAL)



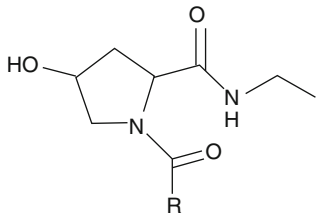
N,N-dimethyl alkanamides (AM)



1,2-dihydroxypropyl
alkanoates (MG)



cis-3-alken-1-ols (CAL)



trans-hydroxyproline-N-alkanamide-
C-ethylamide (HAE)

Fig. 5.2 Chemical structures of the permeation enhancers, R = alkyl chain

enhancers with different polar head groups and enhancers with a carbon–carbon double bond substituting for a single bond in their hydrocarbon chain (the lipophilic moiety). These enhancers were examined employing the experimental strategy described in "Methods" section above, and the experimental details were reported previously (Warner et al. 2003; He et al. 2003). Figure 5.3 presents the aqueous isoenhancement concentrations at $E = 10$ and the octanol/PBS partition coefficients ($K_{\text{octanol/PBS}}$) of the enhancers shown in Fig. 5.2. The isoenhancement concentrations in the plot were interpolated from the E vs. aqueous enhancer concentration plots similar to those in Fig. 5.1, and the *n*-octanol/PBS partition coefficients were determined at the $E = 10$ conditions. In Fig. 5.3, the data points of all enhancers essentially fall on the same line with a slope of –1.

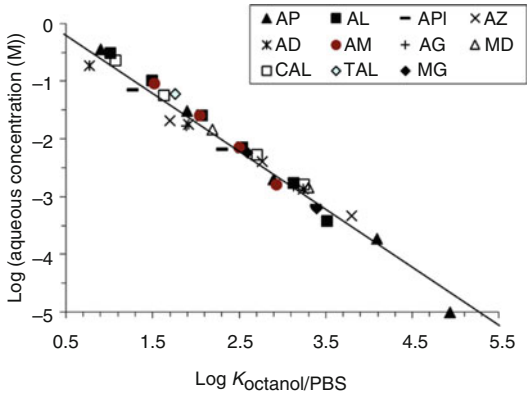


Fig. 5.3 Correlation between aqueous $E=10$ isoenhancement concentration and octanol/PBS partition coefficient ($K_{octanol/PBS}$). The slope of the line in the figure = −1. Each data point represents the average value without showing the standard deviation because the error bar generally lies within the symbol in the log–log plot. Enhancer abbreviations are provided in Fig. 5.2

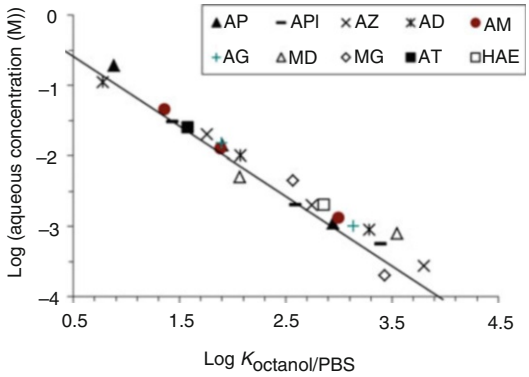


Fig. 5.4 Correlation between the aqueous isoenhancement concentration at $E=4$ and octanol/PBS partition coefficient ($K_{octanol/PBS}$). The slope of the line in the figure = −1. Each data point represents the average value without showing the standard deviation because the error bar generally lies within the symbol in the log–log plot. Enhancer abbreviations are provided in Fig. 5.2

While there are a few moderate outliers, the overall correlation is quite good. Figure 5.4 presents the plot of the $E=4$ isoenhancement concentrations vs. the *n*-octanol/PBS partition coefficients. Similar to Fig. 5.3, there is again a good correlation between the enhancer potency and the *n*-octanol/PBS partition coefficient. Two major conclusions can be deduced from Figs. 5.3 and 5.4. First, the correlations in the enhancer isoenhancement concentration vs. enhancer partition coefficient plots with a slope of around –1 suggest that water-saturated *n*-octanol may represent the chemical microenvironment of the studied enhancers at their site of action in the SC lipid lamellae. Second, these data demonstrate a structure–enhancement relationship of the enhancers, in which the potencies of the enhancers for transport enhancement across the SC lipoidal pathway are related to the enhancer lipophilicities. These results also suggest that the *n*-octanol/PBS partition coefficient is an excellent predictor of enhancer potency (as expressed in terms of the aqueous enhancer concentration) for the skin permeation enhancers studied.

Three other partition coefficient systems (*n*-hexanol/PBS, *n*-decanol/PBS, and *n*-hexane/PBS) were investigated for their ability to provide correlations with the potencies of the enhancers. Here, a main purpose was to further characterize the microenvironment of the enhancer site of action in the transport rate-limiting domain in the SC intercellular lipid lamellae. First, the *n*-hexanol/PBS and *n*-decanol/PBS systems were to test for the degree of selectivity of *n*-octanol among the *n*-alkanols as the organic phase. At the other extreme, the *n*-hexane/PBS system was to test how a pure hydrocarbon environment would represent the microenvironment of the enhancer site of action in the SC transport rate-limiting domain. Figures 5.5, 5.6, and 5.7 show the isoenhancement concentration of $E=10$ vs. the *n*-hexanol/PBS, *n*-decanol/PBS, and *n*-hexane/PBS partition coefficients, respectively. The correlations between enhancer potencies and the partition coefficients of the *n*-hexanol/PBS (Fig. 5.5) and *n*-decanol/PBS (Fig. 5.6) systems (within the scatter of the data) are quite good and comparable to that with the *n*-octanol/PBS system. This suggests that the chemical environment differences among the three *n*-alkanols are probably not large enough for choosing a system among the three to best mimic the chemical microenvironment of the enhancer in the lipid lamellae of the SC. In contrast to the correlation found between the enhancer potencies and the partition coefficients in the studied *n*-alkanol/PBS systems, the correlation between enhancer potency and the partition coefficient with the *n*-hexane/PBS system is poor (Fig. 5.7) suggesting that the microenvironment of the

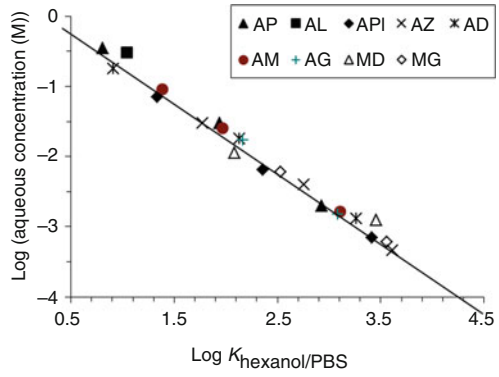


Fig. 5.5 Correlation between the aqueous isoenhancement concentration at $E=10$ and hexanol/PBS partition coefficient ($K_{hexanol/PBS}$). The slope of the line in the figure = −1. Each data point represents the average value without showing the standard deviation because the error bar generally lies within the symbol in the log–log plot. Enhancer abbreviations are provided in Fig. 5.2

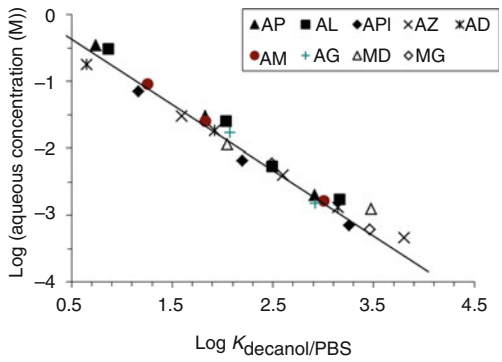


Fig. 5.6 Correlation between the aqueous isoenhancement concentration at $E=10$ and decanol/PBS partition coefficient ($K_{decanol/PBS}$). The slope of the line in the figure = −1. Each data point represents the average value without showing the standard deviation because the error bar generally lies within the symbol in the log–log plot. Enhancer abbreviations are provided in Fig. 5.2

enhancer site of action in the SC lipid lamellae is quite different from a pure hydrocarbon environment. The difference between the poor correlation found in Fig. 5.7 and the good correlations seen in Figs. 5.3, 5.4, 5.5, and 5.6 is an indication of the extent of intermolecular hydrogen bonding of the enhancer with the components of the semipolar microenvironment of the SC lipid lamellae matching rather well to that of the intermolecular hydrogen bonding between the enhancer and the

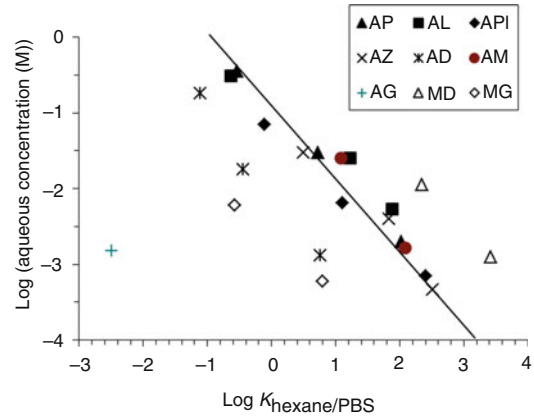


Fig. 5.7 Correlation between the aqueous isoenhancement concentration at $E=10$ and hexane/PBS partition coefficient ($K_{hexane/PBS}$). The slope of the line in the figure = −1. Each data point represents the average value without showing the standard deviation because the error bar generally lies within the symbol in the log–log plot. Enhancer abbreviations are provided in Fig. 5.2

n-alkanol molecules. This match is absent when hexane is the organic phase.

In summary, comparisons of the enhancer potencies with the *n*-hexanol/PBS, *n*-octanol/PBS, *n*-decanol/PBS, and *n*-hexane/PBS partition coefficients showed good correlations for the *n*-alkanol solvents but not for *n*-hexane. A quantitative structure–enhancement relationship has been established with the enhancers. This result supports the hypotheses that (a) the effectiveness of the permeation enhancers is related to their lipophilicity and their ability to partition into the transport rate-limiting domain in the SC intercellular lipid lamellae and (b) the transport rate-limiting domain has a microenvironment with polarity similar to the polarity of water-saturated bulk *n*-hexanol, *n*-octanol, and *n*-decanol.

5.4 Quantitative Structure–Enhancement Relationship Based on Enhancer Concentrations in the Stratum Corneum Intercellular Lipids

The foregoing discussion has provided new insight into the physicochemical factors influencing the potencies of chemical permeation

enhancers (their effectiveness as permeation enhancers). A general quantitative structure–enhancement relationship based on the enhancer aqueous isoenhancement concentration data and their *n*-alkanol/PBS partition coefficients has been established. The discussion up to this point has inferred that because of the good correlation between the aqueous isoenhancement concentration and the *n*-alkanol/PBS partition coefficient, the enhancer target site microenvironment can be well mimicked by liquid *n*-alkanols. However, it would be inappropriate to make any definite conclusions regarding this without information concerning the concentration of the enhancers at their site of action. An independent set of experiments was therefore conducted to quantify enhancer potencies by determining the enhancer concentration in the SC intercellular lipids under the isoenhancement $E=10$ conditions (He et al. 2004). The experimental results obtained from this approach allow for a more critical examination than is possible based on the aqueous isoenhancement concentration data alone. As will be discussed in some detail, the data of the aqueous isoenhancement enhancer concentrations and the corresponding equilibrium enhancer concentrations in the SC intercellular lipids, taken together, will provide a more complete understanding of the enhancer molecular factors associated with enhancer potency as well as further insight into the nature of the enhancer target site microenvironment in the SC.

Figure 5.8 presents the data on enhancer uptake into the intercellular lipid domain of the SC under the $E=10$ isoenhancement conditions for 18 of the enhancers studied in the previous section. The SC intercellular lipid enhancer concentration determined under the isoenhancement condition may be considered as a measure of the intrinsic enhancer potency; the lower this concentration, the higher the intrinsic enhancer potency. Based on the results presented in Fig. 5.8, the potencies of the enhancers are seen to be relatively independent of their octanol/PBS partition coefficients. Note that the *x*-axis and *y*-axis of Fig. 5.8 have the same scales as those in Fig. 5.3. The relatively constant enhancer concentrations in the SC intercellular lipids in

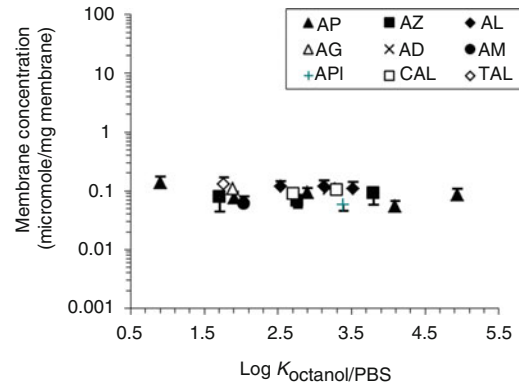


Fig. 5.8 Relationship between enhancer uptake into SC intercellular lipid domain per mg dry SC (μmole/mg) under isoenhancement $E=10$ and octanol/PBS partition coefficient ($K_{octanol/PBS}$). Each data point represents the average value. The standard deviations of the log $K_{octanol/PBS}$ data are not shown because the error bars generally lie within the symbols in the plot. Enhancer abbreviations are provided in Fig. 5.2

Fig. 5.8 are in contrast to the strong dependence between the lipophilicities of the enhancers and their potencies based on the enhancer concentrations in the aqueous phase in Fig. 5.3. This result is quite surprising as one would have expected some enhancer alkyl chain length and/or polar head group dependency on the potencies of the enhancers (based on their concentration in the SC lipid domain). The data in Fig. 5.8, together with those in Fig. 5.3, suggest that (a) the intrinsic potency of the enhancer at its site of action is relatively independent of its alkyl group chain length and the nature of its polar head group, (b) the lipophilicity of the enhancer (both the alkyl chain and the polar head moieties) mainly assists the translocation of the enhancer to its site of action through a free energy of transfer from the bulk aqueous phase to the site, and (c) permeation enhancement is related to the ability of the permeation enhancer to partition into the transport rate-limiting domain, which seems to be well represented by the intercellular lipid "phase."

We will now take a closer look at the data in Fig. 5.8, which support the view that all the studied enhancers (at $E=10$) exhibit essentially the same intrinsic potency. First, the $E=10$ intrinsic potency of *n*-octyl-β-D-glucopyranoside is seen to be essentially the same as that of *n*-octanol.

Considering the large size and polarity of the glucopyranoside group, this suggests that the free volume of the microenvironment of the enhancer site of action in the SC transport rate-limiting domain is insensitive to the differences in the sizes of the enhancer polar head group. This also suggests further that under the conditions of the present study, the SC transport rate-limiting domain may not behave as that of an ordered lipid lamellae in the intercellular region of SC (Bouwstra et al. 2002b; Kuempel et al. 1998; Norlen 2001; White et al. 1988), but more like a conventional, homogeneous, bulk liquid "phase." Second, on the matter of *n*-alkyl group chain length effect, it has been suggested that there may be an optimum chain length (in the range of C9 to C12) for skin permeation enhancers (e.g., Aungst et al. 1986; Lee et al. 1991); yet there is little or no indication of this with the 1-alkyl-2-pyrrolidones (C4 to C12) in the present study. More studies with longer chain 1-alkyl-2-pyrrolidones would be of interest. Finally, it has been hypothesized that an enhancer with unsaturated alkyl chain such as unsaturated alcohols is more potent than an enhancer with saturated alkyl chain, based on the molecular geometry and the presence of kinks in the alkenyl chain of the unsaturated enhancer (Cooper 1984; Aungst et al. 1986; Aungst 1989; Brain and Walters 1993). For a similar reason, the unsaturated enhancer with a *cis* conformation carbon–carbon double bond has been expected to be more potent than the enhancer with a *trans* double bond. However, the results of the present study suggest no influence of a carbon–carbon double bond (in the lipophilic moiety) upon the enhancement effects of the enhancers. Here again, more studies with longer chain enhancers with a double bond located at different positions along the carbon chain would be of interest.

Although the intrinsic potencies of the studied enhancers have been found to be essentially the same and relatively independent of the enhancer molecular structures as revealed by the essentially same SC intercellular lipid "phase" enhancer concentration, it should be pointed out that enhancer lipophilicity is still an important factor because lipophilicity is essential to the translocation of the enhancers to their site of action in the membrane.

In conclusion, it has been somewhat surprising to find that all of the studied enhancers have yielded essentially the same intrinsic potency under the $E = 10$ conditions. As the present study is perhaps the first of its kind on this particular aspect, further work is needed. Despite the need of future testing, the data are so far consistent with the hypothesis that the enhancer polar head and alkyl groups act only to assist in the transfer of the enhancer from the aqueous phase to the SC intercellular lipid lamellae and make the enhancer available for its action in the SC transport rate-limiting domain. The essentially constant enhancer concentration in the intercellular lipid lamellae (Fig. 5.8) and the correlation between the $E = 10$ isoenhancement concentrations and octanol/PBS partition coefficients (Fig. 5.3) also support the interpretation that the microenvironment of the enhancer site of action and the macroscopic SC intercellular lipid "phase" can both be represented by water-saturated liquid *n*-octanol. This is discussed further in the next section.

5.5 Microenvironment of the Site of Enhancer Action

Figure 5.9 is a replot of the data in Figs. 5.3 and 5.8. It shows the relationship of the logarithm of the partition coefficient for enhancer partitioning between the aqueous phase and the intercellular lipid domain (log $K_{\text{SC lipid/PBS}}$) vs. log $K_{\text{octanol/PBS}}$ (He et al. 2004). Data from previous studies with branched alkanols (Chantasart et al. 2004) and 2-phenylethanol (Chantasart et al. 2007) are also included in the figure. The $K_{\text{SC lipid/PBS}}$ values were calculated from enhancer intercellular lipid uptake data and isoenhancement aqueous concentration data (both under $E = 10$ conditions). Arguments are now presented based on Figs. 5.3, 5.8, and 5.9 in support of the hypothesis: the microenvironment for the enhancer site of action is well mimicked by (water-saturated) liquid *n*-octanol. It can be seen in Fig. 5.9 that all enhancers fall essentially on the same line (with modest data scattering). The data correlation is quite good with a regression slope close to unity over a more than 1000-fold range of $K_{\text{octanol/PBS}}$

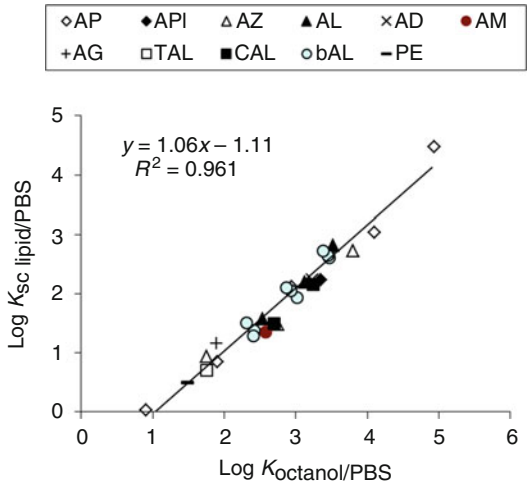


Fig. 5.9 Correlation between the partition coefficient of the enhancer between PBS and the intercellular lipid domain ($K_{SC\ lipid/PBS}$) vs. octanol/PBS partition coefficient ($K_{octanol/PBS}$). Each data point represents the average value without showing the standard deviation because the error bar generally lies within the symbol in the log–log plot. Enhancer abbreviations are provided in Fig. 5.2 except for branched alkanols (bAL) and 2-phenylethanol (PE).

values. Also, there is no significant difference among the correlations of the enhancers with different polar head groups and different alkyl chain length (slope = 1.00-1.30), suggesting that the microenvironment of the site of enhancer action in the SC is essentially the same for all the enhancers. A slope closer to unity in Fig. 5.9 would indicate an even closer similarity between the microenvironment of the enhancer site of action and *n*-octanol. The small positive deviation of the slope from unity may be attributed to (a) somewhat stronger interactions between the studied enhancers and the SC intercellular lipid domain than those of the *n*-octanol phase and (b) the SC intercellular lipid domain being slightly more lipophilic than the *n*-octanol phase. Despite this deviation, the essentially constant slope for the enhancers in the log $K_{SC\ lipid/PBS}$ vs. log $K_{octanol/PBS}$ plot suggests the same microenvironment of the enhancer site of action for the enhancers studied. It is important to emphasize that based on either Fig. 5.3 or 5.8 alone, it would have been inappropriate to conclude that the microenvironment for the enhancer site of action is well mimicked by the *n*-octanol organic phase. The reason is that the SC lipid lamellae are not expected to behave as a conventional, homogeneous liquid phase and there may well be a distribution of regions into which the enhancer may partition (Bouwstra et al. 2002a, b; Kuempel et al. 1998); it would not have been unreasonable to find regions in the lipid lamellae into which the enhancer molecule may partition, but may be relatively ineffective in contributing toward permeation enhancement. However, taken together, Fig. 5.3 and 5.8 would support the argument that a correlation likely exists between the *n*-octanol phase and both the transport rate-limiting domain and the microenvironment of the enhancer site of action in SC.

A point of emphasis is that Fig. 5.9 is evidence that the macroscopic SC intercellular lipid "phase" involved in the $E = 10$ enhancer uptake experiments is well mimicked by liquid (water-saturated) *n*-octanol. It is quite remarkable, considering the diversity of molecular types in this group of enhancers, that such a correlation (over a range of three orders of magnitude) would exist, especially when one recognizes that the SC lipid lamellae would not generally be described as a conventional, homogeneous liquid phase from the standpoint of chemical composition or structural order. One might expect that different regions (domains) in the SC lipid lamellae would favor partitioning of different enhancer molecules differently, especially when such a wide range of molecular types are considered. It may be possible, however, that there is such a distribution of regions (domains), but this distribution can be relatively narrow, especially for amphiphilic molecules (such as our studied enhancers) and especially when the SC lipid lamellae have been fluidized to a significant extent (e.g., at $E \geq 4$).

5.6 Quantitative Structure–Enhancement Relationships of Other Chemical Enhancers and Other Methods

The *n*-alkyl enhancers discussed so far in this chapter include alcohols, amides, dioxolanes, glucopyranosides, monoglycerides, piperidinones, pyrrolidones, azacycloheptanones, and diols as listed in Fig. 5.2. The effects of *cis* and *trans* double bonds and branching in the acyl chains

of permeation enhancers have also been studied (Chantasart et al. 2004; He et al. 2004). As a continuing effort to evaluate enhancer potencies and enhancer quantitative structure–enhancement relationships, permeation enhancers without an acyl chain (Chantasart et al. 2009) and enhancers of high lipophilicity (Warner et al. 2008; Ibrahim and Li 2009) have also been studied recently. Particularly, one of these studies employed the method of direct equilibration of the SC with liquid enhancers (i.e., enhancer equilibration with skin before permeation experiments of aqueous solutions in side-by-side diffusion cells) to examine the relationships between permeation enhancement effects of highly lipophilic enhancers and enhancer concentrations in the SC lipids (Ibrahim and Li 2009, 2010a). The results in this study show that the permeation enhancement effects are strongly related to the enhancer concentrations in the SC lipids: enhancers of higher SC lipid solubilities generally provide larger maximum permeation enhancement effects. This finding supports the hypothesis that enhancer potencies based on enhancer concentrations in the SC lipids are not significantly different from each other. In other words, the potencies of the enhancers based on their concentration in the aqueous phase in the diffusion cell chambers are related to the lipophilicities of the enhancers with the more lipophilic enhancers being more effective permeation enhancers when compared at the same aqueous enhancer concentration in the donor chamber. Recent studies of enhancers without an acyl chain and enhancers of high lipophilicity also support the hypotheses that the microenvironment of the enhancer site of action in the SC can be well mimicked by water-saturated liquid *n*-octanol (Chantasart et al. 2009) and the fluidization of the intercellular lipids in SC is a main mechanism of action of the enhancers (Ibrahim and Li 2010b). Tables 5.1a and 5.1b list all the enhancers studied to date in our laboratory for understanding the quantitative structure–enhancement relationships of permeation enhancers and the microenvironment of their site of action in SC. With the large group of enhancers tested in our studies, the generalization

Table 5.1a List of permeation enhancers used in our quantitative structure–enhancement relationship studies using the symmetric and equilibrium method (symmetric enhancer configuration)

***n*-Alkyl Enhancer**
Ethanol
1-Propanol
1-Butanol
1-Pentanol
1-Hexanol
1-Heptanol
1-Octanol
1-Nonanol
1-Undecanol
N,N-Dimethylhexanamide
N,N-Dimethylheptanamide
N,N-Dimethyloctanamide
N,N-Dimethylnonanamide
2-(1-Butyl)-2-methyl-1,3 dioxolane
2-(1-Hexyl)-2-methyl-1,3 dioxolane
1-Octyl-β-D-glucopyranoside
1-Decyl-β-D-glucopyranoside
1,2-Dihydroxypropyl octanoate
1,2-Dihydroxypropyl decanoate
1-Butyl-2-piperidinone
1-Hexyl-2-piperidinone
1-Octyl-2-piperidinone
1-Ethyl-2-pyrrolidone
1-Butyl-2-pyrrolidone
1-Hexyl-2-pyrrolidone
1-Octyl-2-pyrrolidone
1-Decyl-2-pyrrolidone
1-Butyl-2-azacycloheptanone
1-Hexyl-2-azacycloheptanone
1-Octyl-2-azacycloheptanone
1,2-Hexanediol
1,2-Octanediol
1-2-Decanediol
1,2,3-Nonanetriol
trans-Hydroxyproline-N-decanamide-C-ethylamide

Table 5.1a (continued)

Alkyl Branched and Double-Bond Enhancer
2-Hexanol
3-Hexanol
2-Heptanol
3-Heptanol
4-Heptanol
2-Octanol
3-Octanol
4-Octanol
2-Nonanol
3-Nonanol
4-Nonanol
5-Nonanol
cis-3-Penten-1-ol
cis-3-Hexen-1-ol
cis-3-Octen-1-ol
cis-3-Nonen-1-ol
trans-3-Hexen-1-ol
Other Enhancer
2-Phenoxyethanol
2-Phenylethanol
Benzyl alcohol
2-Ethylhexyl salicylate
Salicylaldehyde
Butylated hydroxyanisole
Thymol
Menthol
Menthone
Carvacrol
Cineole

Table 5.1b List of permeation enhancers used in our quantitative structure–enhancement relationship studies using the direct equilibration method (equilibration of the skin with liquid enhancers and subsequently skin transport experiments in aqueous solutions)

Fatty Acid Enhancer
Decanoic acid
Undecanoic acid
Lauric acid
Tridecanoic acid
Myristic acid
Pentadecanoic acid
Palmitic acid
Stearic acid
Linoleic acid
Oleic acid
Ricinoleic acid
Other Enhancer
Laurocapram (Azone)
Padimate O (PadO)
Oleyl alcohol
Isopropyl myristate
1-Dodecyl-2-pyrrolidone

of these quantitative structure–enhancement relationships to other permeation enhancers is not unreasonable.

5.7 Transdermal Drug Delivery

To apply the quantitative structure–enhancement relationship and the microenvironment data in the development of transdermal drug delivery systems, certain limitations should be noted. They will be discussed as follows.

First, transdermal delivery systems usually employ nonaqueous vehicles and cosolvents. Some components of nonaqueous vehicles are able to partition into SC and alter the polarity of the microenvironment of the SC intercellular lipid domain. They can also act as permeation enhancers in the SC. These effects should be taken into consideration when utilizing the structure–enhancement relationship to predict the effects of enhancers. In the transdermal patch, the nonaqueous vehicles or cosolvents may also alter the thermodynamic activity of the enhancers and therefore the partitioning of the enhancers from the patch vehicle into the SC. This effect is less complicated when the SC is not altered by the vehicle or cosolvents because it can then be predicted with thermodynamics.

The effects of permeation enhancers upon transdermal transport can also be affected by the drug of interest when high drug concentration is used. In a high drug concentration transdermal

delivery system, possible interactions between the drug and the enhancer in the transdermal patch may affect enhancer partitioning into the SC. The presence of drug in high concentration in the SC may also affect the microenvironment of the SC transport pathway, altering the amount of enhancer present at its site of action. These effects can be drug dependent.

One interesting point to note here is the amount of the enhancers in the SC under the $E = 10$ conditions in the present study. The data in Fig. 5.8 show that approximately 0.06 to 0.12 μmol of the enhancers are partitioned into the intercellular lipids of 1 mg of dry SC (SC dry weight measured before enhancer treatment) at $E = 10$. Assuming that the SC intercellular lipids are homogeneous, a simple calculation will indicate that high concentrations of the enhancers are present in the SC intercellular lipids under these conditions (around 5 to 10 %, w/w). Even at these moderately high concentrations (of enhancers of different molecular sizes and polarities), the chemical microenvironment of the enhancer site of action appears to remain the same and similar to liquid *n*-octanol; this is probably not unreasonable in light of the *n*-octanol/PBS partition experiment showing that the *n*-octanol/PBS partition coefficients themselves are not significantly different at these concentrations in the *n*-octanol phase (Warner et al. 2003). In previous studies, the microenvironment of the rate-limiting domains for steroidal permeant transport across SC in buffered saline (control without the presence of enhancers) has been shown to be well mimicked by the *n*-octanol liquid phase (Anderson et al. 1988; Anderson and Raykar 1989; Raykar et al. 1988). Together, this suggests that the microenvironment of the enhancer site of action for permeation enhancement in SC is not significantly altered by the enhancers. This analysis leads one to speculate that (a) the results obtained in the present study will likely hold even in transdermal systems of high drug concentration and with nonaqueous vehicles and (b) the alteration of thermodynamic activity of the enhancer in the transdermal patch system is likely to be the remaining factor.

An important issue has been ignored by intent in the experiments and discussion of the present chapter: the asymmetric enhancer situation in transdermal drug delivery (enhancer concentration gradient in SC). Avoiding such a situation is by design in the present study for the purpose of mechanistic interpretation of the results and identifying a quantitative structure–enhancement relationship without the complications arising from enhancer concentration gradients across the membrane. However, the asymmetric situation is generally encountered in in vivo transdermal delivery, and dealing with the resulting enhancer concentration gradients in the SC would be an important factor in the selection of effective permeation enhancers for transdermal drug delivery. Complex modeling is required in this situation for data analysis. Studies of the relationship between the enhancement effects under the symmetric enhancer condition and the condition of enhancer concentration gradients in SC have been initiated (Chantarsart and Li 2010).

Acknowledgments The authors thank Drs. Kevin S. Warner, Ning He, Doungdaw Chantasart, and Sarah A. Ibrahim for their contributions in the project and the financial support by NIH Grants GM 043181 and GM 063559.

References

Anderson BD, Raykar PV (1989) Solute structure-permeability relationships in human stratum corneum. J Invest Dermatol 93:280–286

Anderson BD, Higuchi WI, Raykar PV (1988) Heterogeneity effects on permeability-partition coefficient relationships in human stratum corneum. Pharm Res 5:566–573

Aungst BJ (1989) Structure/effect studies of fatty acid isomers as skin penetration enhancers and skin irritants. Pharm Res 6:244–247

Aungst BJ, Rogers NJ, Shefter E (1986) Enhancement of naloxone penetration through human skin *in vitro* using fatty acids, fatty alcohols, surfactants, sulfoxides and amides. Int J Pharm 33:225–234

Barry BW (1987) Mode of action of penetration enhancers in human skin. J Control Release 6:85–97

Bouwstra JA, Pilgram GSK, Ponec M (2002a) Does the single gel phase exist in stratum corneum? J Invest Dermatol 118:897–898

Bouwstra JA, Gooris GS, Dubbelaar FER, Ponec M (2002b) Phase behavior of stratum corneum lipid mixtures based on human ceramides: the role of natural and synthetic ceramide 1. J Invest Dermatol 118: 606–617

Brain KR, Walters KA (1993) Molecular modeling of skin permeation enhancement by chemical agents. In: Walters

KA, Hadgraft J (eds) Pharmaceutical skin penetration enhancement. Marcel Dekker, New York, pp 389–416

Chantasart D, Li SK, He N, Warner KS, Prakongpan S, Higuchi WI (2004) Mechanistic studies of branched-chain alkanols as skin permeation enhancers. J Pharm Sci 93:762–779

Chantasart D, Sa-Nguandeekul P, Prakongpan S, Li SK, Higuchi WI (2007) Comparison of the effects of chemical permeation enhancers on the lipoidal pathways of human epidermal membrane and hairless mouse skin and the mechanism of enhancer action. J Pharm Sci 96:2310–2326

Chantasart D, Pongjanyakul T, Higuchi WI, Li SK (2009) Effects of oxygen-containing terpenes as skin permeation enhancers on the lipoidal pathways of human epidermal membrane. J Pharm Sci 98:3617–3632

Chantasart D, Li SK (2010) Relationship between the enhancement effects of chemical permeation enhancers on the lipoidal transport pathway across human skin under the symmetric and asymmetric conditions *in vitro*. Pharm Res 27:1825–1836

Cooper ER (1984) Increased skin permeability for lipophilic molecules. J Pharm Sci 73:1153–1156

Goates CY, Knutson K (1994) Enhanced permeation of polar compounds through human epidermis. I. Permeability and membrane structural changes in the presence of short chain alcohols. Biochim Biophys Acta 1195:169–179

He N, Li SK, Suhonen TM, Warner KS, Higuchi WI (2003) Mechanistic study of alkyl azacycloheptanones as skin permeation enhancers by permeation and partition experiments with hairless mouse skin. J Pharm Sci 92:297–310

He N, Warner KS, Chantasart D, Shaker DS, Higuchi WI, Li SK (2004) Mechanistic study of chemical skin permeation enhancers with different polar and lipophilic functional groups. J Pharm Sci 93:1415–1430

Ibrahim SA, Li SK (2009) Effects of chemical enhancers on human epidermal membrane: structure enhancement relationship based on maximum enhancement (E_{max}). J Pharm Sci 98:926–944

Ibrahim SA, Li SK (2010a) Efficiency of fatty acids as chemical penetration enhancers: mechanisms and structure enhancement relationship. Pharm Res 27:115–125

Ibrahim SA, Li SK (2010b) Chemical enhancer solubility in human stratum corneum lipids and enhancer mechanism of action on stratum corneum lipid domain. Int J Pharm 383:89–98

Kim YH, Ghanem AH, Mahmoud H, Higuchi WI (1992) Short chain alkanols as transport enhancers for lipophilic and polar/ionic permeants in hairless mouse skin: mechanism(s) of action. Int J Pharm 80:17–31

Klimentová J, Kosák P, Vávrová K, Holas T, Hrabálek A (2006) Influence of terminal branching on the transdermal permeation enhancing activity in fatty alcohol and acids. Bioorg Med Chem 14:7681–7687

Kuempel D, Swartzendruber DC, Squier CA, Wertz P (1998) *In vitro* reconstitution of stratum corneum lipid lamellae. Biochim Biophys Acta 1372:135–140

Lee VHL, Yamamoto A, Kompella UB (1991) Mucosal penetration enhancers for facilitation of peptide and protein drug absorption. Crit Rev Ther Drug Carrier Syst 8:91–192

Liu P, Higuchi WI, Ghanem A-H, Kurihara-Bergstrom T, Good WR (1992) Assessing the influence of ethanol in simultaneous diffusion and metabolism of estradiol in hairless mouse skin for the 'asymmetric' situation *in vitro*. Int J Pharm 78:123–136

Norlen L (2001) Skin barrier structure and function: the single gel phase model. J Invest Dermatol 117:830–836

Novotný J, Janůsová B, Novotný M, Hrabálek A, Vávrová K (2009a) Short-chain ceramides decrease skin barrier properties. Skin Pharmacol Physiol 22:22–30

Novotný M, Hrabálek A, Janůsová B, Novotný J, Vávrová K (2009b) Dicarboxylic acid esters as transdermal permeation enhancers: effects of chain number and geometric isomers. Bioorg Med Chem Lett 19:344–347

Ogiso T, Iwaki M, Paku T (1995) Effect of various enhancers on transdermal penetration of indomethacin and urea, and relationship between penetration parameters and enhancement factors. J Pharm Sci 84:482–488

Potts RO, Guy RH (1997) Mechanisms of transdermal drug delivery. Marcel Dekker, New York

Raykar PV, Fung MC, Anderson BD (1988) The role of protein and lipid domains in the uptake of solutes by human stratum corneum. Pharm Res 5:140–150

Smith EW, Maibach HI (1995) Percutaneous penetration enhancers. CRC Press, Inc., Boca Raton

Vávrová K, Zbytovská J, Hrabálek A (2005) Amphiphilic transdermal permeation enhancers: structure-activity relationships. Curr Med Chem 12:2273–2291

Walters KA, Hadgraft J (1993) Pharmaceutical skin penetration enhancers. Marcel Dekker, New York

Warner KS, Li SK, Higuchi WI (2001) Influences of alkyl group chain length and polar head group on chemical skin permeation enhancement. J Pharm Sci 90:1143–1153

Warner KS, Li SK, He N, Suhonen TM, Chantasart D, Bolikal D, Higuchi WI (2003) Structure-activity relationship for chemical skin permeation enhancers: probing the chemical microenvironment of the site of action. J Pharm Sci 92:1305–1322

Warner KS, Shaker DS, Molokhia S, Xu Q, Hao J, Higuchi WI, Li SK (2008) Silicone elastomer uptake method for determination of free 1-alkyl-2-pyrrolidone concentration in micelle and hydroxypropyl-β-cyclodextrin systems used in skin transport studies. J Pharm Sci 97:368–380

White SH, Mirejovsky D, King GI (1988) Structure of lamellar lipid domains and corneocyte envelopes of murine stratum corneum. An x-ray diffraction study. Biochemistry 27:3725–3732

Williams AC, Barry BW (1992) Skin absorption enhancers. Crit Rev Ther Drug Carrier Syst 9:305–353

Yoneto K, Ghanem AH, Higuchi WI, Peck KD, Li SK (1995) Mechanistic studies of the 1-alkyl-2-pyrrolidones as skin permeation enhancers. J Pharm Sci 84:312–317

Yoneto K, Li SK, Higuchi WI, Jiskoot W, Herron JN (1996) Fluorescent probe studies of the interactions of 1-alkyl-2-pyrrolidones with stratum corneum lipid liposomes. J Pharm Sci 85:511–517

Azone® and Its Analogues as Penetration Enhancers

6

Josef Jampílek

Contents

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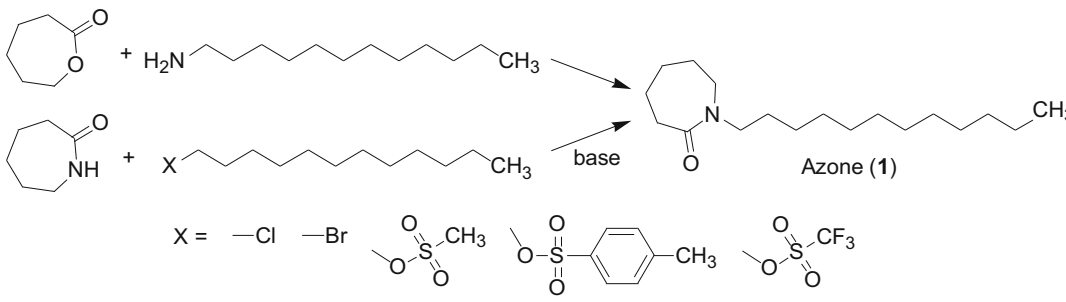
6.1 Azone®

Azone® (1-dodecylazacycloheptan-2-one or laurocapram, **1** see in Scheme 6.1) is one of the most known and the most studied chemical penetration enhancers (CPEs), i.e. excipients facilitating the penetration of drugs through the skin. Azone® analogues contain fragment X-CO-N=, where X is -CH_2–, –NH_2 or-NH- that is characteristic for compounds of the natural moisturising factor (NMF). The NMF is physiologically present in the highly differentiated flattened keratinocytes referred to as corneocytes and being the building blocks of the epidermal barrier. Among the main components of NMF, there are urea, proline, histidine, urocanic acid and 2-pyrrolidone-5-carboxylic acid (Spier and Pascher 1956; Harding and Scott 2002). Azone® belongs to the compounds specifically designed as transdermal penetration enhancers and prepared during the 70s of the twentieth century (Rajadhyaksha et al. 1976a). It contains a lipid alkyl chain and a large polar head group that are thought to be vital for its activity. From the chemical point of view, this compound can be considered to be a hybrid of a cyclic amide (e.g. pyrrolidone structure) with an alkylsulfoxides, but the aprotic sulfoxide group that provides some undesirable properties typical for dimethyl sulfoxide and its derivatives is absent (Williams and Barry 1992; 2004; Moser et al. 2001).

N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_6



Scheme 6.1 Structure and various synthesised ways of 1-dodecylazacycloheptan-2-one (*N*-dodecylcaprolactam, laurocapram, Azone®, **1**)

Generally, Azone® can be synthesised via the base-catalysed (e.g. NaH) coupling of azepan-2-one with 1-dodecylhalide (chloride, bromide) or preferably with a dodecyl chain with a nucleophilic leaving group, for example, mesylate, tosylate, triflate, etc. The reaction is carried out under anhydrous conditions, in an inert atmosphere and in an inert solvent, e.g. toluene (Rajadhyaksha et al. 1976a; Groundwater et al. 1994). In an alternative method, oxepan-2-one is allowed to react with a 1-dodecylamine over the period of 24–48 h at 170–250 °C, see Scheme 6.1 (Rajadhyaksha et al. 1976a).

Azone® is a colourless, odourless liquid with a melting point of −7 °C and provides a smooth, oily but yet non-greasy feel. As it would be expected from its chemical structure, Azone® is a highly lipophilic material with a log $P_{oct/w}$ around 6.2 and is soluble in and compatible with most organic solvents, including alcohols and propylene glycol. The compound has low irritancy and very low toxicity (oral LD_{50} in rat of 9 g/kg) (Williams and Barry 2004).

Azone® was investigated as a CPE for a long time and found to be effective both for lipophilic and hydrophilic drugs but only from a polar medium (Hou and Flynn 1997). In a number of studies, it is used as a standard for evaluation of enhancement effect. As with many penetration enhancers, the effect of Azone® appears to be strongly dependent on its concentration and is also influenced by the choice of vehicle from which it is applied. Azone® is most effective at low concentrations, being employed typically between 0.1 and 5 % (Williams and Barry 2004). Azone® also showed a minimal systemic absorption, being quickly eliminated from circulation (López-Cervantes et al. 2006). Topical formulations with various drugs (5-fluorouracil, morphine hydrochloride, vidarabine, papaverine hydrochloride, arildone, buprenorphine hydrochloride and indapamide) were developed (Sugibayashi et al. 1985, 1989; Komada et al. 1991; Okumura et al. 1991; Baker and Hadgraft 1995; Huang et al. 2002; Ren et al. 2008). Although Azone® proved to be an effective enhancer in different pharmaceutical formulations, its application is not commonly accepted. FDA has not approved its application in pharmaceutical products because of its suspected pharmacological activity or questions about its safety (López-Cervantes et al. 2006). One of the major reasons is the fact that Azone® shows an antiviral effect (Leonard et al. 1987; Afouna et al. 2003). On the other hand, it is included in the Chinese Pharmacopoeia and is widely used in this country not only as a transdermal enhancer but also in insecticides, fungicides, herbicides and as a plant growth regulator. Generally, it is marketed as a successful agrochemical by many Chinese companies.

6.1.1 Mechanism of Action of Azone®

Although Azone® has been investigated for more than 30 years, its mechanism of action has not been quite clarified yet. Extensive discussion concerning the metabolism and fate of Azone® and on its use as a penetration enhancer has been

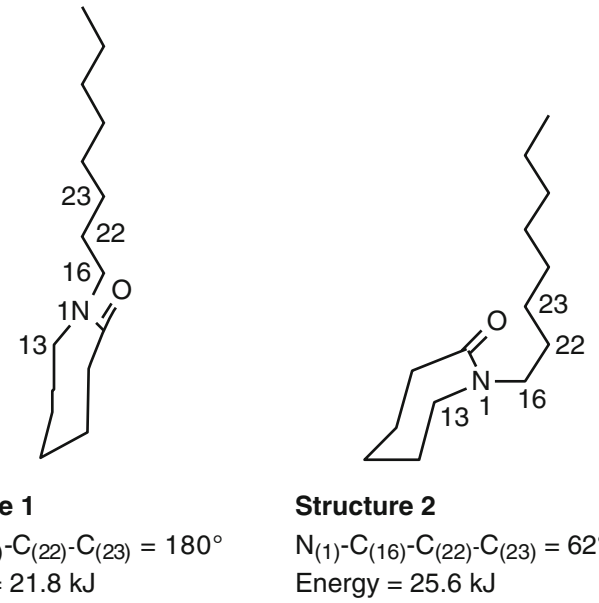


Fig. 6.1 Conformations of Azone® (only eight-chain hydrocarbons shown). $C_{(13)}$-$N_{(1)}$-$C_{(16)}$-$C_{(22)}$ = 90° for both forms (Reprinted and adapted from Hadgraft et al. (1996), Copyright (1996), with permission from Elsevier)

reviewed, and the molecule is still being investigated (Williams and Barry 2004).

Azone® probably exerts its penetration-enhancing effects through interactions with the lipid domains of the SC. Considering the chemical structure of the molecule (possessing a large polar head group and a lipid alkyl chain), it would be expected that the enhancer partitions into the bilayer lipids, disrupting their packing arrangement; integration into the lipids is unlikely to be homogeneous considering the variety of compositional and packing domains within SC lipid bilayers. Thus, Azone® molecules may exist dispersed within the barrier lipids or in separate domains within the bilayers (Williams and Barry 2004).

Various alternative hypotheses of the mechanism of action based on molecular conformation and/or abilities to create *H*-bonds were proposed (Jampílek and Brychtová 2012). The first hypothesis on the enhancement effect of Azone® arises from its ability to exist in a "bent soup spoon" conformation (see Fig. 6.1, structure 1) where the ring is at an angle to the lipophilic chain. It was argued that the increased energy of the angled conformation would be compensated by the removal of the hydrophobic ring methylene groups from the polar region of the lamella back towards the lipophilic hydrocarbon chain region. The minimum energy (21.8 kJ) conformation (the angle of 180° between the polar head group and the aliphatic chain) is essentially planar and would be expected to intercalate between SC ceramides with a minimum of spatial disruption. The "bent spoon" conformation (see Fig. 6.1, structure 2) is obtained by setting the chain–head bond to 62° and has an energy of 25.6 kJ. The energy difference ($\Delta E \approx 4$ kJ) corresponds to a high probability level of about 0.2 for the existence of the "bent spoon" at 37 °C. However, it must be borne in mind that intercalation into a liquid bilayer structure of packed ceramides would provide additional resistance to the existence of this higher energy formation (Hadgraft et al. 1996; Hoogstraate et al. 1991; Lewis and Hadgraft 1990). The second theory discussing the enhancing action of Azone® is based on competition for *H*-bonding sites between SC lipids. The nature of the forces holding the modifiers within the SC (or liposome) bilayers is not known with certainty, but *H*-bonding between head groups is accepted as being an important factor in stabilising ceramide bilayers (Pascher 1976; Jackson et al. 1988). Therefore, Hadgraft et al. (1996; Hadgraft and Pugh 1998) offered an alternative hypothesis that, due to its long carbon chain, Azone® intercalates between ceramides and by means of its carbonyl oxygen moiety competes effectively for *H*-binding

sites in the head of ceramides (disrupts ceramide–ceramide *H*-bonds and binds firmly to a ceramide molecule), the displaced ceramide now being unbound, and on this other/second side where *H*-bonding to the other ceramide molecule is not possible, a "channel" is created. Thus, a region of fluidity appears in the lamella enabling enhanced penetration.

Both theories are probably connected. Recent electron diffraction studies using lipids isolated from the human SC provided good evidence that Azone® exists (or partially exists) as a distinct phase within the SC lipids (Pilgram et al. 2001; Jampílek and Brychtová 2012).

6.1.2 Modification of Azone® Structure

Although Azone® expressed some unfavourable properties (López-Cervantes et al. 2006), it was used as the lead structure, and in the ensuing years, significant efforts were exerted to prepare more effective compounds on the basis of Azone® structure. A number of various derivatives/analogues were prepared and tested to improve Azone® properties. Structural modification of Azone® creates compounds denominated as Azone®-like compounds or Azone® analogues.

Design of new compounds was performed based on the structure–activity relationships (SAR) using standard medicinal chemistry approaches such as: (i) isomerism (structural isomerism, stereoisomerism), (ii) homology (alkyl or alkylene homologous series), (iii) analogy (hydrogenation/dehydrogenation, alkyl, radical, functional groups, rings (cyclic/open analogy), mutual (twin molecules), models) and (iv) isosterism and/or bioisosterism principles (Melichar 1987; Wermuth 2008).

Although Azone® has a simple structure, it offers a huge amount of various modifications. The simplest logical modifications of the structure of Azone® can be the following: (i) in the head group region (ε-lactam ring modifications) and (ii) in the tail region of aliphatic chains (chain modifications) (Jampílek and Brychtová 2012).

The modification of the ε-lactam ring consists especially in the changed number of the atoms of the ring (alkylene homologous) resulting in δ-lactams, γ-lactams or in preparation of open-ring analogues (replacement of a lactam ring by linear alkyl or branched chains). Further modification can be, for example, isosteric replacements of *N*, *O* and *C* atoms, removal or addition of function groups or ring dehydrogenation with introduction of multiple bonds.

The alkyl chain of Azone® allows to apply alkyl homology (chain prolongation/shortening), alkyl analogy (chain branching), dehydrogenation (introduction of multiple bonds) or inserting of function groups. Also isosteric replacement of *N*, *O* or *S* by a carbon atom is possible, i.e. preparation of ethers, thioethers or secondary or tertiary amines.

The modification of the region between the lactam and the aliphatic chain can be noteworthy. The principle of isosterism/bioisosterism or introduction of functional groups or multiple bonds can be applied there (Jampílek and Brychtová 2012).

6.2 Analogues of Azone® as CPEs

Penetration enhancers increase skin permeability by multiple mechanisms, including lipid extraction, phase separation, inverted micelle formation and bilayer fluidization (Menon et al. 1998; Barry 2004). A possible mechanism of penetration enhancement could also involve the formation of a complex between the used penetrating drug and the CPE and/or other components in the pharmaceutical formulation, thus altering the physicochemical properties of the drug and the CPE (Hatanaka et al. 1993; Karande et al. 2007; Drakulic et al. 2008). Principles of modifying the penetration enhancement by CPEs have been investigated by many scientists, for example, by Morimoto et al. (1992), Yamashita et al. (1993), Bando et al. (1996), Manitz et al. (1998), Rim et al. (2005), by Riviere et al. (2005, 2007) or by Bernardo et al. (2008). The above-mentioned studies showed that the penetration enhancement effect depends on the physico-chemical properties of the drug (permeant), the CPE and the vehicle (Williams and Barry 2004; Barry 1987; Kim et al. 2001; Yamashita and Hashida 2003; Degim 2006; Katritzky et al. 2006; Brychtová et al.

2010a, b, 2012). It means that the penetration activity of CPEs is influenced by the selection of a model-penetrating compound and a testing method (Jampílek and Brychtová 2012).

Based on these facts and because various scientist teams used various vehicles, skin of different animals (snake, mice, rat, rabbit, guinea pig, pig, monkey) and human skin as well as different drugs (theophylline, hydrocortisone, vasopressin, indomethacin, 5-fluoruracil, various cardiovascular drugs, antipsychotics, etc.) for investigating transdermal penetration, it is not possible to simply compare values of the penetration enhancement ratio (ER) obtained in different studies.

For all compounds, selected physico-chemical properties such as lipophilicity (log *P*), polar surface area (PSA) and bulk parameter (expressed as molar refractivity (reflecting bulkiness), MR) were calculated and are added to individual Tables 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 6.10, 6.11, 6.12, 6.13, 6.14, 6.15, 6.16, 6.17, 6.18, 6.19, 6.20, 6.21, 6.22, 6.23, 6.24, 6.25, 6.26, 6.27, 6.28, 6.29, 6.30, 6.31, 6.32, 6.33, 6.34, 6.35, 6.36, 6.37, 6.38, 6.39, 6.40, 6.41, 6.42, 6.43 and 6.44. Log *P* and PSA values were calculated by ACD/Percepta version 2012 (Advanced Chemistry Development Inc., Toronto, Canada), and MR values were calculated using CS ChemOffice Ultra version 10.0 (CambridgeSoft, Cambridge, MA, USA). The polar surface area (PSA) is commonly used in medicinal chemistry for the optimisation of an ability of drugs to permeate through cell wall (Ertl 2007); therefore, PSA can be also applied as an important parameter related to penetration of CPEs to the skin and disruption of SC. Also bulk parameter representing the volume of the molecule belongs to molecular descriptors and may often be correlated with biological effect (Wermuth 2008).

The described derivatives are divided into sections according to the ring size (seven-, six- and five-membered cycle), and then mutual analogues are discussed. Open-ring analogues are not mentioned due to the fact that these compounds acquire absolutely different physico-chemical properties by opening of the ring, so completely different structure–activity relationships can be found than for cyclic analogues of Azone®. A number of these open-ring Azone® derivatives were prepared, and investigation of these analogues would require additional comprehensive study. Inside the sections, compounds are classified according to other structure modifications (Jampílek and Brychtová 2012).

6.2.1 Seven-Membered Ring Analogues

In the 1970s, Rajadhyaksha (1976a) prepared and patented *N*-alkyl substituted azacycloheptan-2-ones including Azone® (**1**) and its methylene homologues **2**-**11** (Table 6.1). Hoogstraate et al. (1991) observed that the penetration of

Table 6.1 Series of *N*-alkyl-azacycloheptan-2-ones and their selected physico-chemical properties

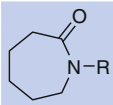
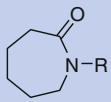
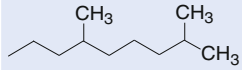
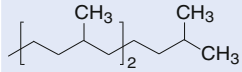
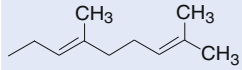
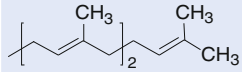
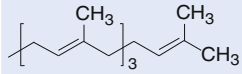
	**R**	**log *P***	**PSA**	**MR** [cm³/mol]
2	$-CH_3$	0.67	20.31	36.22
3	$-C_3H_7$	1.84	20.31	45.62
4	$-C_4H_9$	2.32	20.31	50.22
5	$-C_5H_{11}$	2.98	20.31	54.82
6	$-C_6H_{13}$	3.27	20.31	59.42
7	$-C_7H_{15}$	3.87	20.31	64.02
8	$-C_8H_{17}$	4.37	20.31	68.62
9	$-C_9H_{19}$	4.68	20.31	73.22
10	$-C_{10}H_{21}$	5.10	20.31	77.82
Azone® (**1**)	$-C_{12}H_{25}$	5.92	20.31	87.02
11	$-C_{14}H_{29}$	7.54	20.31	96.22

Table 6.2 Azacycloheptan-2-ones *N*-substituted by branched and/or unsaturated chains and their selected physicochemical properties

	**R**	**log *P***	**PSA**	**MR** [cm³/mol]
12		4.96	20.31	78.17
13		7.11	20.31	105.95
14		4.20	20.31	79.82
15		6.36	20.31	108.41
16		8.16	20.31	132.41
17	$-C_2H_4OH$	0.02	40.54	42.39
18	$-C_3H_6OH$	0.43	40.54	46.99
19	$-CH_2CHOHCH_2OH$	−0.55	60.77	48.45

vasopressin through human SC increased with increasing length of the alkyl side chain in the homologic series of *N*-alkylazacycloheptan-2-ones (see Table 6.1) to C_{12}, i.e. Azone® (**1**), and after that decreased with the increasing chain length. The non-enhanced peptide flux through human SC was 1.6 nmol/cm² per h (peptide concentration in the donor propylene glycol was 6.0 mM). The permeability increased 1.9-fold after the addition of compound **10**, 3.5-fold after the addition of Azone® (**1**) and 2.5-fold after the addition of compound **11**. Michniak et al. (1995a) described the penetration of the suspension of hydrocortisone acetate in propylene glycol (0.003 M) through the hairless mouse skin with the addition of Azone® (**1**) with concentration 0.4 M and ER 19.5 and the penetration of the suspension of hydrocortisone in propylene glycol (0.03 M) through the hairless mouse skin with the addition of Azone® (**1**) with concentration 0.4 M and ER 42.2 (Michniak et al. 1998).

Azacycloheptan-2-ones *N*-substituted by saturated branched alkyl chains, acyclic terpenic chains or alkyl chains substituted by hydroxyl moieties **12**–**19** can be classified as alkyl analogues of Azone® (**1**); see Table 6.2 (Cooper et al. 1985; Okamoto et al. 1988, 1990; Yamashita et al. 1993). Penetration of 6-mercaptopurine through the skin of guinea pigs in vitro was increased tenfold by the use of N-geranylazepan-2-one (**14**) and *N*-farnesylazepan-2-one (**15**) when compared with the control sample without enhancers. Similar compounds with five-membered or six-membered lactam rings are discussed in the below sections. Taking into account the enhancement effect of all these compounds, it can be stated that the substitution of chains by different-size lactam rings did not have any influence on the enhancement activity.

Modification of the connecting linker between the lactam and the aliphatic chains was observed for compounds **20**–**34** (Table 6.3). To the α-position of the alkyl side chain in the Azone® structure, a carbonyl group was introduced, and *N*-acylazepan-2-ones (imides) were obtained. An effect comparable to Azone® (**1**) (Table 6.1, ER = 19.5) was achieved at the penetration of hydrocortisone acetate through the hairless mouse skin in vitro with the application of *N*-tetradekanoylazepan-2-one (**23**, ER = 20.0). The enhancement effect increased with the chain length (ER = 12.2 for **21** and ER = 14.4 for **22**) (Michniak et al. 1993a, 1998).

Table 6.3 Series of *N*-acylazepan-2-ones and their selected physico-chemical properties

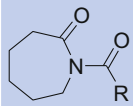
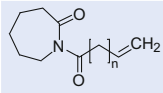
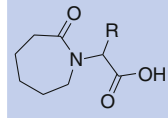
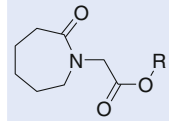
	**R**	**log *P***	**PSA**	**MR** [cm³/mol]
20	-CH_3	1.03	37.38	40.18
21	-C_9H_{19}	5.04	37.38	77.11
22	-$C_{11}H_{23}$	5.70	37.38	86.31
23	-$C_{13}H_{27}$	7.13	37.38	95.21
	***n***	**log *P***	**PSA**	**MR** [cm³/mol]
24	2	1.98	37.38	54.17
25	3	2.46	37.38	58.77
26	4	2.89	37.38	63.37
27	5	3.55	37.38	68.67
28	6	3.69	37.38	72.56
29	7	4.45	37.38	77.16
30	8	4.82	37.38	81.76
31	9	5.11	37.38	86.36
32	11	6.14	37.38	95.56
33	13	7.02	37.38	104.76
34	15	7.93	37.38	113.93

Table 6.4 Series of substituted 2-(2-oxoazepan-1-yl) alkanoic acid and its esters and their selected physico-chemical properties

	**R**	**log *P***	**PSA**	**MR** [cm³/mol]
35	-C_4H_9	1.80	57.61	60.63
36	-C_5H_{11}	2.37	57.61	65.23
37	-C_6H_{13}	2.92	57.61	69.83
38	-C_7H_{15}	3.52	57.61	74.43
39	-C_8H_{17}	3.78	57.61	79.03
40	-C_9H_{19}	4.48	57.61	83.63
41	-$C_{10}H_{21}$	4.82	57.61	88.22
42	-$C_{12}H_{25}$	6.07	57.61	97.42
43	-$C_{14}H_{29}$	7.16	57.61	106.62
44	-$C_{16}H_{33}$	8.17	57.61	115.82
	**R**	**log *P***	**PSA**	**MR** [cm³/mol]
45	-$C_{12}H_{25}$	5.91	46.61	98.57
46	-$C_{14}H_{29}$	7.42	46.61	107.77

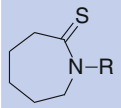
N-Alkenoylazepan-2-ones **24**–**34** (Table 6.3), dehydrogenated analogues of the previously mentioned compounds, were also patented as transdermal penetration enhancers (Minaskanian and Peck 1989a; Peck et al. 1989).

By the shift of the carbonyl group to the β-position of the alkyl chain and isosteric replacement of the oxygen by the terminal carbon, α-(di) substituted 2-(2-oxoazepan-1-yl)alkanoic acids/esters **35**–**46** were prepared; see Table 6.4. 2-(2-Oxoazepan-1-yl)carboxylic acid (**35**) is the starting compound, whose derivatives **36**–**44** that were generated by the homologic lengthening of the alkyl chain were patented. Peck and Minaskanian (1995) noted that all these compounds increased the penetration of tested drugs at least by 76 % in comparison with the penetration without the addition of CPEs. A contrary approach, i.e. the preparation of long-chain esters of 2-(2-oxoazepan-1-yl)acetic acid **45** and **46** (i.e. partial "reverse esters" of **42**, **43**) led to a significant decrease in the activity at the penetration of the suspension of hydrocortisone acetate in propylene glycol through the hairless mouse skin (ER 1.1 for **45** and ER 1.9 for **46**) in comparison with Azone® (**1**, ER = 19.5), imides **22** (ER = 20.0) and **23** (ER = 14.4) (Michniak et al. 1996) and analogues **42** and **43** (Peck and Minaskanian 1995). Much better results were obtained with six-membered and five-membered lactams (alkylene homologues of substances of such type); see next sections.

The change of the position of the alkyl chain from α-acyl carbon to ester oxygen can be understood as so-called analogy of functional groups. This modification concerns only the change of functional groups, and these groups are not isosteric, unlike, for example, ester and amide (COOR/CONHR). Most often, it is dealt with "reverse analogues", in this case reverse esters R-COO-R´/R´-COO-R or reverse amides R-CONH-R´/R´-CONH-R.

The simplest modification of Azone® (**1**) or the structure of its alkyl homologues is isosteric replacement of sulfur by oxygen and preparation of thiolactams **47**–**50** (Table 6.5) (Minaskanian

Table 6.5 Series of *N*-alkylazacycloheptan-2-thiones and their selected physico-chemical properties

	R	log *P*	PSA	MR [cm³/mol]
47	$-C_4H_9$	2.10	35.33	57.40
48	$-C_8H_{17}$	5.08	35.33	75.79
49	$-C_9H_{19}$	5.17	35.33	80.39
50	$-C_{12}H_{25}$	6.59	35.33	94.19

and Peck 1988). These substances were patented as penetration enhancers, but replacement of the carbonyl moiety by the thioxo group leads to a substantial loss of efficiency. N-Dodecylazepan-2-thione (**50**) showed 4.5-fold less effect (ER = 4.3) at penetration of hydrocortisone through the hairless mouse skin in vitro than Azone® (Michniak et al. 1993b). Hadgraft et al. (1996) described the penetration of metronidazole (ethanol solution, 5 μmol/mL) through the human skin, and ER of **50** was 1.4, while ER of Azone® (**1**) was 6.7.

Another simple approach is the introduction of a double bond (dehydrogenation analogues) and/or isosteric replacement of sulfur by carbonyl oxygen as illustrated by compounds **51**–**55** (Table 6.6). Azacycloheptenes/heptadienes were prepared and patented as penetration enhancers for different drugs through the human or animal skin and mucosa (Minaskanian and Peck 1992). Compounds **51**–**55** were recommended for application in different topic formulations at concentration from 1 to 10 %. Unfortunately, no data about their enhancement effect or safety are available; however, it can be supposed that the change of the geometry of lactam caused by introduction of the double bond affects penetration activity. Based on the above presented fact, the enhancement activity of thio-derivatives **52** and **54** was probably not higher than that of compounds **51** and **53** and Azone® (**1**).

By introduction of another carbonyl group (see Table 6.7) to the heterocyclic head of Azone®, *N*-dodecylazacycloheptan-2,5-dione (**56**) was generated (Peck et al. 1987). The change of the position of the second carbonyl group and the substitution of isosteric oxygen by carbon in the $C_{(5)}$ position led to a considerable increase in the acceleration activity of 4-alkyl-1,4-oxazepan-5,7-dione derivatives **57** (ER = 45.0) and **58** (ER = 12.7). 4-Decyl-1,4-oxazepan-5,7-dione (**57**) showed substantially higher activity than Azone® (**1**, ER = 19.5) (Michniak et al. 1993a).

Table 6.6 Series of *N*-alkylazacycloheptenones and their selected physico-chemical properties

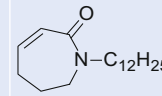
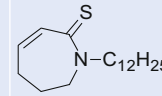
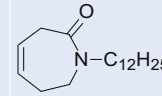
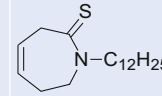
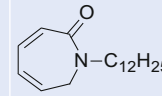
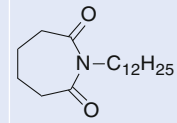
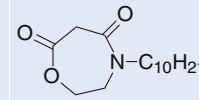
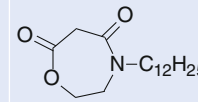
No.	Comp.	log *P*	PSA	MR [cm³/mol]
51		5.53	20.31	88.87
52		6.68	35.33	96.04
53		5.67	20.31	88.38
54		6.31	35.33	95.55
55		5.33	20.31	90.23

Table 6.7 Derivatives of 4-alkyl-1,4-oxazepan-5,7-dione and their selected physico-chemical properties

No.	Comp.	log *P*	PSA	MR [cm³/mol]
56		5.62	37.38	86.31
57		3.55	46.61	75.17
58		4.37	46.61	84.37

The elimination of the carbonyl moiety from the lactam resulted in cyclic amines **59**–**64**; see Table 6.8. Although this radical change of the structure significantly influences physico-chemical properties of the discussed compounds, it could be stated that the reduction of the car-

Table 6.8 Cyclic amines as CPEs and their selected physico-chemical properties

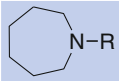
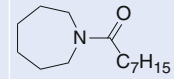
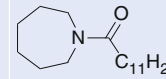
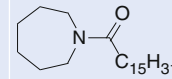
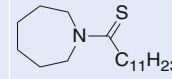
	R	log *P*	PSA	MR [cm³/mol]
59	$-C_{10}H_{21}$	6.03	3.24	78.05
60	$-C_{11}H_{23}$	6.47	3.24	82.65
61	$-C_{12}H_{25}$	6.89	3.24	87.25
62	$-C_{14}H_{29}$	8.37	3.24	96.45
63	$-C_{16}H_{33}$	9.17	3.24	105.65
64	$-C_{18}H_{37}$	9.97	3.24	114.85

Table 6.9 Cyclic amides as CPEs and their selected physico-chemical properties

No.	Comp.	log *P*	PSA	MR [cm³/mol]
65		4.17	20.31	68.62
66		6.05	20.31	87.02
67		8.35	20.31	105.41
68		6.62	35.33	94.19

bonyl group preserved acceleration effect when the enhancement effect of these amines was compared with Azone® homologues. 1-Dodecylazepane (**61**) demonstrated almost the same penetration activity (ER = 15.3) as Azone® (**1**, ER = 19.5) (Michniak et al. 1995a; Minaskanian et al. 1988; Singh 2007). Minaskanian and Peck (1989b) also prepared and patented 2,6-dimethyl analogues of compounds **59**–**64**.

The above-discussed cyclic amines **59**–**64** were transformed to cyclic amides **65**–**68** by the modification of the connecting linker between the ring and the aliphatic chain; see Table 6.9 (Mirejovsky and Takruri 1986). By the change of the position of the carbonyl moiety from the α-position of the lactam to the α-position of the alkyl side chain, the conditions of the analogy of functional groups were met. The discussed cyclic amides **65**–**68** can be classified as reverse amides of Azone® homologues. 1-(Azepan-1-yl)dodecan-1-one (**66**) showed an enhancing activity (ER = 6.4) comparable to Azone® (**1**, ER = 6.7) on penetration of metronidazole (ethanol solution, 5 μmol/mL) through the isolated SC of the human skin (Hadgraft et al. 1996), and a better effect was shown by hydrocortisone at penetration through the hairless mouse skin in vitro (ER of **66** was 34.0, while ER of Azone® (**1**) was 19.5). At the penetration of hydrocortisone, an isostere with a thioxo moiety (**68**) was again less effective (ER = 7.6), the same as derivatives **47**–**50**, compared to compound **66** or Azone® (Michniak et al. 1993b).

Minaskanian and Peck (1989b) also prepared and patented 2-deoxy analogues **69**–**75** of *N*-alkenoylazepan-2-ones **24**–**34** and their 2,6-dimethyl derivatives **76**–**81** (see Table 6.10) as potential CPEs for facilitation of the systemic effect of a number of therapeutic agents. Based on the structural similarity with the excellent CPE **66**, it may be supposed that also compound **71** (terminally dehydrogenated analogue of **66**) can show similar enhancement effect.

The considerable modification of the ring structure (introduction of a double bond to various positions of the ring) and the substitution of the side chain by a carbonyl moiety (generation of imides or reverse amides) were performed and resulted in azacycloheptenes **82**–**85**; see Table 6.11 (Minaskanian and Peck 1992). Unfortunately, no data on the enhancement effect are available. The comparison of the effect of these compounds with their hydrogenated models discussed above would be noteworthy. Based only on the calculated parameters (log *P*, PSA, MR) and structural similarity **82**, **83** with **23** and **84**, **85** with **66**, it can be assumed that high enhancement effect can be expected especially for compounds **84** and **85** (compare: **66** = 6.05, 20.31, 87.02; **84**/**85** = 5.83, 20.31, 88.38).

The maximum changes in comparison with the Azone® structure were performed at design of azacycloheptadienes **86**–**89**; see Table 6.12. They were patented as penetration enhancers for different drugs through the human or animal skin and mucosa similarly as the above-mentioned compounds **82**–**85** (Minaskanian et al. 1992). All these compounds represent noteworthy structures, but unfortunately, no enhancement activity data is available.

Table 6.10 Series of long-chain *N*-acylazepanes and their selected physico-chemical properties

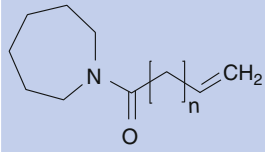
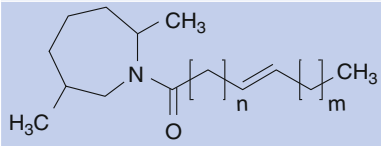
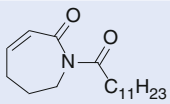
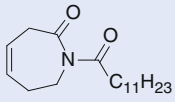
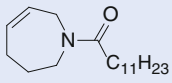
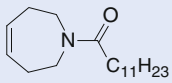
	***n***	**log *P***	**PSA**	**MR** [cm³/mol]
69	6	4.13	20.31	73.27
70	7	4.68	20.31	77.87
71	8	5.03	20.31	82.47
72	11	6.12	20.31	96.27
73	12	6.61	20.31	100.87
74	13	7.15	20.31	105.46
75	15	7.99	20.31	114.66
	***n*/*m***	**log *P***	**PSA**	**MR** [cm³/mol]
76	7/0	5.99	20.31	93.25
77	8/0	6.51	20.31	97.85
78	9/0	6.95	20.31	102.45
79	10/0	7.05	20.31	107.05
80	8/4	8.23	20.31	116.25
81	8/6	9.29	20.31	125.44

Table 6.11 Dehydrogenated azacycloheptane derivatives and their selected physico-chemical properties

No.	**Comp.**	**log *P***	**PSA**	**MR** [cm³/mol]
82		4.93	37.38	88.17
83		5.48	37.38	87.68
84		5.83	20.31	88.38
85		5.83	20.31	88.38

6.2.2 Six-Membered Ring Analogues

Compounds with a six-membered lactam cycle described as potentially effective enhancers were designed, synthesised and evaluated together with ε-lactams. δ-Lactams, or piperidines and piperidine analogues showed mostly less enhancement effect. Enhancers with morpholine/thiomorpholine or piperazine, an isosteric fragment to piperidine, in their structure, proved to be much more significant; therefore, considerable attention is paid to them in this section (Jampílek and Brychtová 2012).

A series of basic *N*-substituted piperidin-2-ones **90–98** (see Table 6.13) very close to azepanone derivatives **1–11**, **17–19** were tested as CPEs by Cooper (1985) and Quan et al. (1990) during the 1980s. These derivatives are alkylene homologues of Azone® (**1**) and its derivatives. An acceleration effect of compounds **90–95** on percutaneous absorption of indomethacin through the skin of newly born pigs in vitro was observed by Quan et al. (1990). *N*-Dodecylpiperidin-2-one (**94**) was the most effective derivative in the series (ER = 48.3) and even comparable to Azone® (**1**, ER = 42.2 at penetration of hydrocortisone through the hairless mouse skin (Quan et al. 1990; Michniak et al. 1998). As was mentioned above, PSA can be used as an important parame-

Table 6.12 Azacycloheptadienes as CPEs and their selected physico-chemical properties

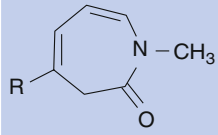
	R	log *P*	PSA	MR [cm³/mol]
86	-Ph	2.06	20.31	61.76
87	-OCH_3	0.26	29.54	43.98
88	-$COCH_3$	0.23	37.38	47.11
89	-$CON(CH_2CH_3)_2$	0.19	40.62	64.98

Table 6.13 Series of *N*-substituted piperidin-2-ones and their selected physico-chemical properties

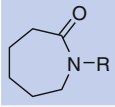
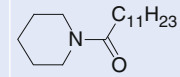
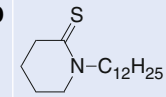
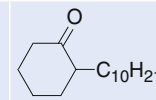
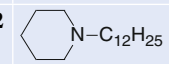
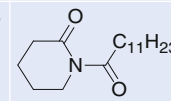
	R	log *P*	PSA	MR [cm³/mol]
90	-C_4H_9	2.02	20.31	45.62
91	-C_6H_{13}	2.95	20.31	54.82
92	-C_8H_{17}	4.18	20.31	64.02
93	-$C_{10}H_{21}$	4.58	20.31	73.22
94	-$C_{12}H_{25}$	5.31	20.31	82.42
95	-$C_{16}H_{33}$	8.00	20.31	100.81
96	-C_2H_4OH	–0.39	40.54	37.79
97	-C_3H_6OH	0.12	40.54	42.39
98	-$CH_2CHOHCH_2OH$	–0.76	60.77	43.85

Table 6.14 Derivatives of six-membered ring analogues of Azone®, their selected physico-chemical properties and enhancement ratios

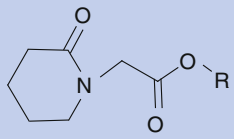
No.	Comp.	log *P*	PSA	MR [cm³/mol]	ER
99		5.50	20.31	82.42	18.9
100		6.26	35.33	89.59	8.6
101		6.24	17.07	74.14	10.3
102		6.54	3.24	82.56	9.9
103		5.32	37.38	81.71	8.6

ter describing penetration of CPEs into the skin. Note that Azone® (**1**) and its cyclic homologues **94** and **212** (see below) possess the same PSA values (20.31), which can correlate with high enhancement effect.

Other simple modifications (see Table 6.14) of effective compound **94**, the closest to Azone® (**1**), can include reverse analogy [1-(piperidin-1-yl) dodecan-1-one (**99**)], isosterism [*N*-dodecylpiperidine-2-thione (**100**) and 2-decylcyclohexanon (**101**)], elimination of the carbonyl moiety [*N*-dodecylpiperidine (**102**)] and the addition of the second carbonyl group and generation of imide [*N*-dodecanoylpiperidine-2-one (**103**)]. As was discussed above, the isosteric substitution of carbonyl oxygen by sulfur did not generate any compound with significant enhancing properties. Thio-derivative **100** was ca. twofold less effective than Azone® (**1**) and compound **94**, but it was ca. twofold more effective than a thio-analogue of Azone® (**50**, ER = 4.3). Decarboxylated compound **102** and 2-decylcyclohexanon (**101**) (isosteric substitution of nitrogen by carbon) showed notably less activity in comparison with Azone® (**1**, ER = 19.5) and in comparison with "parent" *N*-dodecylpiperidin-2-one (**94**, ER = 48.3). Compound **102** showed less activity than its homologue **61** (ER = 15.3). Imide derivative (**103**) was substantially less active than Azone® (**1**) and homologue **22** (ER = 14.4). Amide **99** demonstrated less enhancing effect than compounds **94** and **1** and 1-(azepan-1-yl)dodecan-1-one (**66**) (Michniak et al. 1993b, 1995a, 1998).

In the series of five compounds, the activity decreases as follows: **99** >> **101** ≈ **102** > **100** ≈ **103**. From the study of these modifications, it can be concluded that in general, homologues with a six-membered ring are less effective than compounds

Table 6.15 Series of esters of 2-(2-oxopiperidin-1-yl)acetic acid, their selected physico-chemical properties and enhancement ratios



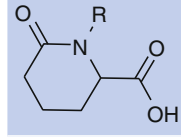
	R	log *P*	PSA	MR [cm³/mol]	ER
104	$-C_8H_{17}$	3.98	46.61	75.58	18.4
105	$-C_9H_{19}$	4.30	46.61	80.17	13.3
106	$-C_{10}H_{21}$	4.62	46.61	84.77	12.7
107	$-C_{11}H_{23}$	5.01	46.61	89.37	36.4
108	$-C_{12}H_{25}$	5.44	46.61	93.97	23.8
109	$-C_{13}H_{27}$	6.72	46.61	98.57	12.0
110	$-C_{14}H_{29}$	7.10	46.61	103.17	37.8

with a seven-membered ring; the absence of a nitrogen atom in the ring or the presence of one more carbonyl group results in a significant loss of activity (Jampílek and Brychtová 2012). From this investigation, it can be concluded that a polar "head" of specific bulkiness of all transdermal enhancers seems to be important for interaction with skin components. The negative effect of the thiocarbonyl moiety at six-membered cycles does not seem to be so significant, while the second carbonyl moiety in **103** was responsible for the negative effect within the six-membered ring, when compared with compound **23**. According to the calculated values of the molecular descriptors, the data of amide **99** are rather close to those of compound **94** and Azone® (**1**).

Esters of 2-(2-oxopiperidin-1-yl)acetic acid **104**–**110** (see Table 6.15) as homologues of inactive long-chain esters of 2-(2-oxoazepan-1-yl) acetic acid **45** (ER = 1.1) and **46** (ER = 1.9) proved to be effective enhancers for penetration of hydrocortisone through the hairless mouse skin; see Table 6.15. The length of the ester chain of the most effective compounds in the series with piperidin-2-one and pyrrolidin-2-one was different. In the case of the six-membered lactam cycle, undecyl (**107**) and tetradecyl esters (**110**) were the most effective (Michniak et al. 1996). The enhancement effect of both esters is comparable with that of Azone® (**1**, ER = 19.5) and compound **94** (ER = 18.9).

Peck and Minaskanian (1995) made an attempt to use analogues with active acids **35**–**44** and prepared and patented several series of carboxylic acid derivatives containing a fragment of piperidin-2-one (**111**–**120**) and piperidine with isosteric sulfur in the position $C_{(3)}$ of the alkyl side chain (**121**–**130**) as transdermal penetration enhancers; see Tables 6.16 and 6.17. All these compounds increased the penetration of the tested drugs at least by 76 % compared with the penetration without the aid of CPEs.

Table 6.16 *N*-substituted derivatives of 6-oxopiperidine-2-carboxylic acid and their selected physico-chemical properties



	R	log *P*	PSA	MR [cm³/mol]
111	$-C_4H_9$	0.82	57.61	51.43
112	$-C_5H_{11}$	1.71	57.61	56.03
113	$-C_6H_{13}$	2.01	57.61	60.63
114	$-C_7H_{15}$	2.69	57.61	65.23
115	$-C_8H_{17}$	3.02	57.61	69.83
116	$-C_9H_{19}$	3.71	57.61	74.43
117	$-C_{10}H_{21}$	4.00	57.61	79.03
118	$-C_{12}H_{25}$	4.59	57.61	88.22
119	$-C_{14}H_{29}$	5.97	57.61	97.42
120	$-C_{16}H_{33}$	7.26	57.61	106.62

Generally, all morpholine/thiomorpholine or piperazine are typical examples of isosteres of piperidine; therefore, it can be stated that derivatives of morpholine **131** and **132** are *O*-isosteres of compound **102** and derivatives of (thio)morpholine **133**–**135** (Table 6.18) are *O*- and/or

Table 6.17 Series of *N*-1-(2-alkylsulfanylethyl)-piperidine-3-carboxylic acids and their selected physico-chemical properties

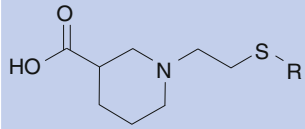
	R	log *P*	PSA	MR [cm³/mol]
121	-C_4H_9	2.84	65.84	68.45
122	-C_5H_{11}	3.40	65.84	73.05
123	-C_6H_{13}	3.96	65.84	77.65
124	-C_7H_{15}	4.34	65.84	82.25
125	-C_8H_{17}	4.88	65.84	86.85
126	-C_9H_{19}	5.29	65.84	91.45
127	-$C_{10}H_{21}$	5.76	65.84	96.04
128	-$C_{12}H_{25}$	6.49	65.84	105.24
129	-$C_{14}H_{29}$	8.02	65.84	114.44
130	-$C_{16}H_{33}$	8.74	65.84	123.64

Table 6.18 Series of (thio)morpholines as potential CPEs, their selected physico-chemical properties and enhancement ratios

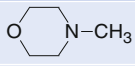
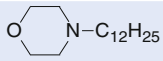
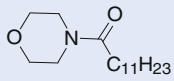
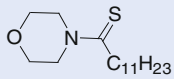
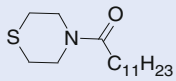
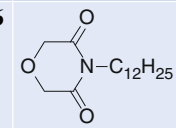
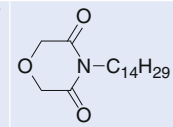
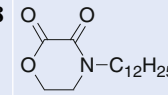
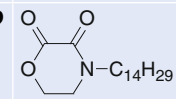
No.	Comp.	log *P*	PSA	MR [cm³/mol]	ER
131		–0.24	12.47	28.99	1.4
132		5.42	12.47	79.78	25.6
133		4.83	29.54	79.54	56.4
134		5.02	44.56	86.72	2.1
135		5.26	45.61	85.66	32.9

Table 6.19 Series of morpholinedione derivatives as potential CPEs, their selected physico-chemical properties and enhancement ratios

No.	Comp.	log *P*	PSA	MR [cm³/mol]	ER
136		4.76	46.61	80.22	10.2
137		5.91	46.61	89.42	14.1
138		4.35	46.61	80.06	7.1
139		5.74	46.61	89.26	5.2

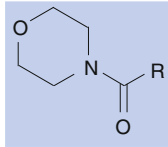
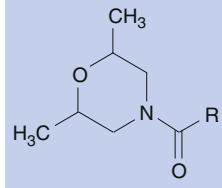
S-isosteres of compound **99**. By the introduction of one more carbonyl group and the isosteric replacement of oxygen by carbon at the $C_{(4)}$ position in compound **103**, morpholinediones **136**–**139** were prepared; see Table 6.19 (Jampílek and Brychtová 2012). All these compounds enhanced transdermal penetration of hydrocortisone. Compounds **132**, **133** and **135** were more active than their "parent" compounds **102** (ER = 9.9) and **99** (ER = 18.9). Derivatives **136** and **137** were more active than compounds **138** and **139**, but they were less active than compounds **57** (ER = 45.0) and **58** (ER = 12.7). Only *N*-dodecylmorpholine (**132**) was more effective than Azone® (**1**, ER = 42.2). Derivative of thiomorpholine **135** showed a lower activity than isostere **133**. 1-Morpholin-4-yl-dodecane-1-thione (**134**) similarly as other thiones (**50**, **100**, **232**) did not demonstrated any enhancement effect (Michniak et al. 1993a, b, 1994, 1995a, 1998).

The abovementioned facts demonstrated that a morpholine scaffold is noteworthy in terms of enhancement activity. Therefore, a huge number of compounds with morpholine in their structure

were patented. Based on the excellent result of compound **133**, analogues of long-chain *N*-acylazepanes **65–81** were prepared. They include 1-morpholin-4-yl-alkan-1-ones (**140–147**) and 1-(2,6-dimethylmorpholin-4-yl)alkan-1-ones (**148–154**) (see Table 6.20), 1-morpholin-4-yl-alkan-1-ones (**155–165**) and 1-(2,6-dimethylmorpholin-4-yl)alken-1-ones (**166–170**) (see Table 6.21) that were patented as potential CPEs for systemically active drugs (Peck et al. 1988; Minaskanian and Peck 1989b).

Within this wide study, also 1-morpholin-4-yl-undec-2-en-1-one (**171**), 1-morpholin-4-yl-deca-2,3-dien-1-one (**172**) and 6-methyl-1-morpholin-4-yl-heptadecan-1-one (**173**) were prepared and patented; see Table 6.22 (Peck et al. 1988). Delmopinol (**174**, known in dentistry as a substance with antiplaque and anti-inflammatory effect, Addy et al. 2007), and its analogues **175** and **176** can be classified as long-chain

Table 6.20 Series of long-chain *N*-acylmorpholines and their selected physico-chemical properties

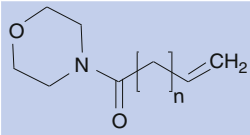
	R	log *P*	PSA	MR [cm³/mol]
140	$-C_9H_{19}$	4.15	29.54	70.73
141	$-C_{10}H_{21}$	4.48	29.54	74.94
142	$-C_{12}H_{25}$	5.20	29.54	84.14
143	$-C_{13}H_{27}$	6.09	29.54	88.74
144	$-C_{14}H_{29}$	6.59	29.54	93.34
145	$-C_{15}H_{31}$	7.03	29.54	97.94
146	$-C_{16}H_{33}$	7.54	29.54	102.54
147	$-C_{17}H_{35}$	8.03	29.54	107.14
	R	log *P*	PSA	MR [cm³/mol]
148	$-C_9H_{19}$	4.77	29.54	79.72
149	$-C_{10}H_{21}$	5.13	29.54	84.32
150	$-C_{11}H_{23}$	5.50	29.54	88.92
151	$-C_{12}H_{25}$	5.85	29.54	93.52
152	$-C_{13}H_{27}$	6.66	29.54	98.12
153	$-C_{15}H_{31}$	7.63	29.54	105.32
154	$-C_{17}H_{35}$	8.63	29.54	116.52

N-morpholine derivatives. All these compounds lack a carbonyl group. Delmopinol, 2-[3-(4-propylheptyl)morpholin-4-yl]ethanol (**174**), its derivative 4-(2-hydroxyethyl)-4-methyl-3-(4-propylheptyl)morpholinium chloride (**175**) and 3-(2-decylmorpholin-4-yl)propan-1-ol (**176**) were patented as enhancers for a wide range of drugs (Table 6.22). It was also determined that these substances were also suitable for enhancement of transport of peptide drugs and anodynes through the mucosa/skin. For example, delmopinol (**174**) increased penetration of morphine sulfate through the human skin. A steady-state flux of this substance was 26 μg/h/cm². The penetration of morphine sulfate without **174** in the vehicle was too low and could not be quantified (Chamber et al. 2008). Compound **175** can evidently have effect on the principle of "phase transfer catalysts" as it belongs to the category of "cationic/invert soaps".

Peck and Minaskanian (1995) were also inspired by effective α-substituted 2-(2-oxoazepan-1-yl) carboxylic acid derivatives **35–44** and patented in 1995 a series of 2-(morpholin-4-yl)alkanoic acids **177–181** (see Table 6.23) as compounds facilitating penetration of antifungal, antibacterial or steroid drugs at least by 76 % compared with the penetration without the aid of CPEs.

As was mentioned above, piperazines as isosteres of piperidine and morpholine were prepared and tested as potential CPEs. Long-chain *N*-acylpiperazines **182–186** and their methyl derivatives **187–196** (Table 6.24) as well as isosteric long-chain *N*-acylmorpholines **140–154** were patented as the most interesting transdermal enhancers. Series of 1,4-bis(1-acyl)piperazines **197–203** (Table 6.25) was also prepared (Peck et al. 1988). 4-Dodecanoylpiperazin-1-ium chloride (**186**) and 1-(3,4-dimethylpiperazin-1-yl) undecan-1-one (**196**) are noteworthy structures underlining the importance of C_{12} chain; nevertheless, again it can be speculated about the enhancement effect of dodecanoyl derivatives **183** (**186**), **192**, **196**. All the calculated molecular descriptors of these compounds are close to morpholine **133**; nevertheless, the behaviour of the second basic piperazine nitrogen in the skin is not clear. There is also a question what compounds

Table 6.21 Series of long-chain *N*-morpholinylalkenones and their selected physico-chemical properties

	***n***	**log *P***	**PSA**	**MR** [cm³/mol]
155	4	1.42	29.54	56.60
156	5	2.29	29.54	61.20
157	6	2.81	29.54	65.80
158	7	3.05	29.54	70.40
159	8	3.81	29.54	75.00
160	9	4.01	29.54	79.60
161	10	4.61	29.54	84.19
162	11	5.06	29.54	88.79
163	12	5.47	29.54	93.39
164	13	5.89	29.54	97.99
165	15	6.67	29.54	107.19
	***n/m***	**log *P***	**PSA**	**MR** [cm³/mol]
166	8/0	**4.12**	29.54	80.91
167	9/0	**4.39**	29.54	85.51
168	10/0	**4.98**	29.54	90.11
169	8/4	**6.21**	29.54	99.31
170	8/6	**7.42**	29.54	108.50

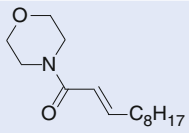
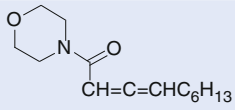
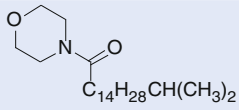
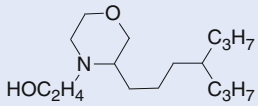
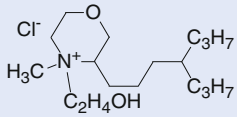
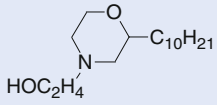
within the series of 1,4-bis(1-acyl)piperazines **197**–**203** may be the most effective. According to the calculated data, bisheptanoyl derivative **198** may have the highest enhancement activity; nevertheless, this is only speculation based on the calculated parameters and their mutual comparison.

6.2.3 Five-Membered Ring Analogues

Pyrrolidin-2-one (**204**) and *N*-methylpyrrolidin-2-one (**205**) are the most studied substances in the group; see Table 6.26. Their effect on the enhancement of the penetration of a number of drugs was investigated. *N*-Methylpyrrolidin-2-one (**205**) is also often used as a cosolvent with other solvents or enhancers (Karande et al. 2006; Furuishi et al. 2007; Rhee et al. 2007; Ren et al. 2008). It is evident that both compounds, based on their properties, can act rather as moisturising factor, and by this way, they enhance penetration through the skin or only to skin; see below.

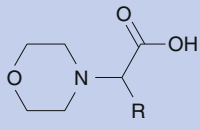
Yoneto et al. (1995, 1998) studied enhancement effects of a series of homologues of *N*-alkylpyrrolidin-2-ones **206**–**210** (see Table 6.26) on the transdermal penetration of steroid hormones (β-estradiol, corticosterone and hydrocortisone) through the hairless mouse skin in vitro. *N*-Octylpyrrolidin-2-one (**210**) was estimated as the most effective. A nearly semilogarithmic linear relationship was obtained between the enhancement potency and the carbon number of the alkyl chain; there was an approximately 3.5-fold increase in the enhancement potency for 1-alkyl-2-pyrrolidone methylene group. An effect of these *N*-alkylpyrrolidin-2-ones **206**–**212** on transdermal penetration of 5-fluorouracil, triamcinolon acetonide, indomethacin and flurbiprofen through the rat skin in vitro was observed by Sasaki et al. (1991). They concluded that the

Table 6.22 Long-chain *N*-acylmorpholines and morpholinoethanol derivatives as potential CPEs and their selected physico-chemical properties

No.	Comp.	log *P*	PSA	MR [cm³/mol]
171		3.25	29.54	76.80
172		2.79	29.54	71.90
173		7.03	29.54	107.32
174		4.02	32.70	81.41
175		NC	NC	NC
176		4.62	32.70	81.24

NC not calculated

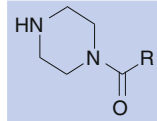
Table 6.23 Series of substituted derivatives of 2-morpholin-4-yl-carboxylic acid and their selected physico-chemical properties

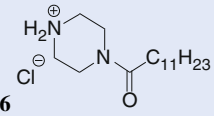


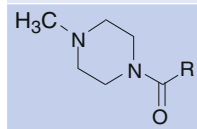
	R	log *P*	PSA	MR [cm³/mol]
177	$-C_4H_9$	1.02	49.77	53.39
178	$-C_6H_{13}$	2.41	49.77	62.59
179	$-C_8H_{17}$	3.40	49.77	71.79
180	$-C_{10}H_{21}$	4.49	49.77	80.99
181	$-C_{12}H_{25}$	5.53	49.77	90.19

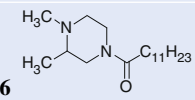
more lipophilic enhancer, i.e. **212**, was more effective in enhancing penetration and solubility of hydrophilic drugs than lipophilic drugs and suggested that an increase of drug solubility in the SC is a possible mechanism of action of investigated enhancers (Sasaki et al. 1991).

Table 6.24 Series of 1-piperazin-1-yl-alkan-1-ones and 1-(4-methylpiperazin-1-yl)-alkan-1-ones and their selected physico-chemical properties



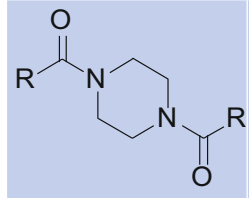
	R	log *P*	PSA	MR [cm³/mol]
182	$-C_4H_9$	0.88	32.34	49.45
183	$-C_{11}H_{23}$	4.98	32.34	81.64
184	$-C_{15}H_{31}$	6.62	32.34	100.04
185	$-C_{16}H_{33}$	7.20	32.34	104.64
186 		NC	NC	NC



	R	log *P*	PSA	MR [cm³/mol]
187	$-C_5H_{11}$	1.94	23.55	59.09
188	$-C_6H_{13}$	2.31	23.55	63.69
189	$-C_7H_{15}$	2.90	23.55	68.29
190	$-C_8H_{17}$	3.22	23.55	72.88
191	$-C_9H_{19}$	4.00	23.55	77.48
192	$-C_{11}H_{23}$	4.67	23.55	86.68
193	$-C_{13}H_{27}$	5.97	23.55	95.88
194	$-C_{15}H_{31}$	6.94	23.55	105.08
195	$-C_{17}H_{35}$	7.95	23.55	114.28
196 		5.00	23.55	91.37

NC not calculated

Table 6.25 Series of 1-(4-acyloylpiperazine-1-yl)alkan-1-ones and their selected physico-chemical properties



	R	log *P*	PSA	MR [cm³/mol]
197	$-C_5H_{11}$	3.40	40.62	82.05
198	$-C_6H_{13}$	4.11	40.62	91.25
199	$-C_7H_{15}$	5.78	40.62	100.44
200	$-C_9H_{19}$	7.83	40.62	118.84
201	$-C_{11}H_{23}$	9.32	40.62	137.24
202	$-C_{15}H_{31}$	13.24	40.62	180.77
203	$-C_{17}H_{35}$	14.99	40.62	199.17

Table 6.26 Series of *N*-alkylpyrrolidin-2-ones and their selected physico-chemical properties

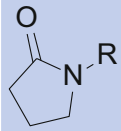
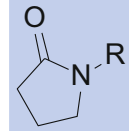
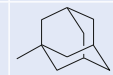
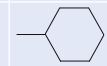
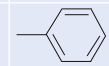
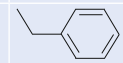
	R	log *P*	PSA	MR [cm³/mol]
204	H	−0.80	20.31	22.02
205	$-CH_3$	−0.34	20.31	27.02
206	$-C_2H_5$	−0.05	20.31	31.83
207	$-C_3H_7$	0.39	20.31	36.42
208	$-C_4H_9$	1.33	20.31	41.02
209	$-C_6H_{13}$	2.56	20.31	50.22
210	$-C_8H_{17}$	3.70	20.31	59.42
211	$-C_{10}H_{21}$	4.27	20.31	68.62
212	$-C_{12}H_{25}$	4.90	20.31	77.82

Table 6.27 *N*-substituted pyrrolidin-2-as CPEs and their selected physico-chemical properties

	R	log *P*	PSA	MR [cm³/mol]
213	$-C_2H_4OH$	−0.74	40.54	33.19
214	$-C_3H_6OH$	−0.27	40.54	37.79
215	$-CH_2CHOHCH_2OH$	−1.22	60.77	39.25
216	$-C_2H_4SC_{10}H_{21}$	5.06	45.61	85.66
217		2.63	20.31	62.35
218		2.18	20.31	48.03
219		1.53	20.31	46.11
220		1.75	20.31	51.52

N-Dodecylpyrrolidin-2-one (**212**), a direct homologue of Azone® (**1**), was also used as an enhancer for penetration of melatonin, and it demonstrated threefold higher effect than *N*-methylpyrrolidin-2-one (**205**) (Rachakonda et al. 2008). It also showed to be very effective for transdermal penetration of hydrocortisone in experiments conducted in vitro on the hairless mouse skin (Godwin et al. 1997). Compound **212** (ER = 60.1) showed to be 1.5-fold more active than Azone® (**1**, ER = 42.2) (Michniak et al. 1998).

Due to the excellent results of these simple homologues of Azone® (**1**), a number of various *N*-substituted pyrrolidin-2-ones were designed as potential CPEs. Other linear or cyclic alkyl chains, chains with the isosteric substitution of sulfur or oxygen by carbon and polyhydroxy chains were proposed as substituents, or radical analogy was applied, and various phenyl or benzyl-substituted pyrrolidin-2-ones (see Table 6.27) were tested and patented. Hydroxy derivatives **213**–**215** that were patented by Cooper can be included among these derivatives (Cooper et al. 1985). The penetration of hydrocortisone through the human skin in vitro was increased ca. eightfold by the use of 1-(2-hydroxyethyl)pyrrolidin-2-one (**213**), when compared with the control sample without enhancer. The addition of *N*-[2-(decylthio)ethyl] pyrrolidin-2-one (**216**) with concentration 3 % increased the penetration of indomethacin (0.5 mg/mL propylene glycol-ethanol 9:1) through the female hairless mice skin ninefold in comparison with the sample without enhancer; so its penetration enhancement was comparable with Azone® (**1**). This compound can also be mentioned as an isoster of compound **212**, and it was proposed and synthesised with the purpose to build in cysteine, a constituent amino acid of hair keratin, to pyrrolidin-2-one structure (Yano et al. 1992). *N*-(adamantan-1-yl)pyrrolidin-2-one (**217**) is a noteworthy derivative of **204** and was prepared and tested as transdermal penetration enhancer similarly as *N*-cyclohexylpyrrolidin-2-one (**218**) and its radical analogues – *N*-phenyl- (**219**) and *N*-benzylpyrrolidin-2-ones (**220**). The penetration of hydrocortisone through the hairless mouse skin in vitro was increased ca. tenfold by the use of compound **220** when compared with the control without enhancer. These compounds showed moderate or no enhancement effect; for example, **217** was fourfold less effective and **218** was 40-fold less active than **212** (Rajadhyaksha et al. 1983; Godwin et al. 1997; Rachakonda et al. 2008).

Modifications in below-discussed derivatives were performed again in the *N*-aliphatic chain. Namely, the introduction of carbonyl group and isosteric replacement of oxygen by carbon were made. Similarly to acetic acid esters **104**–**110**,

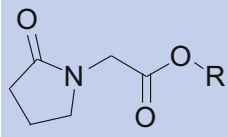
effective enhancers, a homologic series of 2-(2-oxopyrrolidin-1-yl)acetic acid esters **221**–**227**, were prepared and evaluated (see Table 6.28). Dodecyl-2-(2-oxopyrrolidin-1-yl) acetate (**225**, ER = 67.3) was estimated as the best enhancer within the series. Its effect was twofold higher than that of compounds **107** (ER = 36.4) and **110** (ER = 38.8), comparable with that of **212** (ER = 60.1) and by one third higher than the effect of Azone® (**1**, ER = 19.5). These enhancers were proposed as molecules potentially biodegradable by skin esterases (Michniak et al. 1996).

2-(2-Oxopyrrolidin-1-yl)ethyl decanoate (**228**) and 2-(2-oxopyrrolidin-1-yl)ethyl oleate (**229**) (see Table 6.28), which were synthesised as other possible biodegradable enhancers, can be classified as reverse analogues (reverse esters) of compounds **221**–**227**. The principle of the reverse analogy was discussed in Sect. 6.3.1. Also these esters of fatty acids were at least as active as Azone® (**1**), by increasing the in vitro penetration of hydrocortisone through the mouse skin by nearly 500-fold, and showed much lesser irritability than Azone® (**1**) (Lambert et al. 1993).

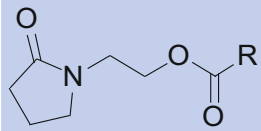
By the isosteric replacement of sulfur by oxygen in compound **212**, *N*-alkylpyrrolidin-2-thiones **230**–**232** (see Table 6.29) were prepared. Dodecyl derivate **232** showed higher enhancement effect (ER = 13.7) than its six-membered and seven-membered homologues, i.e. compounds **50** (ER = 4.3) and **100** (ER = 8.6); however, the isosteric replacement of oxygen by sulfur led to a decrease in enhancement activity in comparison with **212** (Minaskanian et al. 1991). When calculated parameters of discussed thiones **50**, **100** and **232** are compared with each other, the data of compound **232** are the closest to Azone® (**1**).

Compounds **233**–**236** (Table 6.30) can be considered as other simple modifications of effective compound **212**, the closest to Azone® (**1**) and six-membered homologues **99** and **101**–**103** (Table 6.14) (Michniak et al. 1995a, 1998; Godwin et al. 1997). Isosteric 2-decylcyclopentanone (**234**) and decarbonylated *N*-dodecylpyrrolidin (**235**) showed substantially lower activity than Azone® (**1**, ER = 42.2) and "parent" compound **212** (ER = 60.1). Both derivatives possess activity comparable to that of compounds **101** (ER = 10.3) and **102** (ER = 9.9). By the introduction of a carbonyl group to the α-position of the alkyl side chain, *N*-dodecanoylpyrrolidin-2-one (**236**) was generated (Michniak et al. 1998). This imide

Table 6.28 Series of esters of 2-(2-oxopyrrolidin-1-yl)acetic acid, its reverse esters, their selected physico-chemical properties and enhancement ratios



	R	log *P*	PSA	MR [cm³/mol]	ER
221	$-C_8H_{17}$	3.40	46.61	70.98	18.9
222	$-C_9H_{19}$	3.96	46.61	75.58	17.6
223	$-C_{10}H_{21}$	4.30	46.61	80.17	38.2
224	$-C_{11}H_{23}$	4.62	46.61	84.77	15.3
225	$-C_{12}H_{25}$	4.99	46.61	89.37	67.3
226	$-C_{13}H_{27}$	6.29	46.61	93.97	18.0
227	$-C_{14}H_{29}$	6.74	46.61	98.57	25.8



	R	log *P*	PSA	MR [cm³/mol]	ER
228	$-C_9H_{19}$	4.17	46.61	79.48	–
229	$-C_7H_{14}CH=CHC_8H_{17}$	7.61	46.61	117.64	–

Table 6.29 *N*-substituted pyrrolidin-2-thiones and their selected physico-chemical properties

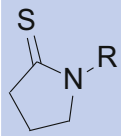
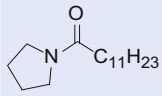
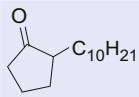
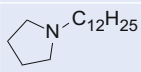
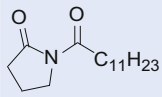
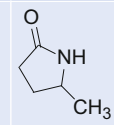
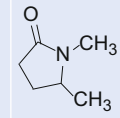
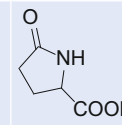
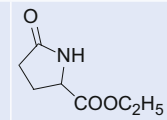
	R	log *P*	PSA	MR [cm³/mol]
230	$-C_4H_9$	1.30	35.33	48.22
231	$-C_8H_{17}$	4.38	35.33	66.59
232	$-C_{12}H_{25}$	5.89	35.33	84.99

Table 6.30 Derivatives of five-membered ring analogues of Azone®, their selected physico-chemical properties and enhancement ratios

No.	Comp.	log *P*	PSA	MR [cm³/mol]	ER
233		5.08	20.31	77.82	45.5
234		5.65	17.07	69.54	8.2
235		6.08	3.24	78.05	5.4
236		4.98	37.38	77.11	12.8

also possesses considerably lower activity than Azone® (**1**) and compound **212**, however comparable to homologue **103** (ER = 8.6). 1-(Pyrrolidin-1-yl)dodecan-1-one (**233**) was prepared as the reverse amide of parent compound **212**. In contrast to the above-mentioned compounds, the enhancement activity of this substance was distinctly higher than that of compound **99** and comparable to Azone® (**1**) and compound **66**, but lower than that of compound **212**. In the series of four compounds, the activity decreases as follows: **233** >>> **236** > **234** > **235**. On the basis of the investigation of these concrete modifications, it can be concluded that in general, five-membered ring homologues are more active than six-membered ring homologues and that the activity of some derivatives is close to or higher than that of a seven-membered ring. It can also be stated that the absence of a nitrogen atom in the ring or the presence of one more carbonyl group results

Table 6.31 Derivatives of 5-substituted pyrrolidin-2-one, their selected physico-chemical properties and enhancement ratios

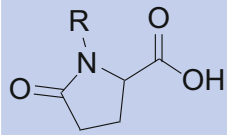
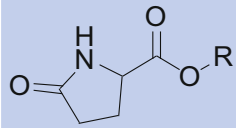
No.	Comp.	log *P*	PSA	MR [cm³/mol]	ER
237		−0.50	29.10	26.71	0.8
238		−0.06	20.31	31.71	1.4
239		−1.76	66.40	27.83	0.7
240		−0.75	55.40	38.07	0.9

in a considerable decrease of activity (Jampílek and Brychtová 2012).

Godwin et al. (1997) prepared also more substituted compounds **237**–**240** (see Table 6.31), alkyl analogues of compound **205**. Nevertheless, none of these compounds demonstrated higher effect than the more lipophilic *N*-dodecylpyrrolidin-2-one (**212**, ER = 42.0). The authors concluded that this disadvantage was probably connected with low lipophilicity. However, the content of hydrocortisone in the skin after the conducted experiments was substantially higher after the use of these more hydrophilic compounds **237**–**240** than after the use of **212**. According to the authors, these derivatives rather showed the ability to accumulate the drug in the skin, which can be used for dermal application or for gradual drug release from the skin to blood capillaries.

5-Oxopyrrolidine-2-carboxylic acid (**239**) is a part of the natural moisturising factor (NMF) present in the human skin (Spier and Pascher 1956; Harding and Scott 2002). However, mainly its *N*-substituted derivatives or its higher esters **241**–**253** were described as enhancers (see Table 6.32). According to Peck and Minaskanian (1995), all these compounds

Table 6.32 5-Oxopyrrolidine-2-carboxylic acids, their esters and their selected physico-chemical properties

	**R**	**log *P***	**PSA**	**MR** [cm^3/mol]
241	-C_4H_9	0.18	57.61	46.83
242	-C_5H_{11}	1.31	57.61	51.43
243	-C_6H_{13}	1.72	57.61	56.03
244	-C_7H_{15}	2.37	57.61	60.63
245	-C_8H_{17}	2.69	57.61	65.23
246	-C_9H_{19}	3.41	57.61	69.83
247	-$C_{10}H_{21}$	3.71	57.61	74.43
248	-$C_{12}H_{25}$	4.29	57.61	83.63
249	-$C_{14}H_{29}$	5.45	57.61	92.82
250	-$C_{16}H_{33}$	6.72	57.61	102.02
	**R**	**log *P***	**PSA**	**MR** [cm^3/mol]
251	-$C_{10}H_{21}$	3.62	55.40	74.86
252	-$C_{12}H_{25}$	4.28	55.40	84.06
253	-$C_8H_{16}CH{=}CHC_8H_{17}$	6.98	55.40	113.02

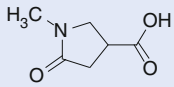
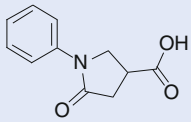
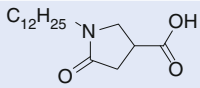
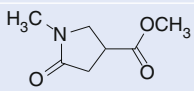
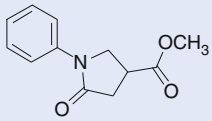
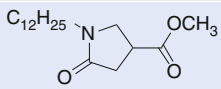
increased the penetration of the tested drugs at least by 76 % compared with the penetration without the aid of CPEs. Compound **248** enhanced the penetration of drugs through the hairless mouse skin by 600 %. This series was much more successful than analogues **35**–**44** or **111**–**120**. *N*-Dodecyl-5-oxopyrrolidin-2-carboxylic acid (**248**) was the most successful from these substituted acids as CPEs of haloperidol (Minaskanian and Peck, 1995), and all esters **251**–**253** increased the penetration of enalapril by 3 %, 9 % and 18 % and clonidine by 7 %, 12 % and 3 % through the snake skin compared with the penetration without CPEs (Alexander et al. 1989) in contrast to esters of 2-(2-oxoazepan-1-yl)acetic acid **45** and **46** that were not active.

As potential CPEs, a series of 5-oxopyrrolidine-3-carboxylic acid derivatives **254**–**259** (Table 6.33) as position isomers of 5-oxopyrrolidine-2-carboxylic acid (**239**) was prepared and tested for transdermal delivery of phenolsulfonphthalein. However, a change of the position of the carboxyl group on the skeleton of *N*-alkylpyrrolidin-2-one resulted in a noticeable decrease of their effect as CPEs, which was consequently intensified by ester generation or *N*-phenyl substitution (Sasaki et al. 1988). Therefore, it can be concluded that the change of the carboxylic position is disadvantageous and the transdermal enhancement effect of this type of compounds is strictly related to the α-position of the pyrrolidine ring of the carboxylic group.

Oligodimethylsiloxanes **260**–**267** containing pyrrolidin-2-one fragment as a terminal group (see Table 6.34) represent highly unusual silicone-based transdermal penetration enhancers. It was established that their enhancing activity depends both on the structure of the polar terminal group and the polymerisation degree. While derivatives of oligodimethylsiloxane with *N*-propylpyrrolidin-2-one **265**–**267** did not have any enhancement effect, a series with *N*-methylpyrrolidin-2-one **261**–**263** and disiloxanes **260** and **264** showed enhancement

effect on penetration of phenazone and indomethacin through the rabbit skin in vitro (Aoyagi et al. 1996).

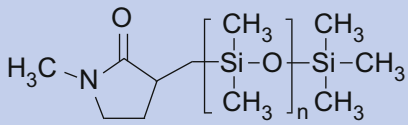
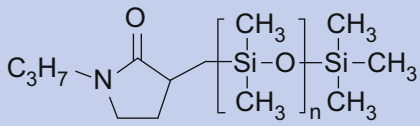
Table 6.33 5-Oxopyrrolidine-3-carboxylic acids, their esters and their selected physico-chemical properties

No.	Comp.	log *P*	PSA	MR [cm³/mol]
254		−1.10	57.61	32.57
255		0.47	57.61	51.66
256		4.39	57.61	83.37
257		−0.48	46.61	38.01
258		1.26	46.61	57.09
259		4.84	46.61	88.80

Derivatives of pyrrolidindion **268–271** were designed by an introduction of another carbonyl moiety on the ring of pyrrolidin-2-one. These compounds were prepared by various scientific teams and evaluated as potential CPEs (see Table 6.35). *N*-Methylpyrrolidin-2,5-dione (**268**) accelerated the penetration of hydrocortisone with ER 3.9 only (Godwin et al. 1997), while *N*-dodecylpyrrolidin-2,5-dione (**269**, ER = 18.8) showed comparable effect to Azone® (**1**, ER = 19.5) (Michniak et al. 1993a). *N*-Geranylpyrrolidin-2,5-dione (**270**) was patented as one of the suitable enhancers for oleocanthal (Feldkamp et al. 2008). A geranyl chain is also present in the molecule of the successful *N*-geranylazepan-2-one (**14**). The position isomer *N*-dodecylpyrrolidin-2,4-dione (**271**) was designed by means of carboxymethyl moiety shifting from the position $C_{(5)}$ to $C_{(4)}$. Compound **271** was patented as a part of transdermal formulations in combination with organic sulfoxides (Singh 2007).

A series of proline esters **272–278** (Table 6.36) were synthesised and tested as CPEs (Fincher et al. 1996; Tenjarla et al. 1999). All the compounds at a concentration of 5 % (w:v) increased the penetration of hydrocortisone (0.03 M in propylene glycol) through the hairless mouse skin.

Table 6.34 Series of oligodimethylsiloxanes and their selected physico-chemical properties

	*n*	log *P*	PSA	MR [cm³/mol]
260	1	3.67	29.54	NC
261	4	6.06	57.23	NC
262	10	10.99	112.61	NC
263	16	18.39	177.22	NC
	*n*	log *P*	PSA	MR [cm³/mol]
264	1	4.33	29.54	NC
265	4	6.66	57.23	NC
266	10	11.67	112.61	NC
267	16	19.07	177.22	NC

NC not calculated

Table 6.35 *N*-substituted pyrrolidinediones designed as potential CPEs and their selected physico-chemical properties

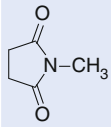
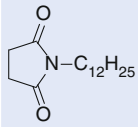
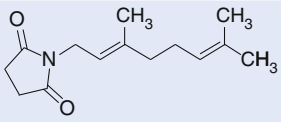
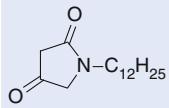
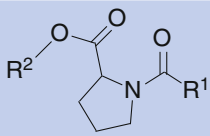
No.	Comp.	log *P*	PSA	MR [cm³/mol]
268		–0.60	37.38	26.32
269		4.83	37.38	77.11
270		2.48	37.38	69.91
271		4.16	37.38	79.10

Table 6.36 Esters of substituted proline, their selected physico-chemical properties and enhancement ratios



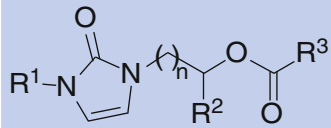
R^1	R^2	log *P*	PSA	MR [cm³/mol]	ER
272	$-CH_3$	$-C_5H_{11}$	2.38	46.61	61.52	14.4
273	$-CH_3$	$-C_8H_{17}$	3.92	46.61	75.32	17.7
274	$-CH_3$	$-C_{10}H_{21}$	4.72	46.61	84.52	18.2
275	$-CH_3$	$-C_{11}H_{23}$	5.14	46.61	89.12	30.6
276	$-CH_3$	$-C_{12}H_{25}$	5.54	46.61	93.72	34.3
277	$-CH_3$	$-C_9H_{17}=C_9H_{18}$	8.41	46.61	127.28	27.1
278	$-C_{11}H_{23}$	$-CH_3$	5.14	46.61	89.12	13.9

The highest fluxes were obtained with **275**–**277** (C_{11}, C_{12} and C_{18} esters) that were comparable to Azone® (**1**) (ER = 22). Compound **277**, which has a *cis*-double bond in the alkyl side chain, also increased the flux significantly. It is noteworthy that compound **278**, i.e. the positional isomer of **275**, showed approximately twofold less enhancement effect than **275**.

Long-chain alkyl esters of *N*,*N*-disubstituted 4-imidazolin-2-ones **279**–**291** (Table 6.37) were prepared as biodegradable CPEs. These cyclic derivatives of urea with concentration of 5 % were tested on penetration of 1 % indomethacin in petrolatum through the snake skin. Compounds **285**, **289** and **291** showed penetration enhancement at least equal to or better than that of Azone®. Prolongation of the linker between imidazolinone and the ester moiety resulted in an increase of the enhancement activity. Carbonates **281**–**283** were inactive in comparison with esters (Higuchi et al. 1989; Wong et al. 1988, 1989).

Substituted 1,3-dioxolanes as pyrrolidine isosteres can also be included in this group of compounds derived from a five-membered ring. 2-Alkyl-1,3-dioxolanes **292**–**294**, 2-alkyl-4-methyl-1,3-dioxolanes **295**–**297**, 4-alkyl-4-

Table 6.37 Series of *N*,*N*-disubstituted 4-imidazolin-2-ones and their selected physico-chemical properties

	***n***	**R^1**	**R^2**	**R^3**	**log *P***	**PSA**	**MR** [cm^3/mol]
279	0	$-CH_3$	H	$-C_9H_{19}$	3.86	49.85	78.53
280	0	$-CH_3$	H	$-C_{11}H_{23}$	4.68	49.85	87.73
281	0	$-CH_3$	H	$-OC_{10}H_{21}$	4.27	59.08	85.15
282	0	$-CH_3$	H	$-OC_{12}H_{25}$	4.96	59.08	94.35
283	0	$-CH_3$	H	$-OC_{14}H_{29}$	6.05	59.08	103.55
284	0	$-CH_3$	$-CH_3$	$-C_{11}H_{23}$	4.97	49.85	92.22
285	0	$-CH_3$	$-CH_3$	$-C_{13}H_{27}$	6.17	49.85	101.42
286	0	$-C_2H_5$	$-CH_3$	$-C_{13}H_{27}$	6.53	49.85	106.22
287	1	$-CH_3$	H	$-C_{11}H_{23}$	4.87	49.85	92.18
288	1	$-CH_3$	$-CH_3$	$-C_9H_{19}$	4.36	49.85	87.67
289	1	$-CH_3$	$-CH_3$	$-C_{11}H_{23}$	5.14	49.85	96.87
290	1	$-CH_3$	$-CH_3$	$-C_{13}H_{27}$	6.37	49.85	106.07
291	2	$-CH_3$	H	$-C_{11}H_{23}$	5.39	49.85	101.38

methyl-1,3-dioxolanes **298–300** and 2-alkyl-2,4-dimethyl-1,3-dioxolanes **301–303** were designed (see Table 6.38) (Michniak et al. 1995b). The penetration activity of all these derivatives was very low and non-comparable to nitrogen isosteres. However, general conclusions of this study can be proposed. The activity also increased with the chain lengthening to nonyl derivative and then decreased again. The lowest effect was shown by trisubstituted dioxolanes **301–303**. Disubstituted compounds **295–297** and **298–300** showed comparable activity, and monosubstituted compounds **292–294** were the most effective; nevertheless, the most effective compound **293** was sevenfold less active than Azone® (**1**) (Jampílek and Brychtová 2012).

The below-mentioned compounds **304–310** (Table 6.39) showed highly specific behaviour. Although all these compounds are similar by their structure to the above-discussed five-membered ring CPEs, all were reported as penetration-inhibiting agents (Peck et al. 1997). These compounds may be formulated into topical compositions that function as barriers to the passage of bioactive compounds and various other agents through the mammalian skin in either direction when applied to the skin. Design of these compounds inspired by observation that compounds with a number of carbonyl moieties interact with the skin components, especially with ceramides, resulted in the "firmer" and less permeable skin barrier (Hadgraft et al. 1996). The structure of these penetration inhibitors is derived from *N*-carbonyl-pyrrolidinediones, where one of carbonyl moieties is always vicinal to nitrogen, and the second carbonyl or carboxylic group can be in the position $C_{(3)}$ or $C_{(4/5)}$. The fact that penetration-inhibiting effect is connected with the presence of more than two carbonyl/carboxylic groups can be confirmed by the matter that effective inhibitors are derivatives of pyrrolidinedione but also of imidazolidine (substance **305**) or oxazolidine (derivatives **306–310**). These heterocycles are isosteric to the starting pyrrolidine.

6.2.4 Mutual Analogues

The principle of "mutual drugs" (twin molecules, twin drug approach) is a combination of two molecules of the same biologically active compound or a combination of two different molecules with similar pharmacodynamic effect, and both molecules can be from the same pharmacological (ATC) class or two different classes. This idea originated in the second half of the nineteenth century as the so-called Salol Nencki Principle.

Table 6.38 Series of substituted 1,3-dioxolanes prepared as potential CPEs, their selected physico-chemical properties and enhancement ratios

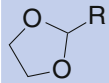
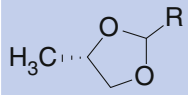
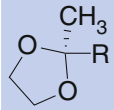
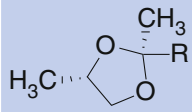
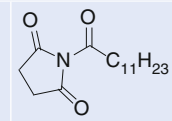
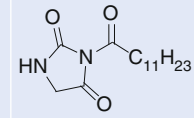
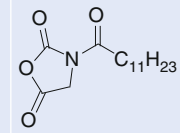
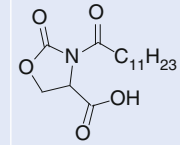
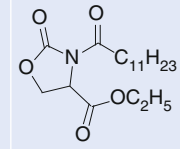
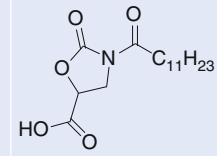
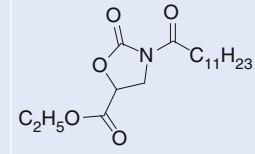
	R	log *P*	PSA	MR [cm³/mol]	ER
292	$-C_7H_{15}$	3.23	18.46	49.48	0.9
293	$-C_9H_{19}$	4.30	18.46	58.68	6.2
294	$-C_{11}H_{23}$	5.16	18.46	67.88	2.6
	R	log *P*	PSA	MR [cm³/mol]	ER
295	$-C_7H_{15}$	3.78	18.46	54.17	0.7
296	$-C_9H_{19}$	4.64	18.46	63.37	2.2
297	$-C_{11}H_{23}$	5.42	18.46	72.57	1.6
	R	log *P*	PSA	MR [cm³/mol]	ER
298	$-C_7H_{15}$	3.72	18.46	54.35	1.0
299	$-C_9H_{19}$	4.79	18.46	63.54	2.4
300	$-C_{11}H_{23}$	5.83	18.46	72.74	2.5
	R	log *P*	PSA	MR [cm³/mol]	ER
301	$-C_7H_{15}$	4.28	18.46	59.04	0.6
302	$-C_9H_{19}$	5.13	18.46	68.23	1.4
303	$-C_{11}H_{23}$	6.25	18.46	77.43	4.2

Table 6.39 Derivatives of pyrrolidine designed as penetration-inhibiting agents (retarders) and their selected physico-chemical properties

No.	Comp.	log *P*	PSA	MR [cm³/mol]
304		4.09	54.45	76.41
305		3.93	66.48	76.48
306		3.99	63.68	74.52
307		4.17	83.91	80.34
308		4.96	72.91	90.57
309		4.12	83.91	80.34
310		4.96	72.91	90.57

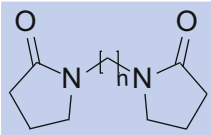
Wilhelm Marceli Nencki (1847–1901, a Polish chemist) prepared phenyl salicylate (named as salol) as antiseptic in 1886 (Melichar 1987). Similar combinations of molecules can be named, for example, salsalate (two molecules of salicylic acid), benorilate (salicylic acid and paracetamol), reproterol (orciprenaline and theophylline), sultamicillin (ampicillin and sulbactam) or sulfasalazine (sulfapyridine and 5-aminosalicylic acid).

Similarly as mentioned above, the Nencki principle was also applied in the field of potential transdermal enhancers. The basic structural scaffolds necessary for transdermal enhancement effect can be connected directly or by means of an aliphatic linker or, for example, using an amide or ester bond, and connected fragments can be the same or different. As discussed below, the most potent mutual CPEs have two "polar heads" connected with C_3 or C_6 linker. It seems that for bifunctional molecules, similar dependence on periodical chain length can be observed as for other Azone® analogues, where, for example, C_{12}, C_{14} and/or C_{16} chains were the most effective. A similar trend can be also found for many classes of drugs. All the discussed bifunctional molecules show a wide range of lipophilicity values, possess large PSA and are bulky.

The simplest approach was chosen by Rajadhyaksha (1976b), who prepared a series of

bis-*N*-pyrrolidinones **311–318** connected by unbranched alkyl chain (see Table 6.40). The most effective CPEs were derivatives linked by C_3 and C_6 chains, i.e. 1,1′-propane-1,3-diyldipyrrolidin-2-one (**311**) and 1,1′-hexane-1,6-diyldipyrrolidin-2-one (**314**) that can be used to enhance the penetration of griseofulvin, clindamycin, neomycin, fluocinolone, etc. (Rajadhyaksha et al. 1983). The penetration of fluocinolone through the hairless mouse skin in vitro was increased ca. twofold by the use of compound **311**, when compared with the control sample without enhancer.

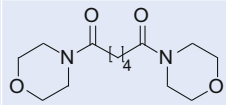
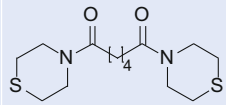
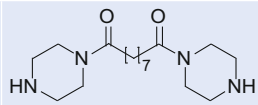
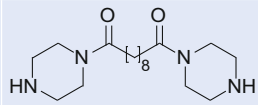
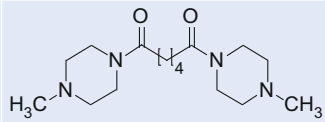
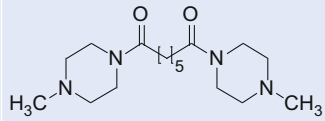
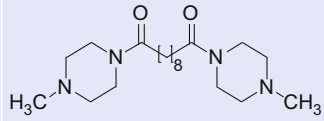
Table 6.40 Bis-*N*-pyrrolidinones as potential CPEs and their selected physico-chemical properties

		***n***	**log *P***	**PSA**	**MR** [cm³/mol]
311		3	–0.28	40.62	56.77
312		4	0.06	40.62	61.37
313		5	0.60	40.62	65.97
314		6	1.22	40.62	70.57
315		7	1.89	40.62	75.17
316		8	2.37	40.62	79.77
317		9	2.66	40.62	84.37
318		10	3.06	40.62	88.97

Peck and Minaskanian (1988) applied a similar simple approach for preparation of bis(thio) morpholinyldiones **319** and **320** and bispiperazinyldiones **321–325** (see Table 6.41). All the twin molecules were used for penetration enhancement of haloperidol.

Breitenbach et al. (2002) synthesised and patented a number of twin molecules connected with

Table 6.41 Bis-*N*-inanyldiones as potential CPEs and their selected physico-chemical properties

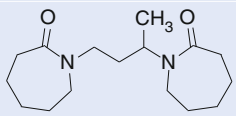
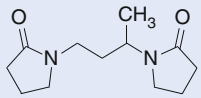
No.	**Comp.**	**log *P***	**PSA**	**MR** [cm³/mol]
319		–0.07	59.08	74.02
320		1.58	91.22	86.25
321		0.72	64.68	92.02
322		1.25	64.68	96.62
323		–0.03	47.10	88.30
324		0.21	47.10	92.90
325		1.67	47.10	106.70

the branched 2-methylpropane linker. As effective fragments azepanone, piperidinone, morpholin-3-one, pyrrolidone and 4-oxazolidinone were used in various combinations, see Fig. 6.2. All the compounds were evaluated for their ability to solubilise drugs (e.g. prednisone, sulfathiazole, trimethoprime, esuprone, paracetamol, oxytetracycline) and enhance their penetration (e.g. ibuprofen, bisabolol) through the skin. 1,1′-Butane-1,3-diyldiazepan-2-one (**326**) and 1,1′-butane-1,3-diyldipyrrolidin-2-one (**327**) seem to be the most effective potential CPEs (see Table 6.42). The fact that 4-oxazolidinones (see Fig. 6.2) expressed enhancement effect supports the theory about the influence of the presence of more than two carbonyl/carboxylic groups in the molecule of a potential CPE on its enhancement effect; see penetration-inhibiting agents (retarders), i.e. compounds **306–310** (Table 6.39).

Kim et al. (2001) prepared and evaluated two series of compounds derived from 3-(2-oxopyrrolidin-1-yl)azepan-2-one, i.e. generated by direct connection of azepanone and pyrrolidinone. One of the series (compounds **328–333**) contained an alkyl side chain and another (compounds **334–339**), an alkyl ester chain (see Table 6.43). Compounds **328–333** are direct analogues of Azone®-like compounds (**1–11**), while esters **334–339** are close to ineffective esters **45** and **46**. The objective of the investigation was to observe the dependence of the enhancing activity on the length of an alkyl/alkyl–ester side chain at permeation of hydrocortisone through the hairless mouse skin in vitro. A parabolic dependence was observed for compounds with the alkyl chain with the maximum activity of 3-(2-oxopyrrolidin-1-yl)-*N*-tetradecylazepan-*2*-one (**332**). Compounds with the alkyl–ester chain showed a linear dependence, i.e. the activity increased with the increasing number of carbons in the chain. Tetradecyl-2-[2-oxo-3-(2-oxopyrrolidin-1-yl) azepan-1-yl]acetate (**339**) was the most effective in the series but less effective than Azone® (**1**, ER = 20.3). If the enhancement activity of the alkyl and alkyl ester series is compared, all the compounds with the alkyl side chain (**328–333**) were more effective than the esters, and compounds **330–332** were more effective than Azone® (**1**, ER = 20.3) (Jampílek and Brychtová 2012).

A series of alkyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(substituted)hexanoates **340–374** (see Table 6.44) were prepared and evaluated recently as potential CPEs (Brychtová et al. 2010a, b, 2012). The structure of these compounds is primarily derived from 6-aminohexanoic acid, whose stability was increased by incorporation of the terminal amino moiety to the pyrrolidine ring, whereby a fragment similar with compound **269** was constructed. The second but the main part of the molecule is derived from α-disubstituted acetates (similar with compounds **35–46**, **104–110**, **177–181**, **221–227**), where five-membered, six-membered and seven-membered lactams and morpholine were used as α-substituents, and a non-branched saturated alkyl chain of the length

Table 6.42 Structures of 1,3-bis(*N*-lactamyl)butanes as potential CPEs and their selected physico-chemical properties

No.	Comp.	log *P*	PSA	MR [cm³/mol]
326		2.19	40.62	79.86
327		0.01	40.62	61.46

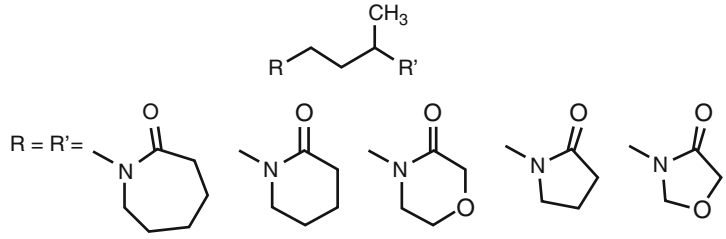
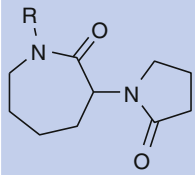
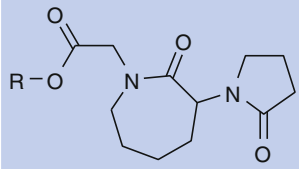


Fig. 6.2 Structures of 1,3-bis (*N*-lactamyl)butanes. R and R´ can be in various combinations

Table 6.43 Series of substituted 3-(2-oxopyrrolidin-1-yl)azepan-2-ones, their selected physico-chemical properties and enhancement ratios

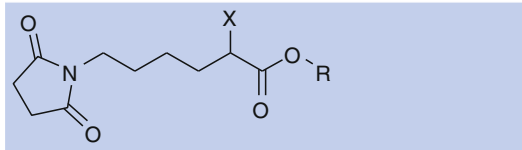
	**R**	**log *P***	**PSA**	**MR** [cm^3/mol]	**ER**
328	$-CH_3$	–0.22	40.62	57.15	1.1
329	$-C_6H_{13}$	2.42	40.62	80.35	4.6
330	$-C_{10}H_{21}$	4.30	40.62	98.75	25.7
331	$-C_{12}H_{25}$	4.99	40.62	107.94	27.5
332	$-C_{14}H_{29}$	6.43	40.62	117.14	40.5
333	$-C_{16}H_{33}$	7.62	40.62	126.34	12.8
	**R**	**log *P***	**PSA**	**MR** [cm^3/mol]	**ER**
334	$-CH_3$	0.00	66.92	68.71	1.2
335	$-C_6H_{13}$	2.43	66.92	91.90	2.3
336	$-C_8H_{17}$	3.58	66.92	101.10	5.9
337	$-C_{10}H_{21}$	4.53	66.92	110.30	10.9
338	$-C_{12}H_{25}$	5.35	66.92	119.50	15.0
339	$-C_{14}H_{29}$	6.73	66.92	128.70	16.1

of C_6 to C_{12} was used in the ester part. Thus, the discussed compounds can be also considered as twin molecules.

The penetration-enhancing effect of the prepared enhancers (20 mg/mL) in propylene glycol/water (1:1 v/v) medium was evaluated. Theophylline (5 mg/mL) was used as a model penetrant. Generally, it can be stated that compounds without α-substitution (C_{10} ester chain **344** and C_{11} ester chain **345**) were the most active followed by seven-membered compounds (C_{12} ester chain **353**) (Brychtová et al. 2012). The rest of the alkyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(substituted) hexanoates showed lower activity. Depending on the substitution by a heterocycle at the α-carbon, the compounds including C_9 to C_{12} alkyl chains were the most effective in a particular series. In the study dealing with six-membered *N*-heterocycles (2-oxopiperidinyl **354–360** or morpholin-4-yl **361–367**) as $C_{(2)}$ substituents, the highest enhancement ratios were exhibited by undecyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopiperidin-1-yl) hexanoates (**359**), while the series with morpholine showed only moderate effects (Brychtová et al. 2010b). In a series of alkyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl)hexanoates, the highest enhancement ratios were exhibited by the nonyl ester chain, compound **371** (Brychtová et al. 2010a).

Ab initio calculations and molecular dynamic simulations were performed, and both in silico techniques predicted the non-planar conformation of the studied compounds **340–374**. However, the conformations of the alkyl and hexanoate chains can change almost freely according to molecular dynamic simulations. When the "Azone® sub-structure" of the compounds is compared with Azone® itself, the most significant difference is the presence of the ester group which, owing to its rigid planar structure, prevents the adoption of the "soup spoon"-like conformation described by Hoogstraate et al. (1991). Therefore, it can be concluded that the significantly lower activities of α-substituted derivatives can be caused by the "non-soup spoon"-like conformations of the *N*-heterocyclic rings (Hoogstraate et al. 1991; Hadgraft et al. 1996; Brychtová et al. 2012).

Table 6.44 Derivatives of alkyl-6-(2,5-dioxopyrrolidin-1-yl)hexanoates, their selected physico-chemical properties and enhancement ratios



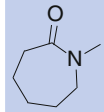
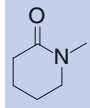
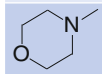
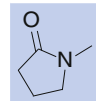
X H / R	R	log *P*	PSA	MR [cm³/mol]	ER
340	-C_6H_{13}	3.27	63.68	78.78	2.7
341	-C_7H_{15}	3.86	63.68	83.38	2.6
342	-C_8H_{17}	4.28	63.68	87.98	2.6
343	-C_9H_{19}	4.75	63.68	92.58	3.1
344	-$C_{10}H_{21}$	5.09	63.68	97.17	3.5
345	-$C_{11}H_{23}$	5.47	63.68	101.77	3.5
346	-$C_{12}H_{25}$	5.90	63.68	106.37	2.9
 R		log *P*	PSA	MR [cm³/mol]	ER
347	-C_6H_{13}	3.19	83.99	108.90	1.9
348	-C_7H_{15}	3.98	83.99	113.50	–
349	-C_8H_{17}	4.36	83.99	118.10	2.2
350	-C_9H_{19}	4.81	83.99	122.70	1.9
351	-$C_{10}H_{21}$	5.15	83.99	127.30	–
352	-$C_{11}H_{23}$	5.51	83.99	131.90	1.8
353	-$C_{12}H_{25}$	5.89	83.99	136.50	2.5
 R		log *P*	PSA	MR [cm³/mol]	ER
354	-C_6H_{13}	2.86	83.99	104.31	2.3
355	-C_7H_{15}	3.52	83.99	108.90	2.2
356	-C_8H_{17}	3.97	83.99	113.50	1.9
357	-C_9H_{19}	4.48	83.99	118.10	1.8
358	-$C_{10}H_{21}$	4.82	83.99	122.70	1.6
359	-$C_{11}H_{23}$	5.17	83.99	127.30	2.4
360	-$C_{12}H_{25}$	5.53	83.99	131.90	1.8
 R		log *P*	PSA	MR [cm³/mol]	ER
361	-C_6H_{13}	2.79	76.15	101.67	2.1
362	-C_7H_{15}	3.39	76.15	106.27	1.3
363	-C_8H_{17}	3.74	76.15	110.87	1.8
364	-C_9H_{19}	4.49	76.15	115.47	1.5
365	-$C_{10}H_{21}$	4.80	76.15	120.07	1.7
366	-$C_{11}H_{23}$	5.13	76.15	124.67	1.6
367	-$C_{12}H_{25}$	5.54	76.15	129.26	1.9

Table 6.44 (continued)

 R		log *P*	PSA	MR [cm³/mol]	ER
368	-C_6H_{13}	2.51	83.99	99.71	2.2
369	-C_7H_{15}	3.10	83.99	104.31	1.9
370	-C_8H_{17}	3.58	83.99	108.90	1.6
371	-C_9H_{19}	4.19	83.99	113.50	2.5
372	-$C_{10}H_{21}$	4.50	83.99	118.10	1.6
373	-$C_{11}H_{23}$	4.84	83.99	122.70	1.9
374	-$C_{12}H_{25}$	5.17	83.99	127.30	2.4

The penetration of the enhancers **340–374** through the skin was also investigated. Only hexyl and heptyl esters of derivatives substituted by a heterocycle at α-carbon were detected in the receptor compartment in 24 h after the start of the experiment. All the other alkyl esters C_8–C_{12} and derivatives without substitution at α-carbon were not detected, and it was assumed that due to their higher lipophilicity, they did not get through the skin barrier or remained incorporated in the skin structures (Brychtová et al. 2010a, b, 2012).

6.3 Structure–Activity Relationships of Azone® Analogues

As was mentioned at the beginning of Sect. 6.3, CPEs increase skin permeability by multiple mechanisms, and their enhancement effect is also influenced by the selection of the model-penetrating compound and testing conditions. Hence, it is practically impossible to conduct a precise investigation of quantitative structure–activity relationship (QSAR) or quantitative structure–property relationship (QSPR) for transdermal enhancers. The investigation of QSAR or QSPR for transdermal enhancers is also influenced by a range of structural differences of CPEs. QSAR/QSPR may be realistic/predictive when the response relies on a single, well-defined mechanism, such as the interaction of a drug with a receptor site (Jampílek and Brychtová 2012).

Under these highly complex circumstances, it is perhaps not surprising that regression analysis cannot quite exactly predict the activity of effective enhancers; nevertheless, today, a comparatively large number of QSAR/QSPR models for prediction of percutaneous drug absorption exist (Hadgraft 2004; Neumann 2008). The predictions are usually performed either using mechanistic (diffusion models) or empirical (data-based models, including artificial neural networks) modelling (Yamashita and Hashida 2003). Recently, purely computational models for predicting skin permeability coefficients were developed as well (Neumann et al. 2006). It is also recognised that QSAR approaches have been applied to the enhancement effect on a single drug. Despite the use of CPEs in many formulations, the number of predictive models that include the effect of CPEs is small. It can be concluded that mechanistic approaches for the prediction of penetration enhancement effects seem to be better than empirical approaches (Jampílek and Brychtová 2012). If logical thinking in combination with mathematical modelling is employed, more interesting relationships between the structure and effect can be found. These approaches take into account lipophilicity and hydrophilicity of the compound as well as its ionisability (pK_a) and pH of the vehicle. Mathematical analysis and modelling membrane interaction between CPEs and phospholipids were recently published (Iyer et al. 2002; Kulkarni et al. 2002; Pugh et al. 2005; Iyer et al. 2007; Kang et al. 2007; Zheng et al. 2008; Notman and Anwar 2012).

As concerns the monitored calculated molecular descriptors, it can be concluded that none of important physico-chemical parameters such as log *P*, PSA or MR (molar refractivity) play the main role, but they are complementary to one another. Compounds with low lipophilicity (log *P* values are negative or up to 1) possess above all skin-moisturising properties, which are typical for compounds **17**–**19**, **96**–**98**, **204**, **205**, **213**–**215**, **237**–**240**, etc. The enhancement activity of compounds with too high lipophilicity (log *P* values higher than 8) subsequently decreases (e.g. **11**, **95**) till complete absence, e.g. **266** and **267**. An optimal range of lipophilicity (log *P*) of effective CPEs containing five- and six-membered ring seems to be from 5 to 6 and that of enhancers containing seven-membered ring from 6 to 7. The PSA 3.24 of enhancement-active amine Azone® analogues is extremely low (e.g. **59**–**64**), while the PSA of other CPEs derived from the structure of lactam, amide, acid or ester is either 20.31 (e.g. **1**, **14**, **15**, **66**, **94**, **233**) or ranged 40-47 (**57**, **107**, **225**, **276**, **332**). It can be stated that when Azone® analogues possess different PSA than the above mentioned, they show probably low or no enhancement effect. Optimal bulk parameters expressed as MR (molar refractivity) seem to be in the range from 80 to 89 cm^3/mol (e.g. **1**, **61**, **66**, **94**, **107**, **225**).

Another molecular-level approach allows identification of dominant molecular features that govern changes in the microscopic organisation of the SC and hence the macroscopic endpoints of CPE activity and membrane safety. Karande et al. (2005) revealed fundamental constrains in optimising the balance between the CPE activity and the membrane safety (irritation potential, IP). They used the high-throughput screening method for evaluation of conductivity enhancement ratio (ER). In the absence of a drug, the use of conductivity ER provides an independent evaluation of enhancement activity. To understand the EP/IP relations, the morphological changes in the skin microenvironment in the presence of CPEs using Fourier transform infrared spectroscopy were explored. The most important contribution of this study is uncovering the fundamental mechanisms and defining the potency and irritation of CPEs (Karande and Mitragotri 2002). The authors applied the theory that CPEs can be divided into two categories: ones that extract the lipids from the SC, labelled "extractors", and ones that partition into the SC lipid bilayers exhibiting a fluidizing effect on the lipid bilayers. They also assumed that the irritation response (IR) of CPEs correlated with the denaturation of the SC proteins. For all of the used CPEs by means of chemical software physico-chemical parameters (molecular descriptors) such as lipophilicity (log *P*), components of solubility parameters related to *H*-bonding, polarity and dispersion were determined. After extensive experiments and calculations, they found that the fluidization potential

of CPEs correlated with their hydrophobicity quantified in terms of log *P* and the extraction potential of CPEs correlated with the ratio of the *H*-bonding component of solubility parameter to the square root of cohesive energy density. Cohesive energy density is the sum of squares of polar, dispersive and *H*-bonding components of the solubility parameter. Finally, it can be assumed that the IP of CPEs correlated with the ratio of *H*-bonding to polarity for extractors and fluidizers. Using this approach, it was found that enhancing activity and irritation are tied together for Azone®-like compounds. Molecular interactions detectable by FT-IR spectrometry can be used to explain that the forces responsible for Azone® analogue's activity and irritation are both proportional to *H*-bonding, which leads to a stronger tie between irritation and potency for these molecules. The irritation behaviour of CPEs is related to the ratio of *H*-bonding to polar interactions. *H*-Bonds are of significant importance in holding the proteins in their native structures. Competitive *H*-bonding from CPEs can potentially change the native *H*-bonding in proteins leading to their unfolding. Consequently, IP scales directly with *H*-bonding ability. Polar interactions scale inversely with the hydrophobicity of a molecule. Hydrophobic molecules may promote partitioning of CPEs in the hydrophobic protein core, resulting in the loss of structural conformations and, hence, the inverse dependence of IP on polarity (Karande et al. 2005).

Due to a large range of structural differences and biological testing models, it is not easy to find a single structure–activity relationship (SAR). Nevertheless, some trends based on SAR may be found, and the most important facts are mentioned below. The individual modifications are again classified into modifications (i) in the head group region and (ii) in the region of chains.

6.3.1 SAR of Polar Head

The absence of the ring, i.e. the polar head, is not investigated in this chapter. Ring opening and subsequent substitution of the amide fragment bring completely different physico-chemical properties and thus the different behaviours of open-ring Azone® derivatives at interaction with skin components as well as drugs (permeants).

Generally, the seven- and five-membered cycles expressed similar enhancement activities. δ-Lactams showed the least effect; nevertheless, the change of δ-lactam to piperidine, morpholine, thiomorpholine or piperazine increased enhancement activity to the level of γ-lactams and ε-lactams.

The nitrogen atom must be present in the ring. Its isosteric replacement by carbon or oxygen causes loss of effect (compare **94/101**, **212/234** or the whole series of dioxolanes **292**–**303**). The isosteric replacement of another carbon in the ring by nitrogen (4-imidazolin-2-ones), by oxygen (1,4-oxazepan or morpholine) or by sulfur (thiomorpholine) does not cause a decrease of activity, in some cases even leads to higher activity (see **102/132**). This replacement should not be performed closer than in the β- or γ-position from the nitrogen in the ring.

It was described above that the ability to create *H*-bonds with ceramides seems to be very important for good enhancement activity. The carbonyl moiety in the polar head of Azone® analogues modifies/disrupts *H*-bonds between ceramides and creates a "channel" between the enhancer and non-bonded ceramides (Hadgraft et al. 1996). Therefore, all active Azone®-like compounds possess an *H*-bonding moiety in their polar head. It seems that only one carbonyl moiety is really needed for enhancement activity, whereas one ring-carbonyl moiety is optimal in the α-position from the nitrogen in the ring. Only for seven-membered rings it was discovered that the effect remains unchanged when the carbonyl is eliminated and amines are generated (see **1/61**). At six- and five-membered rings, enhancement effect decreases (compare, e.g. **94/102**, **94/132** or **212/235**). The move of the carbonyl moiety from the α-position of the cycle to the α-position of the chain does not cause any loss of activity, e.g. **1/66**, **94/133**, **212/233**. Substitution by the second carbonyl or carboxylic moiety of the seven- and five-membered rings at any positions does not have effect on enhancement activity (compare **1** with **56**–**58** or **212** with **248**,**252**,**269**–**271**, **275**, **276**). The second carbonyl/carboxylic moiety of six-membered rings causes a decrease of activity (compare **94** or **132**

with **136–139**). Substitution by the third carbonyl or carboxylic moiety causes total loss of enhancement activity (see **304–310**).

According to Hadgraft's theory, the second carbonyl group in the polar head, i.e. the second carbonyl oxygen, can interact with the hydroxyl moiety of surrounding ceramides and the enhancer becomes a retardant – two *H*-bonding groups on two sides could condense the lamella by cross-linking to ceramides on both sides (Hadgraft et al. 1996). On the other hand, many excellently effective CPEs with two carbonyl moieties situated on both sides of the polar head were described, see, e.g. **57**, **269–271**, **275**, **276**, **328–333**. Here, the conformation of the ring and the spatial proximity of carbonyl with the ceramide hydroxyl moiety are likely to be important.

The isosteric replacement of carbonyl oxygen by sulfur (thiocarbonyl) leads to a decrease of activity (compare **1/50**, **94/100**, **133/134**, **212/232**), probably due to a weak binding strength, and so thio-analogues of Azone® are less efficient at displacing of ceramides (Hadgraft et al. 1996).

The importance of *H*-bonds was confirmed in the work of Karande et al. (2005) (*H*-bonding interactions in CPEs originate from highly electronegative atoms such as *S*, *N* and *O* and compete with water-mediated intermolecular *H*-bonding between the lipid molecules that is responsible for the structural stability of bilayer lamellae). It was observed that the interaction of CPEs between ceramides varies inversely with the square root of cohesive energy density, which is determined by a combination of dispersive interactions arising from temporary induced dipoles, polar interactions arising from dipole–dipole interactions and *H*-bonding interactions. At large values of energy density, the CPE molecules are unlikely to participate in the solvation of lipids (Karande et al. 2005).

It has not been sufficiently supported by biologically data, whether an introduction of double bond/bonds to the ring or additional substitution (another alkyl, aryl, acyl) of the ring by some of the above-discussed groups influences enhancement effect.

Enhancement-effective mutual analogues (twin molecules) contain in their structure two from the above-described "standard" polar heads that are *N*-linked with the aliphatic chain, see, e.g. **311**, **314**, **326** and **327**. It is worth of mentioning that *N*-substituted 7-(2-oxopyrrolidin-1-yl)azepan-2-ones **328**-**333** having a large polar head (two polar heads in the same molecule) did not demonstrate a loss of activity. Nevertheless, according to the above-mentioned statement dealing with the presence of the third carbonyl/carboxylic group, additional substitution of azepanone nitrogen by acetic acid esters (**334–339**) caused again a decrease of enhancement activity.

6.3.2 SAR of Chain

The position of an enhancer in the lamellar structure depends on the balance between its hydrophilic and lipophilic natures. It was shown that Azone® analogues with six carbons in the hydrophobic chain were intercalated in the ceramide bilayer, but the hexyl analogue caused no disordering of the lipid structure. Compounds with small hydrocarbon chains (C_1-C_5), i.e. with low lipophilicity, might be expected to be situated well in the polar region (Hadgraft et al. 1996; Bouwstra et al. 1992) and possess especially properties of NMF, i.e. moisten the skin (show emollient properties) (Speir and Pascher 1956; Godwin et al. 1997; Harding and Scott 2002). Below, some chain-modification relationships to the above-mentioned Azone® analogues are discussed (Jampílek and Brychtová 2012).

Within individual series of Azone®-like compounds in unbranched saturated chains, a parabolic relationship between the activity and the carbon number may be found, see, e.g. compounds **1–11**, **47–50**, **59–64**, **90–95**, **204–212** and **328–333**. The optimum chain length is between C_8 and C_{14} (mostly from C_{10} to C_{12}), which is approximately one half of the length of ceramides and fatty acids in the SC. Incorporation of a compound with a shorter chain would produce a free space within the lamellae; this space would probably be filled by the neighbouring chains, thereby producing lateral fluidization of the tightly packed lamellar lipids. These compounds could also form separate phases within the lipid lamellae, providing a more permeable shortcut for penetrating compounds. It has also been suggested that the chain length of such an enhancer

corresponds to the length of the cholesterol molecule, and, consequently, the enhancer may disrupt the interaction between ceramide and cholesterol or between two cholesterol molecules (Brain 1993; Kanikkannan et al. 2000). In the case that a six- or five-membered head-based enhancer contains more than one polar moiety in its structure, e.g. two carbonyl groups, alkyl chain should be elongated to C_{14} or C_{16}, e.g. **110**, **119**, **120**, **225**, **252**, **253** and **272–277**. When alkyl is used as a linker between two polar heads in the twin molecules, C_3 or C_6 was observed as an optimal length (see **311**, **314**, **326**, **327**).

An introduction of double bond causes a slight decrease of enhancement effect (decrease of lipophilicity); therefore, in comparison with saturated chains, the most active unsaturated derivatives possess mostly longer chain with an optimum of around C_{18} to C_{20} (see, e.g. **14**, **15**, **24–34**, **69–81**, **155–170**, **171**, **172** and **229**). According to the patented molecules, it can be stated that the second double bond may increase the enhancing activity (e.g. **270**), while a greater degree of unsaturation has no further effect. Within Azone® analogues, it has not been sufficiently supported by biologically data whether *cis*-isomers differ significantly from *trans-isomers* in enhancement activity; nevertheless, *cis*-isomer of compound **277** showed enhancement effect comparable with Azone® (**1**).

As concerns the branched chain, it may be assumed that the relatively small degree of branching and/or branching only by a methyl moiety can show a positive enhancement of penetration activity (see compounds **12–16**, **173**, **174**, **289**, **326**, **327**). Substitution by cycloalkane, phenyl, benzyl or other non-aliphatic chain causes a decrease or loss of activity. Compounds substituted with the hydroxyl moiety, e.g. **17–19**, **96–98**, **213–215**, were prepared and patented as potential CPEs; nevertheless, based on the sources it is not possible to make a serious decision about the influence of these hydroxyl moieties on enhancement effect.

Similarly, sufficient quantity of biological results has not been available to make a decision on influence of the isosteric replacement of carbon in the aliphatic chain by another atom; nevertheless, it seems that enhancement effect is not affected (see **121–130**, **216**, **260–264**).

A number of Azone® analogues possess an incorporated carbonyl moiety in the aliphatic chain (in fact ketone-like molecules or esters/amides). These structure changes play an important role in the enhancement effect of potential CPEs. When another carbonyl moiety is introduced, several cases should be distinguished. If the second carbonyl oxygen is introduced to the $C_{(1)}$ or $C_{(2)}$ position of the chain, i.e. α- or β-position, the lactam structure being retained as a polar head, the activity decreases (e.g. **103**, **236**) with the exception of compound **23**. If carbonyl oxygen is introduced to the $C_{(2)}$ position (the $C_{(1)}$ position can be branched or unbranched), the activity is mostly preserved or increased. When CPEs based on the structure of α-(di)substituted acetic acid (or ester) are considered, this modification causes a prolongation of the chain by three atoms, as was discussed above, and does not have any influence on significantly higher enhancement activity compared to non-ester compounds, with exception of compounds **45** and **46**; for example, compare **1** with **42** or **90–95** with **104–110** or **210–212** with **221–225**. If the second carbonyl oxygen is introduced to the $C_{(1)}$ or $C_{(2)}$ position of the chain, or α-(di)substituted acetic acid (or ester) is prepared, but with simultaneous removal of the carbonyl oxygen from the polar head (e.g. reverse analogues are generated, i.e. lactam is transformed to cyclic amine), the activity can be preserved (see compounds **66**, **99**, **133**, **140–158**, **171–173**, **177–181**, **233**). Isosteric replacement of oxygen by sulfur and formation of thiocarbonyl in the $C_{(1)}$ position of the chain (when the carbonyl group is eliminated from the polar head) have negative effect on enhancement activity (see **133** and **134**). If a ring already contains two carbonyl/carboxylic moieties, and the third similar group is incorporated into the chain, a total loss of effect is observed, or potential CPEs are transformed to penetration-inhibiting agents (compare **241–250** and **269–271** with **304–310**).

This decrease of enhancement effect is again caused probably due to the formation of *H*-bonds between carbonyl oxygen in Azone®-like compounds and ceramide hydroxyl, as was discussed above (Bonina et al. 2003). More than two carbonyl/carboxylic groups, sterically and confor-

mally unblocked, create an *H*-bond with the adjacent ceramide polar head group "on the second side of CPE", i.e. *H*-bonds are created on both sides of the CPE, and ceramide-enhancer cross-linking is de facto formed; therefore, paradoxically, penetration through the SC is inhibited (Hadgraft et al. 1996).

Conclusion

Azone® analogues were classified according to their structure in terms of the approach of medicinal chemistry. Some QSAR approaches and their results were briefly mentioned as well. General SAR for the design of enhancing-effective Azone®-like molecules can be the following: ε- or γ-lactams as a head group and alkyl chain involving approximately 12 or (in case the ester group is inserted) 16 members, lipophilicity approx. 6, an unbranched (low branched) chain and low polar surface area (PSA approx. 20 or 40–47), i.e. a limited number of polar atoms and molecular volume till 89 cm^3/mol.

It was found that with high probability, Azone® analogues represent compounds that show direct correlation between enhancement effect and irritation potential, and therefore it can be stated that the most active Azone®-like compounds described herein possess optimal irritation potential at maximum enhancing activity, and it is doubtful that any new CPEs within this group with better enhancement effect and irritation potential ratio could be found. Based on these facts, it can be finally concluded that the family of Azone®-like compounds will not probably provide any compound with significant enhancement activity.

Acknowledgment This study was supported by the Grant Agency of the Czech Republic (Czech Science Foundation): project number GAČR P304/11/2246.

References

Addy M, Moran J, Newcombe RG (2007) Meta-analyses of studies of 0.2% delmopinol mouth rinse as an adjunct to gingival health and plaque control measures. J Clin Periodontol 34:58–65

Afouna MI, Fincher TK, Zaghloul AAA, Reddy IK (2003) Effect of Azone upon the in vivo antiviral efficacy of cidofovir or acyclovir topical formulations in treatment/prevention of cutaneous HSV-1 infections and its correlation with skin target site free drug concentration in hairless mice. Int J Pharm 253:159–168

Alexander J, Higuchi T, inventors; Merck & Co., Inc., assignees (1989) Pyroglutamic acid esters used as dermal penetration enhancers for drugs. United States patent US 4847250

Aoyagi T, Tadenuma R, Nagase Y (1996) Novel silicones for transdermal therapeutic system. 6. Preparation of oligodimethylsiloxane containing 2-pyrrolidone moiety as a terminal group and its enhancing effect on transdermal drug penetration. Macromol Chem Phys 197:677–686

Baker EJ, Hadgraft J (1995) In vitro percutaneous absorption of arildone, a highly lipophilic drug, and the apparent no-effect of the penetration enhancer Azone in excised human skin. Pharm Res 12:993–997

Bando H, Sahashi M, Mohri S, Yamashita F, Takakura Y, Hashida M (1996) In vivo skin penetration enhancement of acyclovir by theoretical design of prodrug-enhancer combination. Int J Pharm 145:103–113

Barry BW (1987) Mode of action of penetration enhancers in human skin. J Control Release 6:85–97

Barry BW (2004) Breaching the skin's barrier to drugs. Nat Biotechnol 22:165–167

Bernardo FP, Saraiva PM (2008) A theoretical model for transdermal drug delivery from emulsions and its dependence upon formulation. J Pharm Sci 97:3781–3809

Bonina F, Santagati NA, Puglia C (2003) Ketoprofen 1-alkylazacycloalkan-2-one esters as dermal prodrugs: In vivo and in vitro evaluations. Drug Dev Ind Pharm 29:181–190

Bouwstra JA, Gooris GS, Brussee J, Salomons-de Vries MA, Bras W (1992) The influence of alkyl-azones on the ordering of the lamellae in human stratum corneum. Int J Pharm 79:141–148

Brain KA (1993) Walters KA. In: Walters KA, Hadgraft J (eds) Pharmaceutical skin penetration enhancement. Marcel Dekker, New York, pp 389–416

Breitenbach J, Lang S, Kratz D, inventors; BASF, assignees (2002) 1,3-bis-(N-Lactamyl)propane and the pharmaceutical and cosmetic use thereof. United States patent US 6497886

Brychtová K, Jampílek J, Opatřilová R, Raich I, Farsa O, Csőllei J (2010a) Synthesis, physico-chemical properties and penetration activity of alkyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl) hexanoates as potential transdermal penetration enhancers. Bioorg Med Chem 18:73–79

Brychtová K, Opatřilová R, Raich I, Kalinowski D, Dvořáková L, Plaček L et al (2010b) Investigating the activity of 2-substituted alkyl-6-(2,5-dioxopyrrolidin-1-yl)hexanoates as skin penetration enhancers. Bioorg Med Chem 18:8556–8565

Brychtová K, Dvořáková L, Opatřilová R, Raich I, Káčerová S, Plaček L et al (2012) Investigation of sub-

stituted 6-aminohexanoates as skin penetration enhancers. Bioorg Med Chem 20:86–5
Chamber O, Thelin B, inventors; Sinclair Pharmaceuticals Ltd. assignees (2008) Morpholines and piperidines as transdermal and transmucosal drug delivery enhancers. WO 2008139170 A2
Cooper EG, inventors; The Procter & Gamble Company, assignees (1985) Penetrating topical pharmaceutical compositions containing 1-dodecyl-azacycloheptan-2-one. United States patent US 5447934
Degim IT (2006) New tools and approaches for predicting skin permeability. Drug Discov Today 11:517–523
Drakulic BJ, Juranic IO, Eric S, Zloh M (2008) Role of complexes formation between drugs and penetration enhancers in transdermal delivery. Int J Pharm 363:40–49
Ertl P (2007) Polar Surface Area. In: Mannhold R (ed) Molecular drug properties. Wiley-VCH, Weinheim, pp 111–126
Feldkamp JR, Koenig DW, Wenzel SW, Joseph WR, inventors; Kimberly-clark Worldwide, Inc., assignees (2008) Transdermal delivery of oleocanthal for relief of inflammation. WO 2008001240 A2
Fincher TK, Yoo SD, Player MR, Sowell JW, Michniak B (1996) In vitro evaluation of a series of N-dodecanoyl-L-amino acid methyl esters as dermal penetration enhancers. J Pharm Sci 85:920–923
Furuishi T, Oda S, Saito H, Fukami T, Suzuki T, Tomono K (2007) Effect of permeation enhancers on the in vitro percutaneous absorption of pentazocine. Biol Pharm Bull 30:1350–1353
Godwin DA, Michniak BB, Player MR, Sowell JW (1997) Transdermal and dermal enhancing activity of pyrrolidinones in hairless mouse skin. Int J Pharm 155:241–250
Groundwater PW, Hadgraft J, Harrison JE, Watkinson AC (1994) The synthesis of perdeuterated Azone (d_{35}-1-dodecylhexahydro-2H-azepin-2-one). J Labelled CompRadiopharm 34:1047–1053
Hadgraft J (2004) Skin deep. Eur J Pharm Biopharm 58:291–299
Hadgraft J, Pugh WJ (1998) The selection and design of topical and transdermal agents. A review. J Investig Dermatol Symp Proc 3:131–135
Hadgraft J, Peck J, Williams DG, Pugh WJ, Allan G (1996) Mechanisms of action of skin penetration enhancers/retarders: Azone and analogs. Int J Pharm 141:17–25
Harding CR, Scott IR (2002) Stratum corneum moisturizing factors. In: Leyden JJ, Rawling AW (eds) Skin moisturization. Informa Health Care, London, pp 61–80
Hatanaka T, Shimoyama M, Sugibayashi K, Morimoto Y (1993) Effect of vehicle on the skin permeability of drugs: polyethylene glycol 400-water and ethanol-water binary solvents. J Control Release 23:247–260
Higuchi T, Higuchi KW, Wong, O. inventors; IPRX Inc., assignees (1989) Imidazolin-2-ones. United States patent US 4845233
Hoogstraate AJ, Verhoef J, Brussee J, Ijzerman AP, Spies F, Bodde HE (1991) Kinetics, ultrastructural aspects and molecular modeling of transdermal peptide flux enhancement by N-alkylazacycloheptanones. Int J Pharm 76:37–47
Hou SY, Flynn GL (1997) Influences of 1-dodecylazacycloheptan-2-one on permeation of membranes by weak electrolytes. 1. Theoretical analysis of weak electrolyte diffusion through membranes and studies involving silicone rubber membranes. J Pharm Sci 86:85–91
Huang B, Gu MN, Li GF, Lin CS, Guo D (2002) Experimental study of in vitro buprenorphine hydrochloride transdermal permeation through hairless mouse skin. Di Yi Jun Yi Da Xue Xue Bao 22:895–897
Iyer M, Mishra R, Han Y, Hopfinger AJ (2002) Predicting blood-brain barrier partitioning of organic molecules using membrane-interaction QSAR analysis. Pharm Res 19:1611–1621
Iyer M, Zheng T, Hopfinger AJ, Tseng YJ (2007) QSAR analyses of skin penetration enhancers. J Chem Inf Model 47:1130–1149
Jackson M, Johnston DS, Chapman D (1988) Differential scanning calorimetric and Fourier transform infrared spectroscopic investigations of cerebroside polymorphism. Biochim Biophys Acta 944:497–506
Jampílek J, Brychtová K (2012) Azone analogues: classification, design, and transdermal penetration principles. Med Res Rev 32:907–947
Kang L, Yap CW, Lim PFC, Chen YZ, Ho PC, Chan YW et al (2007) Formulation development of transdermal dosage forms: quantitative structure–activity relationship model for predicting activities of terpenes that enhance drug penetration through human skin. J Control Release 120:211–219
Kanikkannan N, Kandimalla K, Lamba SS, Singh M (2000) Structure-activity relationship of chemical penetration enhancers in transdermal drug delivery. Curr Med Chem 7:593–608
Karande P, Mitragotri S (2002) High throughput screening of transdermal formulations. Pharm Res 19: 655–660
Karande P, Jain A, Ergun K, Kispersky V, Mitragotri S (2005) Design principles of chemical penetration enhancers for transdermal drug delivery. Proc Natl Acad Sci U S A 102:4688–4693
Karande P, Jain A, Mitragotri S (2006) Insights into synergistic interactions in binary mixtures of chemical permeation enhancers for transdermal drug delivery. J Control Release 115:85–93
Karande P, Jain A, Arora A, Ho MJ, Mitragotri S (2007) Synergistic effects of chemical enhancers on skin permeability: a case study of sodium lauroylsarcosinate and sorbitan monolaurate. Eur J Pharm Sci 31:1–7
Katritzky AR, Dobchev DA, Fara DC, Hur E, Tamm K, Kurunczi L et al (2006) Skin permeation rate as a function of chemical structure. J Med Chem 49:3305–3314
Kim N, El-Kattan AF, Asbill CS, Kennette RJ, Sowell JW, Latour R, Michniak BB (2001) Evaluation of derivatives of 3-(2-oxo-1-pyrrolidine)hexahydro-1H-azepin-

2-one as dermal penetration enhancers: side chain length variation and molecular modeling. J Control Release 73:183–196

Komada F, Imanidis G, Miyajima M, Okano T, Durrani MJ, Higuchi WI, Kim SW, Shannon WM, Baker DC (1991) Design of 9-beta-D-arabinofuranosyladenine (ara-A) transdermal delivery system for animal studies: regulation of drug concentration in vivo. J Pharm Sci 80:935–941

Kulkarni A, Han Y, Hopfinger AJ (2002) Predicting Caco-2 cell permeation coefficients of organic molecules using membrane interaction QSAR analysis. J Chem Inf Comput Sci 42:331–342

Lambert WJ, Kudla RJ, Holland JM, Curry JT (1993) A biodegradable transdermal penetration enhancer based on N-(2-hydroxyethyl)-2-pyrrolidone. I. Synthesis and characterization. Int J Pharm 95:181–192

Leonard MF, Kumar A, Murray DL, Beaman DC (1987) Inhibitory effect of Azone (1-dodecylazacycloheptan-2-one) on herpes simplex viruses. In vivo and in vitro studies. Chemotherapy 33: 151–156

Lewis D, Hadgraft J (1990) Mixed monolayers of dipalmitoylphosphatidylcholine with Azone or oleic acid at the air-water interface. Int J Pharm 65:211–218

López-Cervantes M, Márquez-Mejía E, Cázares-Delgadillo J, Quintanar-Guerrero D, Ganem-Quintanar A, Ángeles-Anguiano E (2006) Chemical enhancers for the absorption of substances through the skin: Laurocapram and its derivatives. Drug Dev Ind Pharm 32:267–286

Manitz R, Lucht W, Strehmel K, Weiner R, Neubert R (1998) On mathematical modeling of dermal and transdermal drug delivery. J Pharm Sci 87:873–879

Melichar B (ed) (1987) Chemical drugs, 3rd edn. Avicenum, Prague

Menon GK, Lee SH, Roberts MS (1998) Ultrastructural effects of some solvents and vehicles on the stratum corneum and other skin components: Evidence for an 'extended mosaic-partitioning model of the skin barrier'. In: Roberts MS, Walters KA (eds) Dermal absorption and toxicity assessment. Marcel Dekker, New York, pp 727–751

Michniak BB, Player MR, Chapman JM, Sowell JW (1993a) In vitro evaluation of a series of Azone analogs as dermal penetration enhancers I. Int J Pharm 91:85–93

Michniak BB, Player MR, Fuhrman LC, Christensen CA, Chapman JM, Sowell JW (1993b) In vitro evaluation of a series of Azone analogs as dermal penetration enhancers. II. (Thio)amides. Int J Pharm 94:203–210

Michniak BB, Player MR, Chapman JM Jr, Sowell JW (1994) Azone analogues as penetration enhancers: effect of different vehicles on hydrocortisone acetate skin permeation and retention. J Control Release 32:147–154

Michniak BB, Player MR, Godwin DA, Phillips CA, Sowell JW (1995a) In vitro evaluation of a series of Azone analogs as dermal penetration enhancers. IV Amines Int J Pharm 116:201–209

Michniak BB, Player MR, Godwin DA, Sowell JW (1995b) Investigation of enhancer structure activity relationships in congeners of 2-(1-nonyl)-1,3-dioxolane. Drug Deliv 2:117–122

Michniak BB, Player MR, Sowell JW (1996) Synthesis and in vitro transdermal penetration enhancing activity of lactam N-acetic acid esters. J Pharm Sci 85:150–154

Michniak BB, Player MR, Godwin DA, Lockhart CC, Sowell JW (1998) In vitro evaluation of Azone analogs as dermal penetration enhancers: V. Miscellaneous compounds. Int J Pharm 161:169–178

Minaskanian G, Peck JV, inventors; Nelson Research & Development Co., assignees (1988) Azacycloalkane, -ketone, and -thione derivatives as penetration enhancers for transdermal delivery of systemic agents. PCT Int Appl WO 88 02626 A1

Minaskanian G, Peck JV inventors; Nelson Research & Development Co., assignees (1989a) Topical compositions comprising 1-oxo- or thiohydrocarbyl-substituted azacycloalkanes as penetration enhancers. PCT Int Appl WO 8902735 A1

Minaskanian G, Peck JV inventors; Nelson Research & Development Co., assignees (1989b) Penetration enhancers for trandermal delivery of systemic agent. United States patent US 4879275

Minaskanian G, Peck JV, Nelson EL, inventors; Witby Research Inc., assignees (1991) Compositions comprising 1-substituted azacycloalkanes. United States patent US 4992422

Minaskanian G, Peck JV, inventors; Witby Research Inc., assignees (1992) Penetration enhancers for transdermal delivery of systemic agents. United States patent US 5142044

Minaskanian G, Peck JV, inventors; Witby Research Inc., assignees (1995) Penetration enhancers for transdermal delivery of systemic agents. United States patent US 5444075

Mirejovsky D, Takruri H (1986) Dermal penetration enhancement profile of hexamethylenelauramide and its homologues: In vitro versus in vivo behavior of enhancers in the penetration of hydrocortisone. J Pharm Sci 75:1089–1093

Morimoto Y, Hatanaka T, Sugibayashi K, Omiya H (1992) Prediction of skin permeability of drugs: comparison of human and hairless rat skin. J Pharm Pharmacol 44:634–639

Moser K, Kriwet K, Naik A, Kalia YN, Guy RH (2001) Passive skin penetration enhancement and its quantification in vitro. Eur J Pharm Biopharm 52:103–112

Neumann D (2008) Modeling transdermal absorption. In: Ehrhardt C, Kim KJ (eds) Drug absorption studies: In situ, in vitro and in silico models. Springer, New York, pp 459–485

Neumann D, Kohlbacher O, Merkwirth C, Lengauer T (2006) A fully computational model for predicting percutaneous drug absorption. J Chem Inf Model 46:424–429

Notman R, Anwar J. (2013) Breaching the skin barrier – Insights from molecular simulation of model membranes. Adv Drug Del Rev 65:237–250

Okamoto H, Hashida M, Sezaki H (1988) Structure-activity relationship of 1-alkyl- or 1-alkenylazacycloalkanone derivatives as percutaneous penetration enhancers. J Pharm Sci 77:418–424

Okamoto H, Muta K, Hashida M, Sezaki H (1990) Percutaneous penetration of acyclovir through excised hairless mouse and rat skin: effect of vehicle and percutaneous penetration enhancers. Pharm Res 7:64–68

Okumura M, Nakamori Y, Sugibayashi K, Morimoto Y (1991) Enhanced skin permeation of papaverine by a medium chain glyceride. Drug Des Deliv 7:147–157

Pascher I (1976) Molecular arrangements in sphingolipids. Conformation and hydrogen bonding of ceramides and their implication on membrane stability and permeability. Biochim Biophys Acta 455:433–451

Peck JV, Minaskanian G, inventors; Nelson Research & Development Co., assignees (1987) Use of azacycloalkanes as penetration enhancers for pharmaceutical and agrochemical preparations. WO 8704594 A1

Peck JV, Minaskanian G, inventors; Research Triangle Pharmaceuticals Ltd. assignees (1988) Morpholine and piperazine derivatives as penetration enhancers for transdermal delivery of systemic agents. European patent EP 268222 A2

Peck JV, Minaskanian G, inventors; Nelson Research & Development Co., assignees (1989) Penetration enhancers for transdermal delivery of systemic agents. United States patent US 4879275

Peck JV, Minaskanian G, inventors; Witby Research Inc., assignees (1995) Carboxylic acid derivatives as transdermal penetration enhancers. United States patent US 5472946

Peck JV, Minaskanian G, Sleevi MC, inventors; Durham Pharmaceuticals LLC., assignees (1997) Cyclic amides and derivatives thereof. United States patent US 5703104

Pilgram GSK, van der Meulen J, Gooris GS, Koerten HK, Bouwstra JA (2001) The influence of two azones and sebaceous lipids on the lateral organization of lipids isolated from human stratum corneum. Biochim Biophys Acta-Biomembr 1511:244–254

Pugh WJ, Wong R, Falson F, Michniak BB, Moss GP (2005) Discriminant analysis as a tool to identify compounds with potential as transdermal enhancers. J Pharm Pharmacol 57:1389–1396

Quan D, Higuchi RI, Takayama K, Higashiyama K, Nagai T (1990) Enhancing effect of piperidone derivatives on the percutaneous absorption of indomethacin. Drug Des Deliv 6:61–71

Rachakonda VK, Yerramsetty KM, Sundararajan VM, Robinson RL, Gasem KAM (2008) Screening of chemical penetration enhancers for transdermal drug delivery using electrical resistance of skin. Pharm Res 25:2697–2704

Rajadhyaksha VJ, inventors; Nelson Research & Development Company, assignees (1976a) Vehicle composition containing 1-substituted azacycloheptan-2-ones. United States patent US 3989816

Rajadhyaksha VJ, inventors; Nelson Research & Development Company, assignees (1976b) Novel N-bis-azacyclopentan-2-onyl alkanes. United States patent US 3989815

Rajadhyaksha VJ, inventors; Nelson Research & Development Company, assignees (1983) Vehicle composition containing 1-substituted azacyclononan-2-ones. United States patent US 4415563

Ren C, Fang L, Li T, Wang M, Zhao L, He Z (2008) Effect of permeation enhancers and organic acids on the skin permeation of indapamide. Int J Pharm 350:43–47

Rhee YS, Huh JY, Park CW, Nam TY, Yoon KR, Chi SC, Park ES (2007) Effects of vehicles and enhancers on transdermal delivery of clebopride. Arch Pharm Res 30:1155–1161

Rim JE, Pinsky PM, van Osdol WW (2005) Finite element modeling of coupled diffusion with partitioning in transdermal drug delivery. Ann Biomed Eng 33:1422–1438

Riviere JE, Brooks JD (2005) Predicting skin permeability from complex chemical mixtures. Toxicol Appl Pharmacol 208:99–110

Riviere JE, Brooks JD (2007) Prediction of dermal absorption from complex chemical mixtures: incorporation of vehicle effects and interactions into a QSPR framework. SAR QSAR Environ Res 18:31–44

Sasaki H, Kojima M, Mori Y, Nakamura J, Shibasaki J (1988) Enhancing effect of pyrrolidone derivatives on transdermal drug delivery I. Int J Pharm 44: 15–24

Sasaki H, Kojima M, Mori Y, Nakamura J, Shibasaki J (1991) Enhancing effect of pyrrolidone derivatives on transdermal penetration of 5-fluorouracil, triamcinolone acetonide, indomethacin and flurbiprofen. J Pharm Sci 80:533–538

Singh J, inventor; Nuvo Research Inc., assignees (2007) Transdermal drug delivery formulation comprising an organic sulfoxide, a fatty acid ester, a fatty acid, and Azone-related compound. WO 2007016766 A1

Spier HW, Pascher G (1956) Analytical and functional physiology of the skin surface. Hautarzt 7:55–60

Sugibayashi K, Hosoya K, Morimoto Y, Higuchi WI (1985) Effect of the absorption enhancer, Azone, on the transport of 5-fluorouracil across hairless rat skin. J Pharm Pharmacol 37:578–580

Sugibayashi K, Sakanoue C, Morimoto Y (1989) Utility of topical formulations of morphine hydrochloride containing Azone and N-methyl-2-pyrrolidone. Sel Cancer Ther 5:119–128

Tenjarla SN, Kasina R, Puranajoti P, Omar MS, Harris WT (1999) Synthesis and evaluation of N-acetylprolinate esters – novel skin penetration enhancers. Int J Pharm 192:147–158

Wermuth CG (ed) (2008) The practice of medicinal chemistry, 3rd edn. Academia Press/Elsevier, London

Williams AC, Barry BW (1992) Skin absorption enhancers. Crit Rev Ther Drug Carrier Syst 9:305–353

Williams AC, Barry BW (2004) Penetration enhancers. Adv Drug Deliv Rev 56:603–618

Wong O, Huntington J, Konishi R, Rytting JH, Higuchi T (1988) Unsaturated cyclic ureas as new nontoxic

biodegradable transdermal penetration enhancers I: Synthesis. J Pharm Sci 77:967–971

Wong O, Tsuzuki N, Nghiem B, Kuehnhoff J, Itoh T, Masaki K, Huntington J, Konishi R, Rytting JH, Higuchi T (1989) Int J Pharm 52:191–202

Yamashita F, Hashida M (2003) Mechanistic and empirical modeling of skin permeation of drugs. Adv Drug Deliv Rev 55:1185–1199

Yamashita F, Yoshioka T, Koyama Y, Okamoto H, Sezaki H, Hashida M (1993) Analysis of skin penetration enhancement based on a two-layer skin diffusion model with polar and nonpolar routes in the stratum corneum: dose-dependent effect of 1-geranylazacycloheptan-2-one on drugs with different lipophilicities. Biol Pharm Bull 16:690–697

Yano T, Higo N, Furukawa K, Tsuji M, Noda K, Otagiri M (1992) Evaluation of new penetration enhancer 1-[2-(decylthio)ethyl]azacyclopentan-2-one. J Pharmacobiodyn 15:527–533

Yoneto K, Ghanem AH, Higuchi WI, Peck KD, Li SK (1995) Mechanistic studies of the 1-alkyl-2-pyrrolidones as skin permeation enhancers. J Pharm Sci 84:312–317

Yoneto K, Li SK, Higuchi WI, Shimabayashi S (1998) Influence of the permeation enhancers 1-alkyl-2-pyrrolidones on permeant partitioning into the stratum corneum. J Pharm Sci 87:209–214

Zheng T, Hopfinger AJ, Esposito EX, Liu J, Tseng YJ (2008) Membrane-interaction quantitative structures-activity relationship (MI-QSAR) analyses of skin penetration enhancers. J Chem Inf Model 48:1238–1256

Aromatic Iminosulfuranes, A Novel Class of Transdermal Penetration Enhancers

7

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7.1 Introduction

The stratum corneum (SC) is the outermost layer of the skin and the major barrier to transdermal and topical drug delivery (Scheuplein and Blank 1971). Despite the development of new strategies for skin penetration enhancement in the past few decades, overcoming the skin barrier in a safe and effective way still remains a great challenge. One of the most promising and most widely investigated techniques to facilitate drug permeation through the skin is the use of chemical enhancers. An ideal enhancer would have the following properties: pharmacologically inert, chemically stable, nontoxic, nonirritant, nonallergic, and without irreversible effects on the skin (Sinha and Kaur 2000; Barry 2006). Many researchers have studied the mechanisms of action of chemical enhancers, and the following have been suggested as possible explanations for the enhancement of permeation of compounds through the SC: (a) disorganization of SC

N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_7

structure due to the interaction of the chemical enhancer with SC intercellular lipids resulting in the fluidization of the lipid environment, (b) interaction with intracellular proteins contained within the corneocytes, and (c) increasing partitioning and solubility of the drug in the SC (Barry 2006; Marjukka Suhonen et al. 1999; Williams and Barry 1991).

Dimethyl sulfoxide (DMSO) is one of the oldest and most extensively studied penetration enhancers in topical and transdermal pharmaceutical formulations (Marren 2011). Its mechanism of enhancement is believed to occur through the denaturation of proteins that results in conformational changes in the intracellular keratins (Oertel 1977) and extraction of intercellular lipids (Elfbaum 1968). Generally, high concentration of DMSO (>60 %) is required to generate enhancement activity, and at these high concentrations, it is known to cause skin irritation (Kim et al. 1999).

Moreover, studies on structure-activity relationships of several enhancers revealed that the presence of a cyclic structure plays a significant role in the penetration enhancement activity. Thus, recently Kim et al. (Kim et al. 1999) and Strekowski et al. (1999) investigated skin penetration enhancement efficacy of a group of aromatic iminosulfuranes. These classes of iminosulfuranes are isoelectronic with DMSO and possess at least one aromatic ring in their structure. Thus, they were expected to show high potency in transporting drugs through the skin while causing minimum or no skin irritation (Kim et al. 1999). In this chapter, the chemical structure of aromatic iminosulfuranes, their toxicity, their biological effectiveness as penetration enhancer, and their mechanisms of enhancement will be discussed.

7.2 Structure

Structurally, aromatic iminosulfuranes are divided into three classes. As shown in Fig. 7.1, in class I, arylsulfonyl group is attached to the nitrogen atom of the backbone structure. Class II and class III consist of derivatives of *N*-aroyliminosulfurane and *N*-aryliminosulfurane, respectively (Kim et al. 1999).

Iminosulfuranes were synthesized from the reaction of activated DMSO by trifluoroacetic anhydride with an amino derivative such as arylsulfonamide or arylamide (Sharma and Bobek 1975; Kim et al. 1999). In Fig. 7.2 the synthesis of class II iminosulfuranes is illustrated (Strekowski et al. 1999). The yield was reported to be up to 90 %, and proton nucleic magnetic resonance (^{1}H NMR) and carbon-13 NMR (^{13}C NMR) spectroscopy were performed to confirm the chemical structures. Iminosulfuranes possess a similar backbone structure to DMSO, in which the polarity of S-O bound in DMSO is modified by changing oxygen to nitrogen substitute to obtain *S,S*-dimethyl-*N*-iminosulfurane backbone structure (Kim et al. 1999; Strekowski et al. 1999).

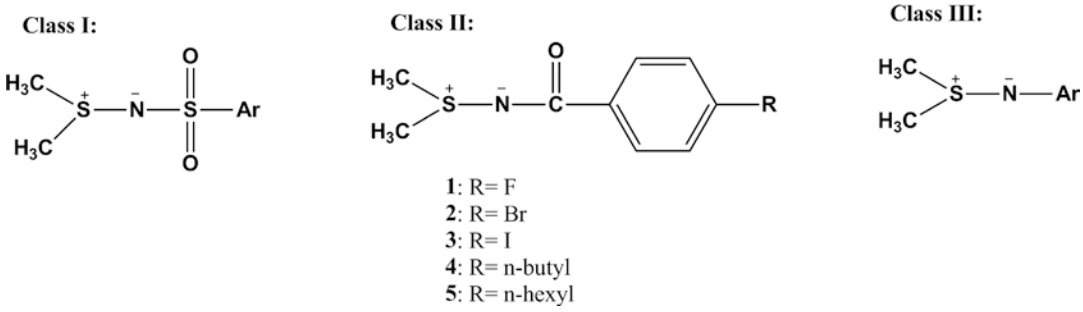

Fig. 7.1 Chemical structures of three classes of aromatic iminosulfuranes (Kim et al. 1999)

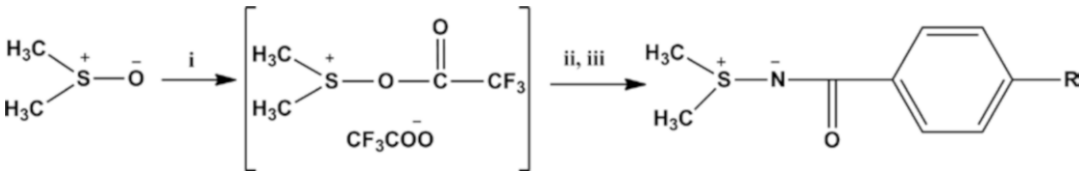

Fig. 7.2 Synthesis of second class of iminosulfuranes. (i) $(CF_3CO)_2O$ in dichloromethane was added slowly to DMSO in dichloromethane at −60 °C; (ii) 4-R-$C_4H_4CONH_2$ was added slowly to the resultant mixture and was stirred at −50 °C for 3 h; (iii) quenching with 10 % aqueous NaOH at −50 °C was followed by extraction with dichloromethane, and finally, the residue was crystallized from ether or ether/pentanes (Strekowski et al. 1999)

7.3 Penetration Enhancement Activity of Iminosulfuranes

The penetration enhancement activities of a series of iminosulfuranes were studied by evaluating the compound effects on the flux of a model drug through the skin *in vitro*. Hydrocortisone and caffeine were selected as a hydrophobic and a hydrophilic drug, respectively. In a preliminary study performed by Kim et al. (1999), penetration enhancement effects of three series (class I–III) of iminosulfuranes on hydrocortisone permeation were evaluated using hairless mouse skin. All iminosulfuranes were applied to skin at 0.4 M in propylene glycol to ensure their maximum thermodynamic activity. A saturated suspension of model drug (e.g., hydrocortisone) in propylene glycol was applied after pretreatment of skin with iminosulfuranes. Among the three series of compounds tested, *N*-(4-bromobenzoyl)-*S,S*-dimethyliminosulfurane (compound **2**) in *N*-aroyliminosulfuranes (class II compounds) showed statistically significant activity-enhancing hydrocortisone permeation through mouse skin compared to control (propylene glycol). The amount of drug permeating through the mouse skin in 24 h (Q_{24}) was 996.2 ± 192.5 μg/cm^2, and flux value (J) was 42.9 ± 7.5 μg/cm^2/h. This finding was consistent with another study that was performed on human cadaver skin in which compound **2** showed significant enhancement effect as well as highest Q_{24} and J for hydrocortisone among several analogs tested (Fig. 7.3) (Song et al. 2005). Compound **2** also showed significantly lower lag time (T_{lag}) compared to control. Moreover, the penetration enhancement effect of compound **2** on hydrocortisone and caffeine was shown to be effective at a lower concentration of 0.2 M, which was the nontoxic concentration obtained from cytotoxicity assays on fibroblasts and keratinocytes (Song et al. 2005). Additionally, the enhancement effect of compound **2** was found to be higher with the hydrophobic drug than with the hydrophilic drug. The mechanism of enhanced drug permeation was suggested to result from an increase in partition coefficient and solvent effect from the use of the enhancer. The partition coefficient of hydrocortisone and caffeine was increased by 5.4-fold and 1.4-fold, respectively, in the presence of 0.4 M of compound **2**. The flux J can be described as (Sinko 2006):

$$J = P\left(C1 - C2\right) \text{ and } P = \frac{DK}{h}$$

where P is the permeability coefficient, D is the diffusion coefficient, K is the partition coefficient, $C1$–$C2$ is the concentration difference between the donor ($C1$) and receiver solutions ($C2$), and h is the length of permeation path. Hence, J is directly proportional to P and indirectly proportional to K, and the increase of partition coefficient for both hydrophobic and hydrophilic model drugs by compound **2** contributed to the increased flux values. Another possible explanation for the enhancement activity of compound **2** is the change in diffusivity across the skin via interaction of the enhancer with intercellular lipids in SC. T_{lag} can be described as:

$$T_{lag} = \frac{h^2}{6D}$$

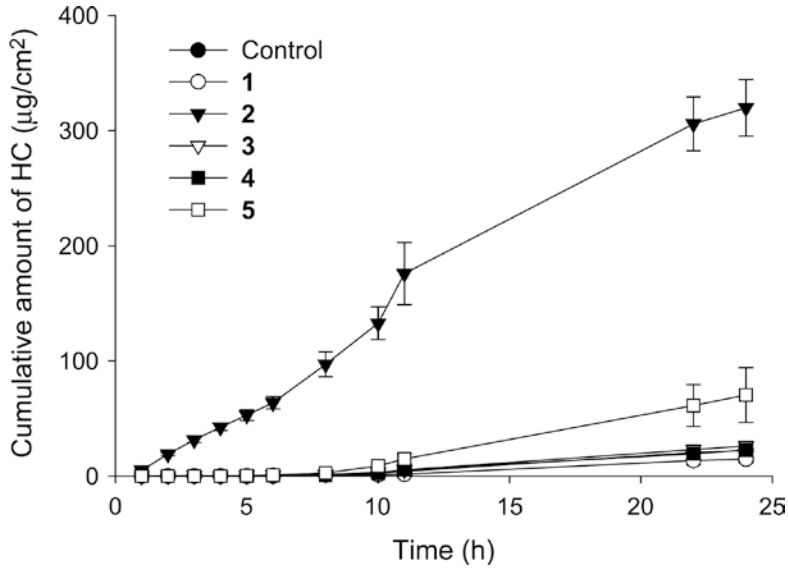

Fig. 7.3 Penetration profile of hydrocortisone through human cadaver skin treated with 0.4 M *S,S*-dimethyl-*N*-(4-R-benzoyl)iminosulfurane (class II *N*-aroyliminosulfuranes); where compound 1: R = F; 2: R = Br; 3: R = I; 4: R = n-butyl; 5: R = n-hexyl; control = propylene glycol only. Each point represents means ± standard deviation ($n = 5$) (With permission, Song et al. 2005)

where h is the length of permeation path and D is the diffusion coefficient. T_{lag} is directly proportional to h squared and inversely related to D. Since T_{lag} of hydrocortisone was decreased 7.4-fold by 0.4 M compound **2**, it was suggested that the enhancer also affected membrane fluidity and this resulted in the decrease of T_{lag}.

In conclusion, these findings suggest that compound **2** acts as a potent enhancer for the transdermal delivery of both hydrophobic and hydrophilic model drugs.

7.4 Cytotoxicity of Iminosulfuranes

An ideal penetration enhancer should reversibly reduce SC's barrier properties while not decreasing cell viability. Song et al. (2005) investigated the cytotoxicity of iminosulfuranes on normal human skin cells (fibroblasts and keratinocytes) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay to determine "safe" concentrations. Briefly, skin cells were treated with varying concentrations of iminosulfuranes in 1 % DMSO and were incubated for 24 h at 37 °C. Then the media was replaced by the MTT solution and incubated for 3 h before measuring absorbance at 595 nm. Cells that were treated with 1 % DMSO were used as a control. Cytotoxicity of iminosulfuranes was found to be concentration dependent in both fibroblasts and keratinocytes. The concentration range that resulted in 50 % cell viability (IC_{50}) was 0.7–0.9 M and there was no statistically significant decrease of cell viability below 0.2 M for *S,S*-dimethyl-*N*-(4-fluorobenzoyl) iminosulfurane (compound **1**), compound **2**, *S,S-dimethyl-N*-(4-iodobenzoyl)iminosulfurane (compound **3**), and *S,S*-dimethyl-*N*-(4-n-hexylbenzoyl) iminosulfurane (compound **5**). Therefore, it was suggested that iminosulfuranes were relatively nonirritant for application to skin at concentrations below 0.2 M.

7.5 Mechanistic Studies Using Differential Scanning Calorimetry, Nuclear Overhauser Effect Spectroscopy, and Nuclear Magnetic Resonance

Possible correlations of the biological effectiveness of class II halogen-substituted iminosulfuranes and their interaction with lipid model systems comprising either unilamellar or multilamellar lipid bilayers were studied by Barrow et al. (2005). Various techniques such as differential

scanning calorimetry (DSC), nuclear magnetic resonance (NMR) spectroscopy, and nuclear overhauser effect spectroscopy (NOESY) were used for investigating the mechanism of partitioning and interaction of iminosulfuranes with L-α-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC).

Calorimetry is a method that allows us to establish a correlation between temperature and physical properties of materials. DSC measures the changes of physical properties of a sample plotted as temperature versus time (Haines et al. 1998). Multilamellar vesicles of DMPC containing 10 mol% or 1 mol% iminosulfurane were used for DSC studies. Class II halogen-substituted iminosulfuranes each affected in varying degrees the gel-to-liquid crystal phase transition of DMPC. Compound **2** that had showed the higher enhancement activity in skin permeation studies caused the greatest perturbation of the gel-to-liquid crystal phase transition of DMPC and decreased the phase transition temperature (T_m) and therefore, was found to destabilize DMPC bilayer. The T_m values of DMPC vesicles containing 10 mol% halogen-substituted iminosulfuranes were reduced compared to that of the control DMPC preparations without iminosufurane (Barrow et al. 2005).

NMR spectroscopy is another analytical technique that takes advantage of the magnetic properties of atomic nuclei to identify physicochemical properties such as structure, dynamics, reaction state, and the environment in which the atoms and molecules exist (Ohno et al. 2011). NOESY is a type of two-dimensional NMR that is used to detect spins undergoing cross-relaxation (Martin and Zektzer 1988). The NMR spectroscopy and NOESY studies of Barrow et al. showed strong cross-peaks from the interaction of the phenyl protons of both bromo and iodo derivatives with the fatty acid methylene and choline N-methyl protons of DMPC. Cross-peaks from the phenyl protons of the chloro derivative to the DMPC protons were much weaker than those observed in the spectrum of DMPC vesicles with compound **2** (DMPC-2), suggesting less penetration of chloro derivative into the lipid layers. Strong cross-peaks between the phenyl protons of compound **2**/compound **3** and those of choline N-methyl groups of DMPC and also cross-peak to the acyl terminal methyl group observed only in DMPC-2 spectrum indicated a deeper penetration of compound **2** into the DMPC bilayer. There were no detectable cross-peaks in the spectrum of DMPC vesicles with 10 mol% *S,S-dimethyl-N*-(benzoyl) iminosulfurane (non-halogen-substituted iminosulfurane), nor in the spectrum of DMPC with 1 mol% of compound **2** or **3** (Barrow et al. 2005).

Additionally, quantitative structure-activity relationship studies of the iminosulfuranes showed that hydrophobicity of these compounds and the number of hydrogen bonds found in their molecules have a positive correlation to their enhancement activity. The maximum enhancement efficacy was also controlled by a range of optimal molecular size (Song et al. 2005).

7.6 Cutaneous Biotransformation of the Bromo Derivative of Iminosulfuranes and Its Intermediate Product

It has been found that conversion of compound **2** to its intermediate product, 4-bromobenzamide (Fig. 7.4), occurs rapidly in physiologic buffer, and even faster in skin tissue. In order to understand the mechanism of penetration enhancement of compound **2** and 4-bromobenzamide, their metabolic elimination was investigated in presence of skin from various species. Briefly, skin pieces from mouse rat and pig were mixed with phosphate buffer saline (PBS) with pH 4, 5, 6, or 7.4 and were incubated at 37° in presence of compound **2**. In most of the cases, 70–80 % decrease in the compound concentration was observed in 10 min followed by a slow decline of 0.35–0.50 μg/h (Sintov et al. 2009).

To evaluate whether the metabolism of compound **2** is paralleled with enhancement activity, Sintov et al. used iodine and alkylating agents, such as N-ethylmaleimide (NEM) and iodoacetic acid (IAA) to inhibit their elimination (Sintov et al. 2009). A concentrated solution of iodine in ethanol, water, and propylene glycol was prepared

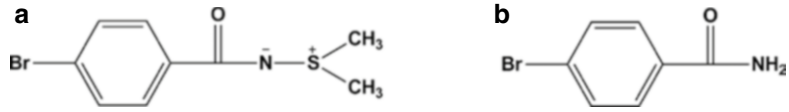

Fig. 7.4 The chemical structure of (**a**) N-(4-bromobenzoyl)-S,S-dimethyliminosulfurane and (**b**) 4-bromobenzamide (Sintov et al. 2009)

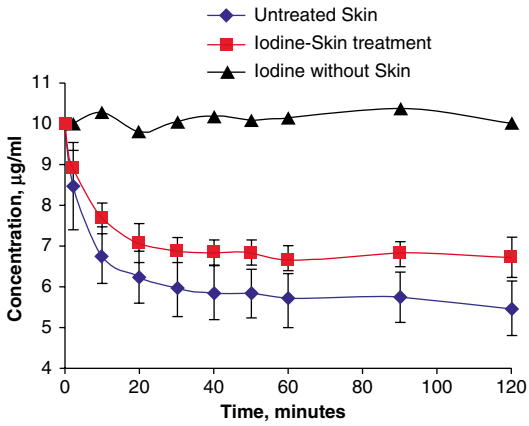

Fig. 7.5 *In vitro* elimination profiles of 4-bromobenzamide using pig skin, with and without iodine pretreatment of the skin ($n = 7$) (With permission, Sintov et al. 2009)

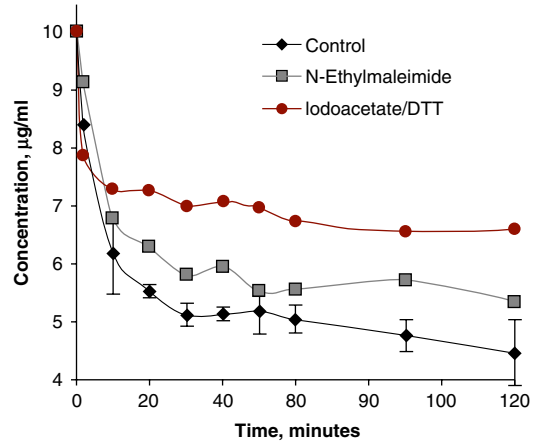

Fig. 7.6 *In vitro* metabolism of 4-bromobenzamide in the presence of rat skin. The kinetic profile of 4-bromobenzamide elimination is illustrated in presence of iodoacetate/dithiothreitol and N-ethylmaleimide (With permission, Sintov et al. 2009)

and added to the skin PBS system and allowed to incubate at 37 °C. Iodine is a mild oxidant and can change activity of proteins by oxidizing the free thiol groups in cysteinyl residues (Trundle and Cunningham 1969). Figure 7.5 depicts the influence of iodine on the metabolism of 4-bromobenzamide in the presence of pig skin. Since iodine was unable to completely inhibit biotransformation of 4-bromobenzamide, it is believed that this chemical is involved in certain steps of enzymatic process but not all. In order to confirm this conclusion, I_2/KI solution was applied on the surface of the rat skin mounted between vertical Franz diffusion cell donor and receptor compartment. The receptor compartment was filled with PBS pH 7.4 and maintained at 37 °C. After 2 h of iodine application, receptor media was replaced with 10 µg/ml 4-bromobenzamide in PBS. HPLC analysis of samples withdrawn from receptor compartment at different time points determined that metabolism of 4-bromobenzamide was significantly decreased (30 %) in iodine-treated skin.

The inhibition mechanism of iodine on 4-bromobenzamide metabolism was believed to be related to reaction of iodine with cysteinyl residues of the metabolic enzyme; therefore, Sintov et al. (2009) tested whether covalent binding of alkylating agents with free sulfhydryls could be a more effective way to block the enzyme. Iodoacetic acid (IAA) and N-ethylmaleimide (NEM) are two S-alkylating agents which are known to react with thiol groups of cysteinyl residues. The kinetic profile of 4-bromobenzamide in the presence of alkylating agents is demonstrated in Fig. 7.6. Iodoacetate/dithiothreitol (IAA/DTT) and NEM were separately added to the reaction mixture of 4-bromobenzamide and rat skin in PBS, and a significant inhibition of the metabolism was observed. A greater inhibition of enzymatic reactions was obtained with IAA/DTT than NEM. This may be a consequence of using DTT, which can enhance the availability of thiol groups

to S-carboxymethylation by reducing existing disulfide bonds to free thiols (Sintov et al. 2009; Zoeller et al. 1997).

Conclusion

The search for an effective and marketable skin penetration enhancer with "ideal" properties still continues. The relatively high chemical stability and low toxicity of the aromatic iminosulfuranes make them good candidates for being used as enhancer compounds. Due to their similar chemical structure with that of DMSO, they were synthesized and tested by the Michniak research group as percutaneous penetration enhancers. From all three classes of iminosulfuranes reviewed in this chapter, compound **2**, classified in the second class of iminosulfuranes, stands out with respect to its high enhancer effects and its high interaction with the lipid bilayers in the skin. A mechanism of enhancement was investigated for compound **2**, in which this nontoxic and potent enhancer was activated by its own metabolism in skin tissue, and 4-bromobenzamide played a major role in generating the high penetration enhancement effect.

For the first time, in this series of studies by Michniak et al. and collaborative groups, we introduce the concept of the "pro-enhancer" – an equivalent term for "prodrug." The positive data suggest that we should continue the studies of novel compounds which may be good but not excellent "enhancers" themselves but, once applied to the skin, are transformed by enzymes within the skin layers to increase the degree of transdermal drug delivery enhancement.

References

Barrow DJ Jr, Chandrasekaran S, Heerklotz HH, Henary MM, Michniak BB, Nguyen PM et al (2005) Mechanistic studies on percutaneous penetration enhancement by N-(4-halobenzoyl)-S,S-dimethyliminosulfuranes. J Lipid Res 46(10):2192–2201

Barry BW, editor (2006) Penetration enhancer classification. In: Percutaneous penetration enhancers, 2nd edn. Taylor and Francis, Boca Raton

Elfbaum SG (1968) The effect of dimethyl sulfoxide on percutaneous absorption: a mechanistic study. Part II. J Soc Cosmetic Chem 19:841–847

Haines PJ, Reading M, Wilburn FW (eds) (1998). Differential thermal analysis and differential scanning calorimetry. In: Handbook of thermal analysis and calorimetry. Elsevier Science, Amsterdam

Kim N, El-Khalili M, Henary MM, Strekowski L, Michniak BB (1999) Percutaneous penetration enhancement activity of aromatic S, S-dimethyliminosulfuranes. Int J Pharm 187(2):219–229

Marjukka Suhonen T, Bouwstra JA, Urtti A (1999) Chemical enhancement of percutaneous absorption in relation to stratum corneum structural alterations. J Control Release 59(2):149–161

Marren K (2011) Dimethyl sulfoxide: an effective penetration enhancer for topical administration of NSAIDs. Phys Sportsmed 39(3):75–82

Martin GE, Zektzer AS (1988) Introduction and practical considerations of two-dimentional NMR spectroscopy. Two-dimensional NMR methods for establishing molecular connectivity. A chemist's guide to experiment selection, performance, and interpretation. Wiley-VCH, New York

Oertel RP (1977) Protein conformational changes induced in human stratum corneum by organic sulfoxides: an infrared spectroscopic investigation. Biopolymers 16(10):2329–2345

Ohno A, Inomata K, Tochio H, Shirakawa M (2011) Application of NMR spectroscopy in medicinal chemistry and drug discovery. Curr Top Med Chem 11(1):68–73

Scheuplein RJ, Blank IH (1971) Permeability of the skin. Physiol Rev 51(4):702–747

Sharma RA, Bobek M (1975) Synthesis of 5-vinyluridine and 5-vinyl-2'-deoxyuridine as new pyrimidine nucleoside analogs. J Org Chem 40(16):2377–2379

Sinha VR, Kaur MP (2000) Permeation enhancers for transdermal drug delivery. Drug Dev Ind Pharm 26(11):1131–1140

Sinko PJ (2006) Martin's physical pharmacy and pharmaceutical sciences physical chemical and biopharmaceutical principles in the pharmaceutical sciences, 5th edn. Lippincott Williams & Wilkins, Philadelphia

Sintov AC, Zhang PJ, Michniak-Kohn BB (2009) Cutaneous biotransformation of N-(4-bromobenzoyl)-S,S-dimethyliminosulfurane and its product, 4-bromobenzamide, leading to percutaneous penetration enhancement of drugs: initial evidence using hydrocortisone. J Control Release 133(1):44–51

Song Y, Xiao C, Mendelsohn R, Zheng T, Strekowski L, Michniak B (2005) Investigation of iminosulfuranes as novel transdermal penetration enhancers: enhancement activity and cytotoxicity. Pharm Res 22(11):1918–1925

Strekowski L, Henary M, Kim N, Michniak BB (1999) N-(4-bromobenzoyl)-S, S-dimethyliminosulfurane, a potent dermal penetration enhancer. Bioorg Med Chem Lett 9(7):1033–1034

Trundle D, Cunningham LW (1969) Iodine oxidation of the sulfhydryl groups of creatine kinase. Biochemistry 8(5):1919–1925

Williams AC, Barry BW (1991) Terpenes and the lipid-protein-partitioning theory of skin penetration enhancement. Pharm Res 8(1):17–24

Zoeller RT, Fletcher DL, Butnariu O, Lowry CA, Moore FL (1997) N-ethylmaleimide (NEM) can significantly improve in situ hybridization results using 35S-labeled oligodeoxynucleotide or complementary RNA probes. J Histochem Cytochem 45(7):1035–1041

Alaptide as Transdermal Permeation Modifier

8

Josef Jampílek and Jiří Dohnal

Contents

8.1 Introduction

Within investigation of biological properties of piperazine-2,5-diones, a series of spirocyclic cyclodipeptides containing 1-amino-1-cycloalkylcarboxylic acid were synthesized by the group of Kasafírek at the Research Institute for Pharmacy and Biochemistry (former Czechoslovakia, now the Czech Republic) in the 1980s of the twentieth century (Kasafírek et al. 1984, 1986; Kasafírek et al. 1992a, b; Šturc and Kasafírek 1992; Vinšová et al. 1993, 1994). These compounds were designed as analogues of melanocyte-stimulating hormone release-inhibiting factor (MIF), also known as Pro-Leu-Gly-NH_2, i.e. L-prolyl-L-leucylglycinamide (Celis et al. 1971; Petersson and Uvnäs-Moberg 2004). (*S*)-8-Methyl-6,9-diazaspiro[4.5]decan-7,10-dione (VUFB-15754), known by the international non-proprietary name (INN) "alaptide" (**1**) (Fig. 8.1), was selected from the series of prepared spiro derivatives as the most advantageous MIF analogue from the point of view of enzymatic stability and its pharmacodynamic profile. Alaptide showed significant curative effect in different therapeutic areas on experimental animal models (Vanžura et al. 1986; Rádl et al. 1990; Kasafírek et al. 1992c, 1994; Nedvídková et al. 1994; Hliňák et al. 2008).

The synthesis and the structure of alaptide are shown in Fig. 8.1. For example, it can be prepared by

Dr. Evžen Kasafírek, the "parent" of the alaptide molecule who tragically left us, was an excellent medicinal chemist who was undervalued in his lifetime. He left us many results of his creative work. He educated experts who hold top positions in the world's largest pharmaceutical companies. We dedicate this article to his memory.

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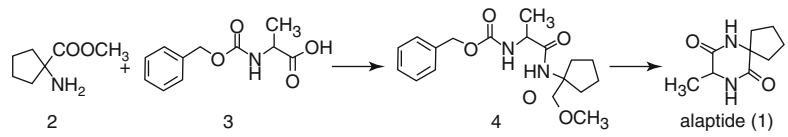
N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_8

Fig. 8.1 Synthesis of alaptide



condensation of methyl 1-aminocyclopentane-1-carboxylate (**2**) with *N*-benzyloxycarbonyl-L-alanine (**3**), which gave methyl 1-(benzyloxycarbonyl-L-alanyl)aminocyclopentane-1-carboxylate (**4**). Debenzylation of **4** followed by a cyclization yielded alaptide (**1**) (Rádl et al. 1990; Šturc and Kasafírek 1992; Kasafírek et al. 1992b; Vinšová et al. 1993). It was found that when optically pure starting compounds are used, no change of configuration (racemization) occurs, and a chirally pure final product is obtained (VUFB Internal report, 1988 unpublished data).

Alaptide is a white crystalline compound with melting point 308–312 °C. It is poorly soluble; its solubility in water is 0.1104 g/100 mL, in ethanol 0.1011 g/100 mL, in the mixture water/ethanol (1:1) 0.3601 g/100 mL and in hexane 0.0024 g/100 mL. Its log $P_{W/Oct}$ is 1.39. Alaptide possesses one centre of chirality; its specific optical rotation $[\alpha]_D^{20}$ is −21.9 (0.2 M methanol). The substance is stable in the sunlight and it can be stored at ambient temperature (Rádl et al. 1990; Jampílek et al. 2011; 2014a). A liquid chromatographic method for enantiopurity control of alaptide was developed (Douša and Lemr 2011). Besides the recently published X-ray powder diffraction data revealing the unit cell parameters and the space group of alaptide crystals (Maixner et al. 2009), the absolute configuration of the molecule was also determined by electronic circular dichroism spectroscopy (Julínek et al. 2010). Conformational analysis at the molecular mechanics level that allowed to change simultaneously the dihedral angles within both rings and their mutual orientation disclosed five possible geometries. For all five conformers, the central six-membered ring adopts exclusively the conformation of a slightly twisted boat. Depending on the position of the methyl group, the resulting conformers can be divided into two groups: the first one, comprising **1a**, **1b** and **1c**, and the second one, comprising **1d** and **1e**, with the methyl group being in the equatorial and axial positions, respectively, see Fig. 8.2. The detailed structure of all the conformers is depicted in Fig. 8.3. The relative populations of all conformers of alaptide determined according to ΔG at room temperature are as follows: 23.4 % (**1a**), 37.9 % (**1b**), 13.8 % (**1c**), 10.2 % (**1d**) and 14.8 % (**1e**) (Julínek et al. 2010). The conformation of alaptide seems to be important for its skin-affecting properties, i.e. permeation-enhancing ability.

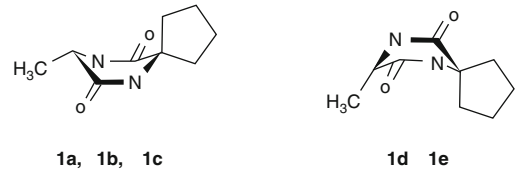


Fig. 8.2 Schematic representation of twisted boat conformation of alaptide with methyl group in equatorial (conformers **1a**, **1b** and **1c**) and axial (conformers **1d** and **1e**) positions. Hydrogen atoms are omitted for clarity (Reprinted and adapted from Julínek et al. (2010), Copyright (2010), with permission from Elsevier)

During biological assays, it was found that alaptide affects behaviour and learning abilities of rodents, particularly rats and mice, i.e. it can be classified as nootropic (Krejčí et al. 1986; Hliňák et al. 1990; Hliňák and Krejčí 1991, 1992; Kasafírek et al. 1994; Nedvídková et al. 1994; Hliňák et al. 1996, 2008; Hliňák and Krejčí 2005). Alaptide also expressed anti-proliferative activity (Vanžura et al. 1986, 1993) and immunomodulatory effect (Blažíčková et al. 1996). Alaptide also reduced the number and extent of experimental gastric ulcers. The gastric juice quantity, acidity and enzymatic activity were not influenced (Korbová et al. 1988, 1994). Alaptide was also tested on diploid cell line of human embryonic lung LEP-19, where it expressed stimulating effect on growth and breeding of cells without transformation changes in their morphology. In

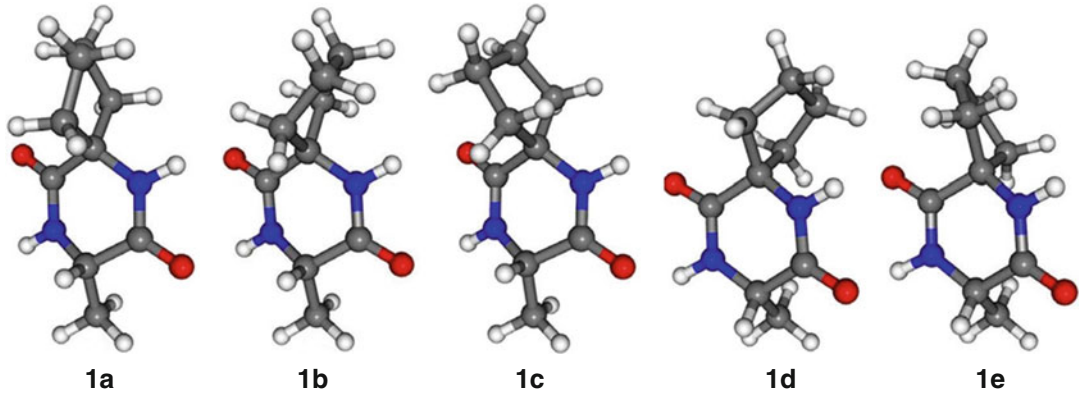


Fig. 8.3 Five possible conformers of alaptide (Reprinted and adapted from Julínek et al. (2010), Copyright (2010), with permission from Elsevier)

vitro tests also showed that alaptide increased cell proliferation, and both above-mentioned effects can significantly contribute to the curative effect of alaptide. An influence of alaptide on epidermal regeneration was investigated in a number of tests. In vivo experiments were performed using domestic pigs, to which alaptide was applied on experimental injury, and faster skin regeneration was observed after alaptide application. Similarly, alaptide accelerated curing of experimental skin injuries in rats (Rádl et al. 1990; Kasafírek et al. 1992c). Alaptide probably negatively affects the inhibition of the release of melanocyte-stimulating hormone, and thus it increases the concentration of melanocytes in epidermis. Melanocytes significantly influence the creation and function of keratinocytes by means of melanosomas (McGrath et al. 2004; James et al. 2006). Metabolic studies in rats showed that alaptide is readily absorbed from the gastrointestinal tract and permeates the blood-brain barrier. The maximum concentration in brain is reached within 1 h and then the level slowly decreases (Vanžura et al. 1986; Rádl et al. 1990). It is excreted unchanged, mostly via urine (90 %); a similar metabolic profile was also found in man (Lapka 1991). Alaptide also demonstrated very low acute toxicity in rats and mice; in female rats the dose of 1 g/1 kg caused only 20 % mortality. The evaluation of subchronic and chronic toxicity was carried out in rats in the dose of 20 mg/mL and in dogs in the dose of 10 mg/mL, and no toxic, genotoxic, teratogenic and embryotoxic effects were observed (Vanžura et al. 1986; Kosař and Vanžura 1988; Rádl et al. 1990). Alaptide enantiomers were tested in metabolic and induction studies on primary human hepatocyte cultures. It was found that alaptide enantiomers do not induce biotransformation enzymes CYP1A1, CYP1A2 and CYP1B1 in hepatocytes. These biotransformation enzymes are critical in bioactivation of procarcinogens and are upregulated also by ultraviolet-B radiation (UVB) in the skin. Based on this study it can be stated that alaptide both in enantiomeric pure forms and in racemate can be safely utilized for local applications (Jampílek et al. 2011, 2014a; Opatřilová et al. 2013).

In the 1990s of the twentieth century, clinical trials until the second phase were performed for verification of alaptide effect as a nootropic (in tablets), an antiulcerant (in tablets) and a dermatological agent (in ointments and collagen coating plates) (VUFB Internal report, 1998, unpublished data).

8.2 Alaptide as Modifier of Permeation

The permeation through the least permeable layer of the skin, the *stratum corneum* (SC), is a limiting process. The skin barrier for facilitation of drug permeation can be mostly affected by

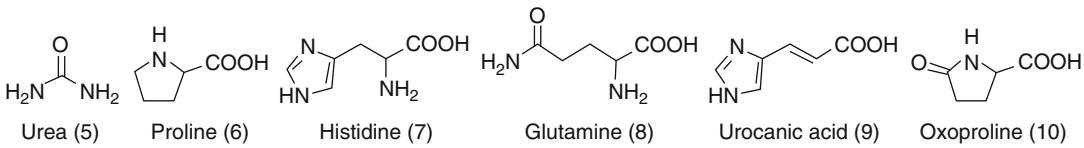


Fig. 8.4 Structures of selected components of natural moisturizing factor (NMF)

various so-called chemical permeation enhancers (CPEs), which are excipients that facilitate the permeation of drugs through the skin. CPEs interact mainly with lipid components of the SC or specifically affect intercellular space between corneocytes or modify corneocytes by hydration or denaturation of keratin (Jampílek and Brychtová 2012). From the chemical point of view, the group of CPEs is very heterogeneous. However, it is possible to observe certain common elements in their structure. They often contain a fragment of the basic natural moisturizing factor (NMF). The NMF is physiologically present in highly differentiated flattened keratinocytes (referred to as corneocytes) that are the building blocks of the epidermal barrier. The NMF derived from profilaggrin (highly phosphorylated histidine-rich polypeptide) is a mixture of hygroscopic compounds that help to maintain skin hydration. The NMF is capable of retaining water in the horny layer. These substances, contained in the NMF, with a special water-binding capacity are derived from sweat and sebaceous oils, e.g. urea (**5**), as well as from the cornification process, e.g. proline (**6**). Other components of the NMF include free amino acids such as histidine (**7**) and glutamine (**8**), which are further converted to urocanic acid (**9**) and 2-pyrrolidone-5-carboxylic acid (oxoproline, pyroglutamic acid, **10**), as well as ornithine, citrulline, arginine, glutamic acid, aspartic acid, glycine, alanine, serine, phenylalanine, tyrosine, lysine, valine, threonine, leucine, etc. (see Fig. 8.4). The result of the ability of these compounds to bind water is slowed-down water loss due to evaporation (Spier and Pascher 1956; Harding and Scott 2002; Kezic et al. 2009; Cork et al. 2009; McGrath 2012).

In Fig. 8.4 it can be observed that NMF components contain a characteristic fragment of heteroatoms X–CO–N=, where X is $-CH_2-$, $-NH_2$ or –NH–. This fragment can be found in many CPEs; see Fig. 8.4. A hypothesis was proposed that small polar molecules may break the intermolecular *H*-bonds that hold the ceramide molecules together in the SC. Also small molecules derived from NMF components can increase the water-binding capacity so that to moisten the skin and to facilitate permeation of compounds through the skin (Jampílek and Brychtová 2012). Based on the aforementioned characteristics and epidermal regeneration properties of alaptide (see Sect. 8.1), the knowledge of the structure and properties of CPEs, the hypotheses of CPE mechanism of action and the previous experience with several other groups of CPEs (Brychtová et al. 2010a, b, 2012; Jampílek and Brychtová 2012), we decided to evaluate alaptide as a potential transdermal permeation enhancer. Alaptide as well as some other potential CPEs contain the above-discussed characteristic fragment of the NMF (see Fig. 8.4) as it is illustrated in Fig. 8.5 (Minaskanian and Peck 1992; Rachakonda et al. 2008; Godwin et al. 1997). In a number of in vitro studies, it was demonstrated that alaptide is very effective as transdermal permeation modifier (Jampílek et al. 2012a, b, 2013a, b, 2014b; Černíková et al. 2014a, b; Opatřilová and Jampílek 2014).

As was discussed above, alaptide and its analogues show poorly aqueous solubility, which represents the essential disadvantage of the development of these compounds. One of the ways how to increase the solubility of the spirocyclic dipeptides was synthesis of *N*-prolonged peptides as prodrugs (Kasafírek et al. 1992a, b). The second, physical, approach was used later, and micronized alaptide was used in a mixture with various surfactants or complexing agents, or nanoparticles of alaptide were prepared. Alaptide nanoparticles were obtained by milling alaptide with emulsifiers and other stabilizers. Alaptide nanoparticles were generated by dispergation using a technique of wet milling in the aqueous solution of a surface modifier. This method assumes pulverization of all major micrometer particles to

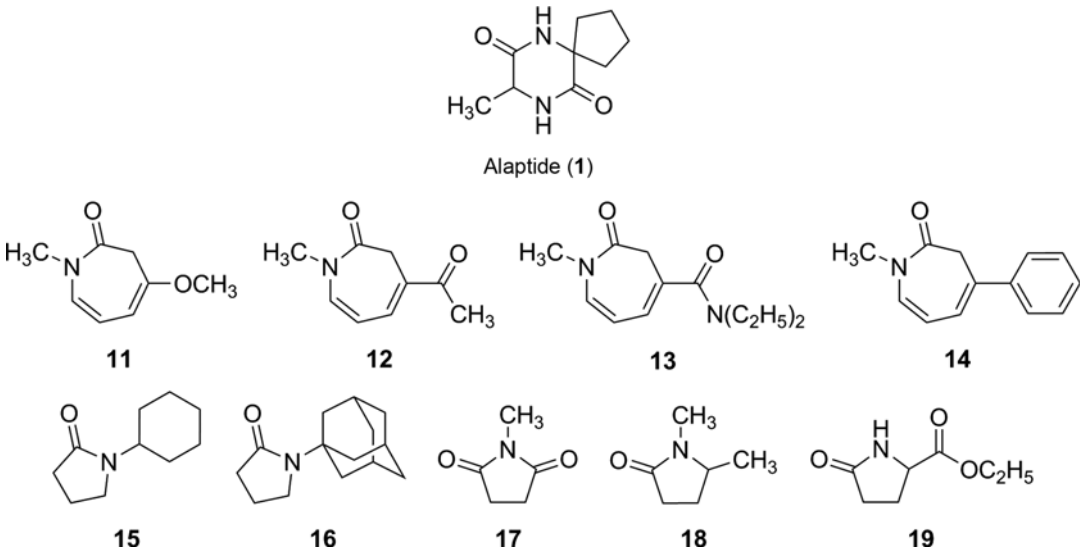


Fig. 8.5 Comparison of structural features of alaptide (**1**) and selected potential CPEs: **11**–**14** (Minaskanian and Peck 1992), **15**, **17**–**19** (Godwin et al. 1997) and **16** (Rachakonda et al. 2008)

nanoparticles (Jampílek et al. 2011, 2012a, b, 2013a, b, 2014a, b; Opatřilová et al. 2013; Opatřilová and Jampílek 2014; Černíková et al. 2014a).

Modified alaptide was obtained by the above-mentioned physical "technological" routes and showed considerably higher solubility than that obtained using the chemical way – preparation. Alaptide modified in this way can be used as a chemical transdermal permeation modifier, i.e. as pharmaceutical adjuvant (excipient), in pharmaceutical formulations, such as oleo-ointments, hydro-ointments, oleo-creams, hydro-creams, gels or transdermal therapeutic systems, where it influences the absorption/permeation of various compounds into the skin and/or through the skin, increasing their concentration at the place of administration and/or their systemic concentration.

8.2.1 Conditions of In Vitro Screening

Alaptide synthesized by the standard procedure (Rádl et al. 1990) was micronized. The particle-size distribution of the used micronized alaptide was 50–80 % up to ten maximum Feret diameters. It was measured by a microscope Nikon Optiphot 2 with a digital camera VDS CCD-1300F (USA). The suspension of micronized alaptide, polyvinylpyrrolidone and purified water was initially mixed at ambient temperature and then filtered through a mill sieve. The milling procedure was performed using a nanomill Netzsch (Germany) with glass beads. The total time of milling was 57.5 h. The content of alaptide in the suspension was 38.76 g/L. The particle size of the prepared nanonized alaptide was measured by Sympatec Nanophox 0138 P (Germany), and the particle size x_{50}–x_{90} was up to 900 nm (Jampílek et al. 2011, 2012a, b, 2013a, b, 2014a, b; Opatřilová and Jampílek 2014; Černíková et al. 2014a).

A number of various active pharmaceutical ingredients (APIs) from almost all anatomical therapeutic chemical (ATC) groups were evaluated. Nevertheless in this contribution only selected examples are discussed. The permeation of the API alone through the skin from propylene glycol/water (1:1) medium or phosphate buffer (pH 7.4) and the API from formulations (ointment, cream, gel) without alaptide was monitored and served as control. Subsequently the permeation of the API with various concentrations of micronized or nanonized alaptide (nanonized alaptide in the amount corresponding to the concentration of micronized alaptide) from propylene glycol/water medium or phosphate buffer was investigated. Also the permeation of

the API from pharmaceutical formulations (ointment, cream, gel) with various concentrations of micronized or nanonized alaptide (nanonized alaptide in the amount corresponding to the concentration of micronized alaptide) was evaluated (Jampílek et al. 2012a, b, 2013a, b, 2014b; Opatřilová and Jampílek 2014; Černíková et al. 2014a).

In vitro experiments on permeation of various APIs or APIs in formulations without or with the presence of alaptide as a chemical permeation modifier was performed using a vertical Franz diffusion cell (SES Analysesysteme, Germany). Full-thickness skin from porcine ear (*Sus scrofa f. domestica*) was used as a model membrane. The porcine ear skin is a suitable in vitro model for the human skin (Franz 1975; Jacobi et al. 2007) and has been used in numerous percutaneous absorption studies (Meyer et al. 1978; Herkenne et al. 2006; Brychtová et al. 2010a, b, 2012; Mrózek et al. 2011, 2013; Coufalová et al. 2013; Jampílek and Brychtová 2012; Jampílek et al. 2012a, b, 2013a, b, 2014b; Opatřilová et al. 2013; Opatřilová and Jampílek 2014; Černíková et al. 2014a, b) The porcine skin has shown to be histologically and biochemically similar to the human skin (Sekkat et al. 2002).

The investigated samples (see above) were applied in the form of solution, suspension, emulsion, gel, cream or ointment, always with the API concentration of 10 mg/mL. The skin was mounted with the epidermal side up between the donor and the receptor compartments of the diffusion cell. The receptor compartment was filled with phosphate-buffered saline (pH 7.4) and maintained at 37 ± 0.5 °C. Most of the studies involved the use of propylene glycol or its mixture with water as a donor vehicle. Previous studies have indicated that propylene glycol by itself (or a propylene glycol/water co-solvent system) does not interfere with membranes, but rather exhibits a synergistic effect in combination with other permeation enhancers (Williams and Barry 1989; Yamane et al. 1995; Evrard et al. 2001; Jampílek and Brychtová 2012). Therefore mostly propylene glycol/water (1:1) system was used as a donor vehicle for evaluation of permeation of API alone (API without pharmaceutical formulation).

The receptor compartment was continuously stirred using a magnetic stirring bar. Than a sample was applied to the skin surface, and the donor compartment of the cell was covered. Samples were taken from the receptor phase in time intervals, and the cell was refilled with an equivalent volume of the fresh buffer. For each API, a minimum of three skin samples was used, which was obtained from a minimum of 2 animals. The concentration of the permeated API was chromatographically determined (Jampílek et al. 2012a, b, 2013a, b, 2014b; Opatřilová and Jampílek 2014; Černíková et al. 2014a, b).

8.2.2 Effect of Alaptide on Modification of APIs Permeation

The primary screening of alaptide effectivity as an excipient that influences permeation of different compounds through the skin was performed with the model compound theophylline. Theophylline was used as a model drug of medium polarity (log P −0.06; log D_8 −0.05) (Ni et al. 2000; Katz et al. 2006) as it had been extensively studied in transdermal permeation experiments (Sloan et al. 1998; Fang et al. 2004; Jampílek and Brychtová 2012). Based on the results with theophylline, other experiments with different APIs from various ATC classes were performed. The results are illustrated in the below presented Figs. 8.6, 8.7, 8.8, 8.9, 8.10, 8.11, 8.12, 8.13, 8.14, 8.15, 8.16, 8.17, 8.18, 8.19, 8.20, 8.21, 8.22, 8.23 and 8.24 as the dependences of the permeated amount of drug [%] on time [min], whereas 100 % is the amount of the applied API in a sample. The concentration of the API was 10 mg/mL. The used concentration of alaptide [%] is related to API amount (w/w). Unless otherwise indicated below, alaptide was added in the amount of 0.1 % (w/w in relation to API amount).

Permeation of theophylline was evaluated from water as a donor vehicle with the addition of 0.01 and 0.1 % of alaptide in relation to theophylline amount; see Fig. 8.6. Of the total amount of theophylline in water, max. 0.24 % permeated without the addition of alaptide within 1 h.

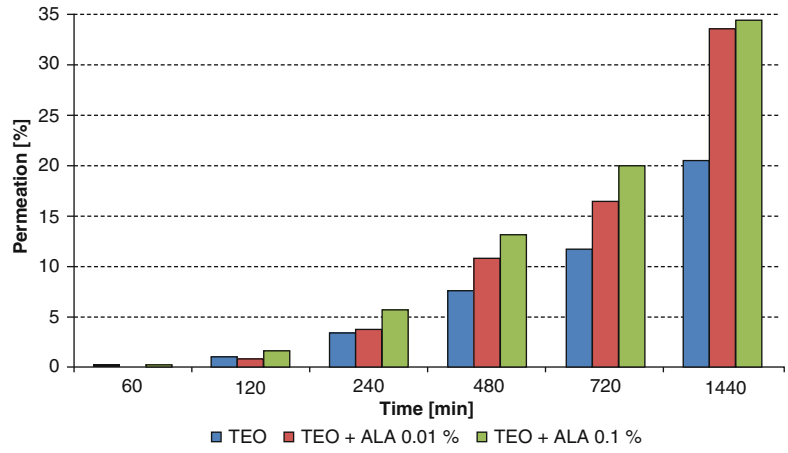


Fig. 8.6 Permeation of theophylline (*TEO*) through the skin from water depending on amount of micronized alaptide (*ALA*) used in concentrations 0.01, 0.1 % in time

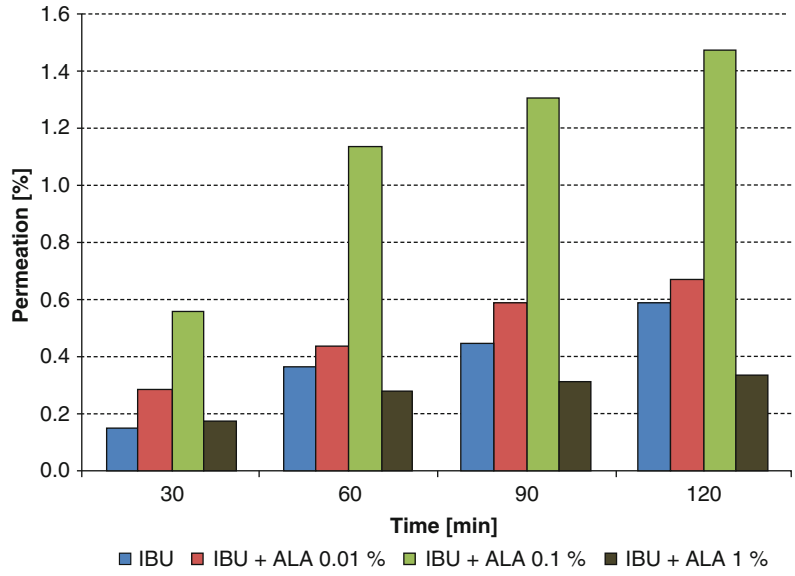


Fig. 8.7 Permeation of ibuprofen (*IBU*) through the skin from propylene glycol/water medium depending on amount of micronized alaptide (*ALA*) in concentrations 0.01, 0.1 and 1 %

The addition of 0.01 % of alaptide did not have any significant effect on theophylline permeation, but the addition of 0.1 % of alaptide increased theophylline permeation through the skin by 54 % within 1 h and by 70 % within 2 h. After 24 h the effect of the higher amount of alaptide was not apparent.

After that permeation of ibuprofen through the skin from the propylene glycol/water (1:1) medium was tested depending on the amount of micronized alaptide in concentrations 0.01, 0.1 and 1 % (in relation to ibuprofen amount); see Fig. 8.7. Of the total amount of the used ibuprofen, max. 0.36 % permeation without alaptide within 1 h. The addition of 0.01 % of alaptide increased the permeation of ibuprofen by 87 % within 30 min and by 20 % within 1 h compared to the permeation of ibuprofen without alaptide. The highest permeation was detected after the addition of 0.1 % of alaptide: the permeation was increased by 270 % within 30 min and by 213 % within 1 h compared to the permeation of ibuprofen without alaptide. The further addition of alaptide in the amount up to 1 % decreased the permeation of ibuprofen. The permeation of ibuprofen through the skin from hydroxypropyl cellulose gel depending on the amount of micronized alaptide in time is illustrated in Fig. 8.8. The

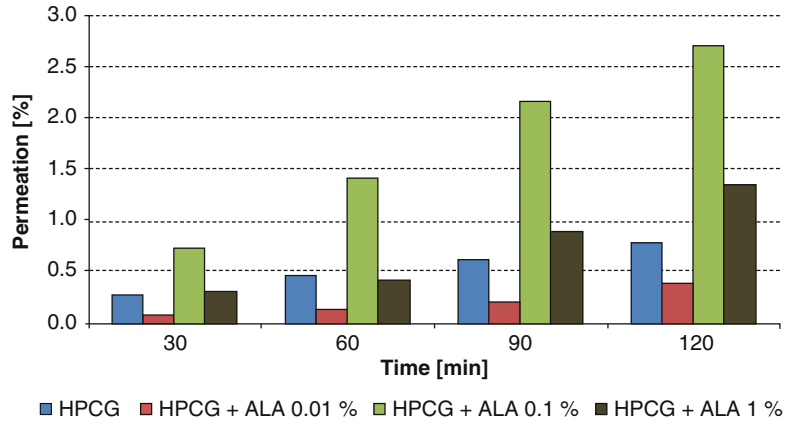


Fig. 8.8 Permeation of ibuprofen through the skin from hydroxypropyl cellulose gel (*HPCG*) depending on amount of micronized alaptide (*ALA*) in time

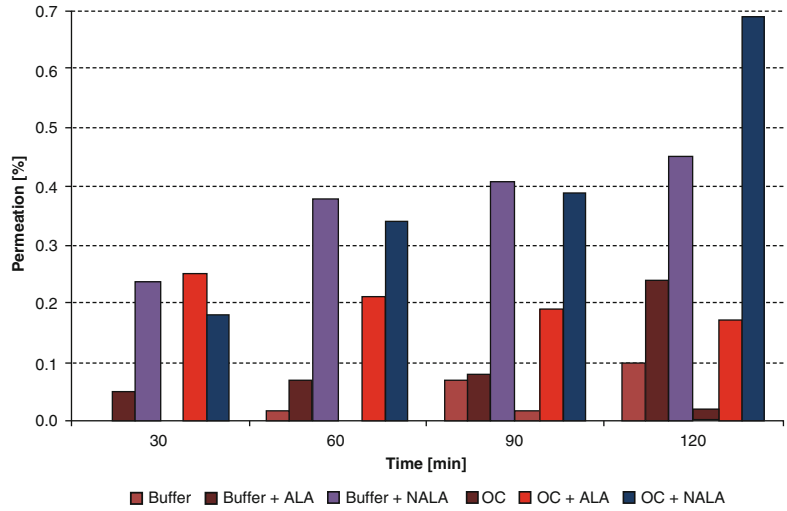


Fig. 8.9 Comparison of Permeation of nimesulide through the skin from various media without and with presence of micronized or nanonized alaptide (*ALA*, *NALA*) from phosphate buffer and from oleo-cream (OC) in time

hydroxypropyl cellulose gel with 1 % of ibuprofen was prepared, and micronized alaptide in concentrations 0.01, 0.1 and 1 % (in relation to ibuprofen amount) was added. Of the total amount of ibuprofen, max. 0.78 % permeated without alaptide within 2 h. The highest permeation was observed after the addition of 0.1 % of alaptide; the permeation was increased by 200 % within 1 h and by 246 % within 2 h compared to the permeation of ibuprofen without alaptide. The further addition of alaptide up to 1 % increased ibuprofen permeation by 74 % within 2 h compared to the permeation of ibuprofen without alaptide.

The comparison of the permeation of nimesulide through the skin from the phosphate buffer (pH 7.4) and from oleo-cream without and with the presence of 0.1 % of micronized or nanonized alaptide in time is shown in Fig. 8.9. The permeation from the cream with micronized alaptide was 0.25 % of the total amount of nimesulide after 30 min, and the permeation with nanonized alaptide was 0.18 % of the total amount of nimesulide after 30 min; nimesulide was not detected without alaptide after 30 min. The highest permeation from the cream was detected after the addition of nanonized alaptide; the permeation was increased by 3400 % within 2 h compared to the permeation of nimesulide without alaptide. The permeation of meloxicam through the skin from the phosphate buffer with the addition of nanonized alaptide was by 383 % higher after 30 min than without alaptide; see Fig. 8.10.

The permeation of diclofenac through the skin from the phosphate buffer or from propylene

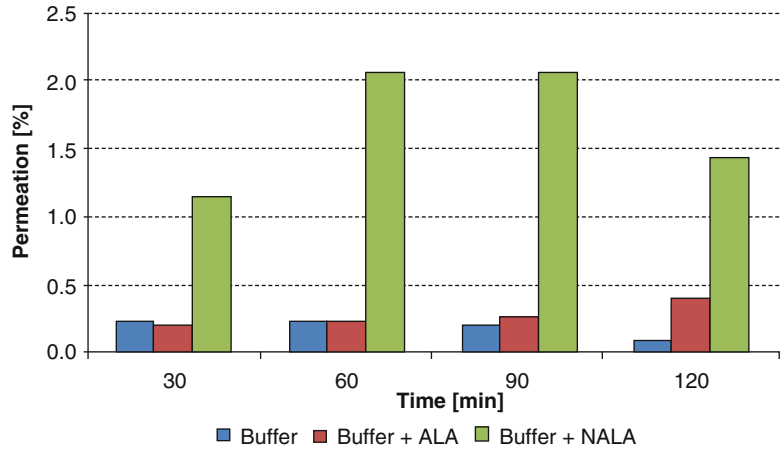


Fig. 8.10 Comparison of permeation of meloxicam through the skin from phosphate buffer without and with presence of micronized or nanonized alaptide (*ALA*, *NALA*) in time

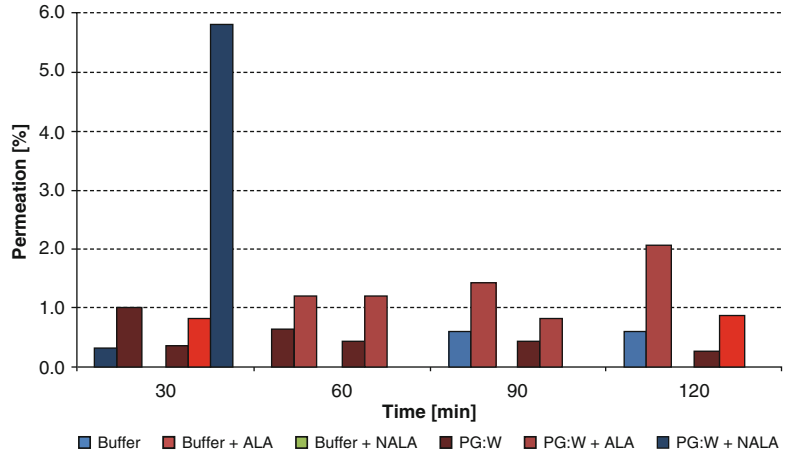


Fig. 8.11 Comparison of permeation of diclofenac through the skin from phosphate buffer or from propylene glycol/water (*PG*:*W* 1:1) medium without and with presence of micronized or nanonized alaptide (*ALA*, *NALA*) in time

glycol/water medium without and with the presence of micronized or nanonized alaptide in time is illustrated in Fig. 8.11. The addition of micronized alaptide to propylene glycol/water medium increased the permeation of diclofenac by 124 %, and the addition of nanonized alaptide increased the permeation of diclofenac by 1445 % after 30 min in comparison with the permeation of diclofenac without alaptide. Diclofenac from carbomer gel with the application of nanonized alaptide permeated by 77 % more after 30 min than without alaptide; see Fig. 8.12.

The permeation of dexamethasone through the skin both from the buffer and the propylene glycol/water medium was possible within 30 min only after the addition of nanonized alaptide. Without the addition of nanonized alaptide, dexamethasone permeated from neither of the media; see Fig. 8.13. The permeation of fluocinolone acetonide through the skin from hydro-cream or oleo-cream without and with the presence of micronized alaptide in time is shown in Fig. 8.14. The highest permeation of fluocinolone acetonide was observed from the oleo-cream; the permeation from the hydro-cream was on the average by 14 % less. The permeation of fluocinolone acetonide from hydro-cream was on the average by 240 % more within 4–8 h and from oleo-cream by 260 % more within 4–8 h and by 140 % more within 20–24 h than without utilization of alaptide.

The addition of micronized alaptide to the buffer increased the permeation of triamcinolone through the skin by 174 % after 30 min compared

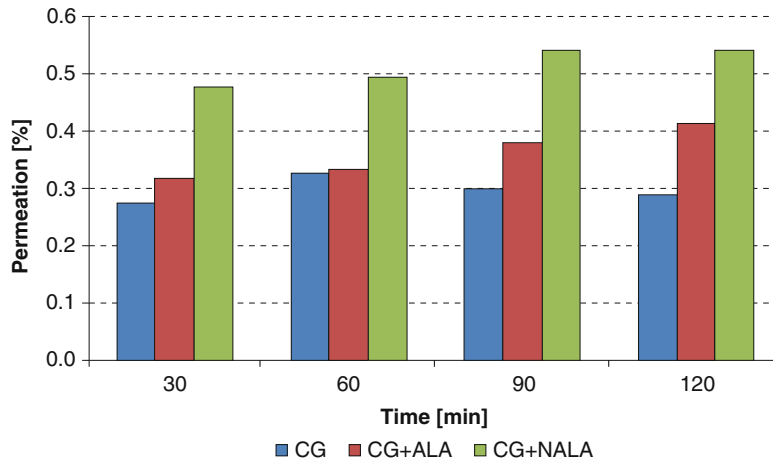


Fig. 8.12 Comparison of permeation of diclofenac through the skin from carbomer gel (*CG*) without and with presence of micronized or nanonized alaptide (*ALA*, *NALA*) in time

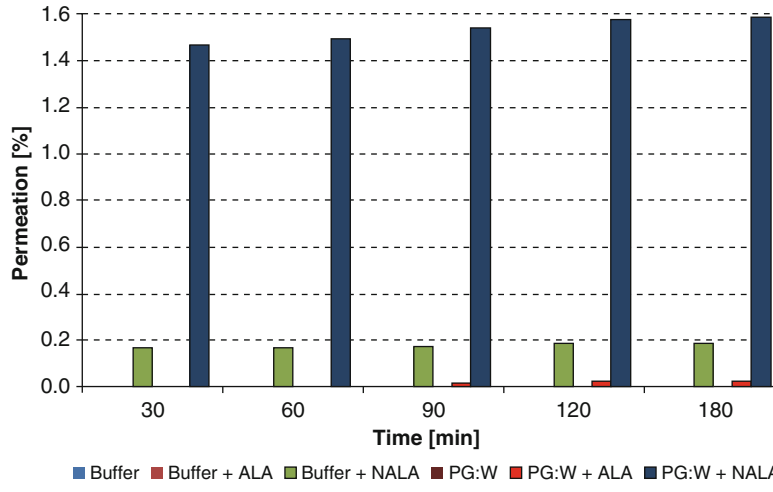


Fig. 8.13 Comparison of permeation of dexamethasone through the skin from various media without and with presence of micronized or nanonized alaptide (*ALA*, *NALA*) from the propylene glycol/water (*PG:W*) medium and from phosphate buffer in time

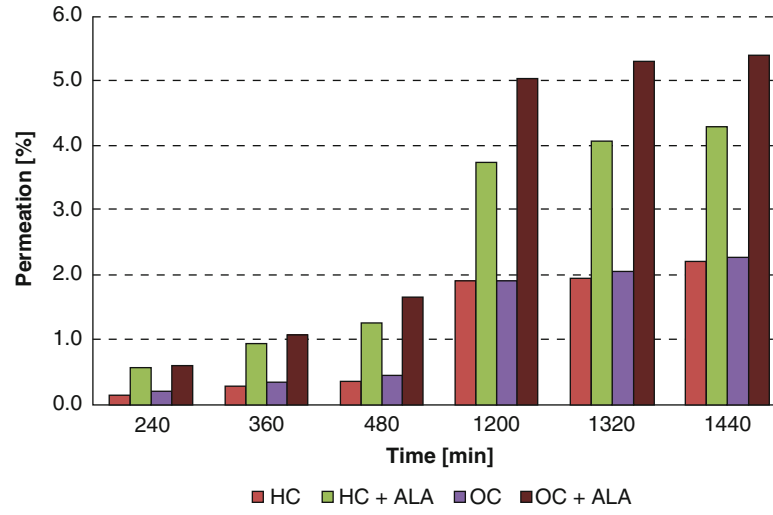


Fig. 8.14 Comparison of permeation of fluocinolone acetonide through the skin from hydro-cream (*HC*) and oleo-cream (OC) without and with presence of micronized alaptide (*ALA*) in time

to the permeation of triamcinolone without alaptide, and from the propylene glycol/water medium containing the micronized alaptide, triamcinolone permeated by 267 % more after 30 min compared to the permeation of triamcinolone without alaptide; see Fig. 8.15. Without the addition of alaptide, triamcinolone acetonide permeated through the skin from the propylene glycol/water medium and from the buffer in 90 or 120 min, see Fig. 8.16. After the addition of micronized alaptide to the buffer, the permeation of triamcinolone acetonide increased by 5 % in 90 min compared to the permeation of triamcinolone acetonide without alaptide. The addition of nanonized alaptide to the propylene glycol/water medium enabled the permeation of triamcinolone acetonide already in 30 min compared to the permeation of triamcinolone acetonide without alaptide, and the addition of micronized alaptide increased the permeation by 790 % in 30 min compared to the addition of nanonized alaptide. Without the presence of alaptide, triamcinolone acetonide from oleo-ointment was not observed in the sample within 3 h, but after the addition of micronized alaptide, triamcinolone acetonide was detected already after 30 min. The addition of nanonized alaptide increased the permeation of triamcinolone acetonide through the skin from the oleo-ointment by 273 % after 30 min compared to the permeation of triamcinolone acetonide with micronized alaptide; see Fig. 8.17.

The permeation of benzylpenicillin (penicillin G) through the skin from propylene glycol/water medium and from buffer without and with

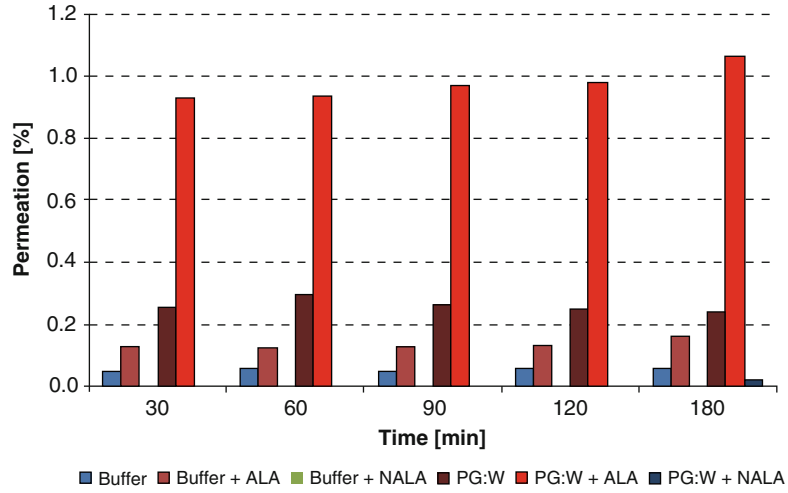


Fig. 8.15 Comparison of permeation of triamcinolone through the skin from the propylene glycol/water (*PG*:*W*) medium and from phosphate buffer without and with presence of micronized or nanonized alaptide (*ALA*, *NALA*) in time

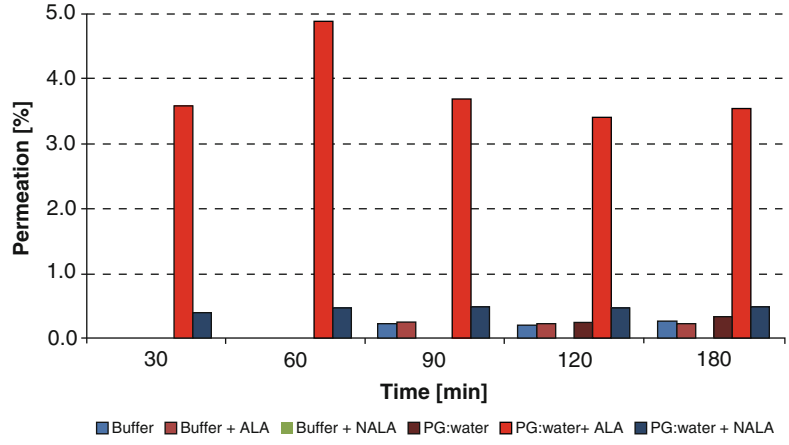


Fig. 8.16 Comparison of permeation of triamcinolone acetonide through the skin from the propylene glycol/water (*PG*:*W*) medium and from phosphate buffer without and with presence of micronized or nanonized alaptide (*ALA*, *NALA*) in time

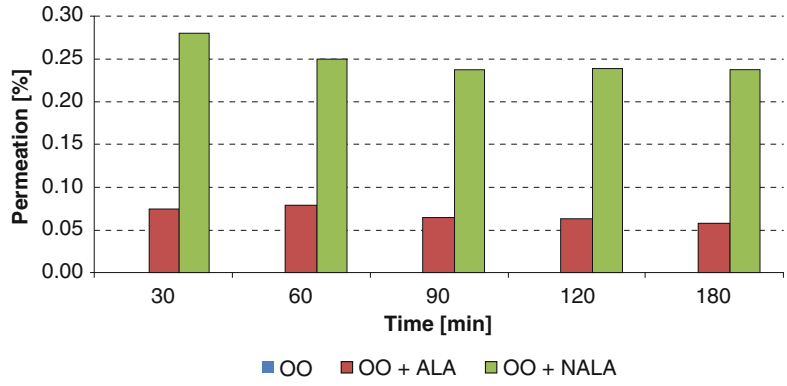


Fig. 8.17 Comparison of permeation of triamcinolone acetonide through the skin from oleo-ointment (OO) without and with presence of micronized or nanonized alaptide (*ALA*, *NALA*) in time

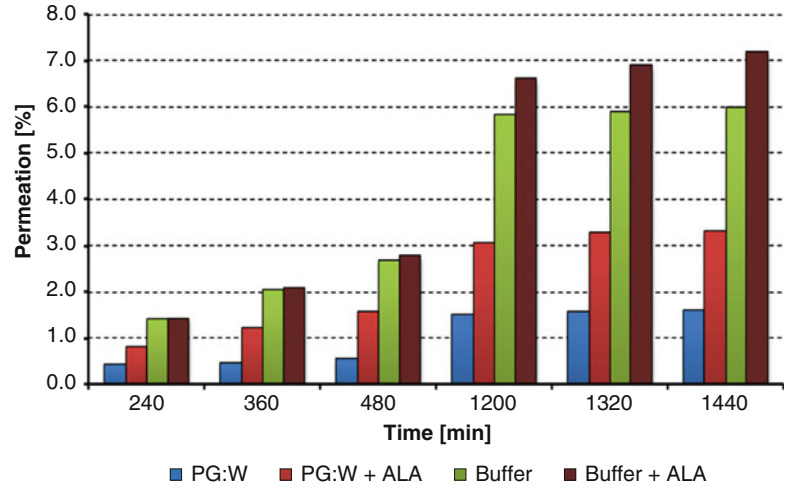


Fig. 8.18 Comparison of permeation of benzylpenicillin (penicillin G) through the skin from propylene glycol/water (*PG*:*W*) medium and from phosphate buffer without and with presence of micronized alaptide (*ALA*) in time

the presence of micronized alaptide in time is illustrated in Fig. 8.18. The permeation of penicillin G from the propylene glycol/water medium was increased by 170 % within 8 h after the addition of alaptide compared to the permeation of penicillin G without alaptide. The permeation of penicillin G from the buffer after the addition of alaptide was on the average by 20 % higher within 24 h compared to the permeation of penicillin G without alaptide. The addition of alaptide increased the permeation of phenoxymethylpenicillin (penicillin V) from the propylene glycol/water medium by 65 % within 8 h and on the average by 45 % within 20–24 h compared to the permeation of penicillin V without alaptide. The permeation of penicillin V from the buffer after the addition of alaptide was on the average by 50 % more within 8 h compared to the permeation of penicillin V without alaptide; see Fig. 8.19.

The permeation of mupirocin through the skin from hydro-ointment without and with the presence of micronized or nanonized alaptide in time is shown in Fig. 8.20. The permeation of mupirocin was increased after the addition of micronized alaptide by 144 % already after 30 min and by 400 % after 60 min compared to the permeation of mupirocin without alaptide; thus alaptide significantly accelerated the permeation of mupirocin through the skin. On the contrary, the addition of nanonized alaptide significantly inhibited (decreased by 160 % within the 1st hour) permeation through the skin (compared to the permeation of mupirocin without alaptide), so mupirocin acted only on the skin surface.

The addition of micronized alaptide to the buffer increased the permeation of pyrazinamide by 240 % after 8 h and by 300 % after 24 h compared to the permeation of pyrazinamide without

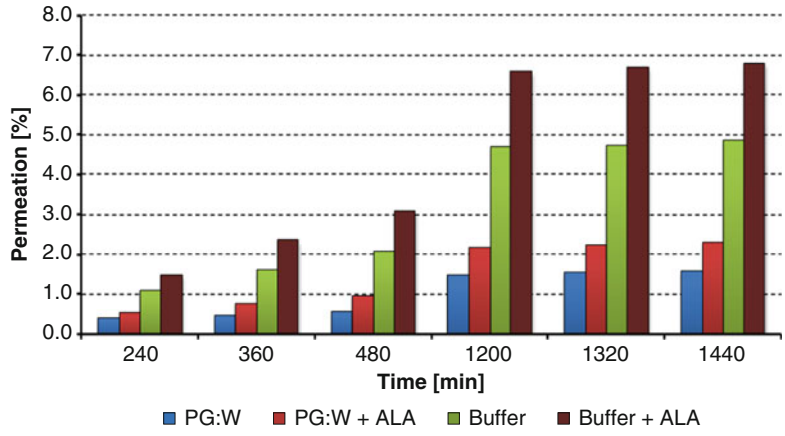


Fig. 8.19 Comparison of permeation of phenoxymethylpenicillin (penicillin V) through the skin from the propylene glycol/water (*PG*:*W* 1:1) medium and from phosphate buffer without and with presence of micronized alaptide (*ALA*) in time

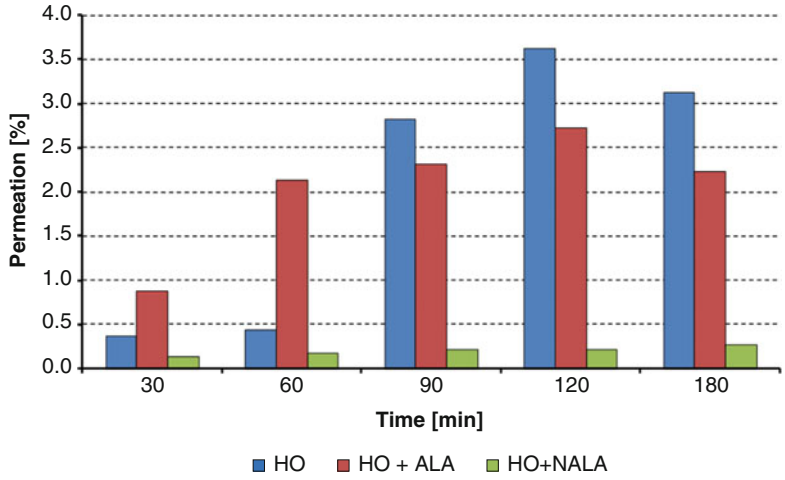


Fig. 8.20 Comparison of permeation of mupirocin through the skin from hydro-ointment (*HO*) without and with presence of micronized or nanonized alaptide (*ALA*, *NALA*) in time

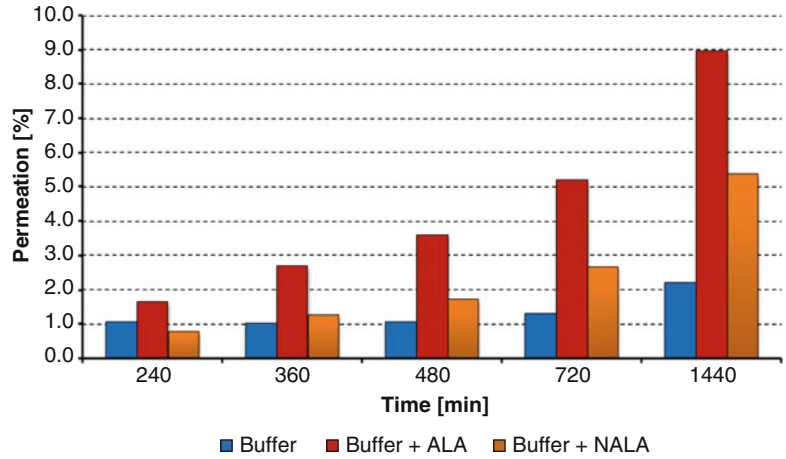


Fig. 8.21 Comparison of permeation of pyrazinamide through the skin from phosphate buffer without and with presence of micronized or nanonized alaptide (*ALA*, *NALA*) in time

alaptide, and the addition of nanonized alaptide increased the permeation of pyrazinamide by 145 % within 24 h compared to the permeation of pyrazinamide without alaptide; see Fig. 8.21.

The permeation of fluconazole through the skin from oleo-ointment and hydro-cream without and with the presence of nanonized alaptide in time is shown in Fig. 8.22. The permeation of

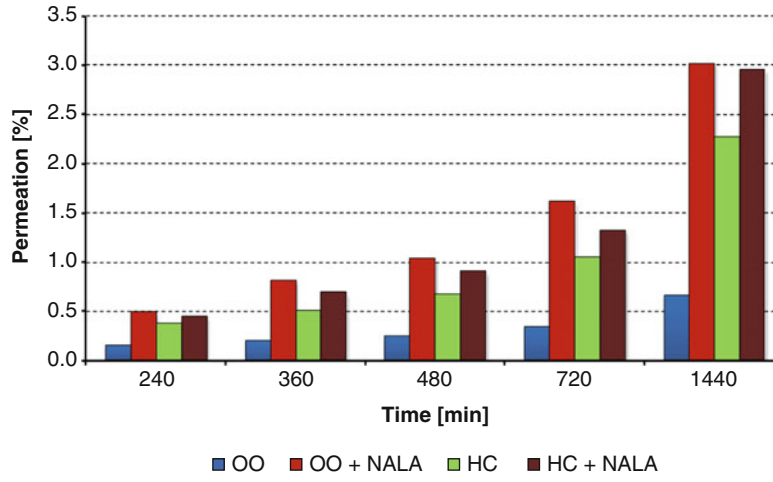


Fig. 8.22 Comparison of permeation of fluconazole through the skin from oleo-ointment (OO) and hydro-cream (*HC*) without and with presence of nanonized alaptide (*NALA*) in time

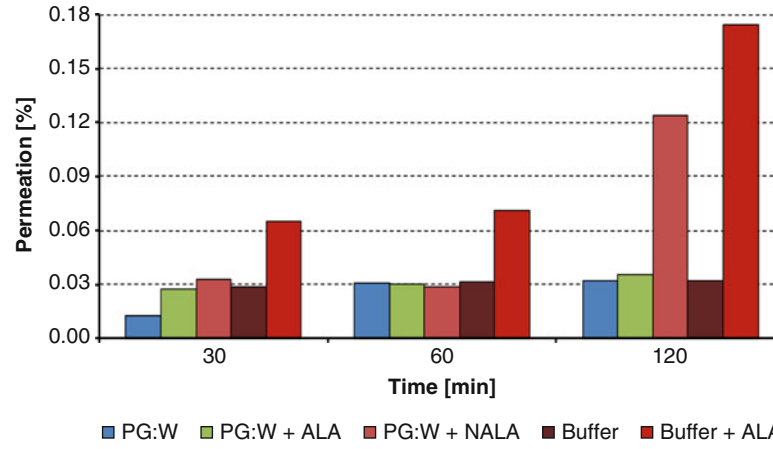


Fig. 8.23 Comparison of permeation of acyclovir through the skin from propylene glycol/water (*PG*:*W*) medium and from phosphate buffer without and with presence of micronized or nanonized alaptide (*ALA*, *NALA*) in time

fluconazole from ointment after the addition of nanonized alaptide increased on the average by 300 % within 8 h and by 350 % within 12–24 h compared to the permeation of fluconazole without alaptide. The addition of nanonized alaptide increased the permeation of fluconazole from cream by 28 % within 12 h in comparison with the formulation without alaptide.

The addition of micronized alaptide to the propylene glycol/water medium containing acyclovir caused an increase in the permeation of acyclovir by 110 % already after 30 min, and the addition of nanonized alaptide to the propylene glycol/water medium containing acyclovir increased the permeation of acyclovir on the average by 158 % after 30 min and by 280 % after 2 h compared to the permeation of acyclovir without alaptide. The addition of micronized alaptide to the buffer increased the permeation of acyclovir by 126 % already after 30 min and by 440 % after 2 h compared to the permeation of acyclovir without alaptide; see Fig. 8.23. The addition of nanonized alaptide increased the permeation of acyclovir through the skin from hydro-cream by 25 % already after 30 min, and the addition of micronized alaptide increased the permeation of acyclovir from carbomer gel on the average by 37 % after 30 min compared to the permeation of acyclovir without alaptide; see Fig. 8.24.

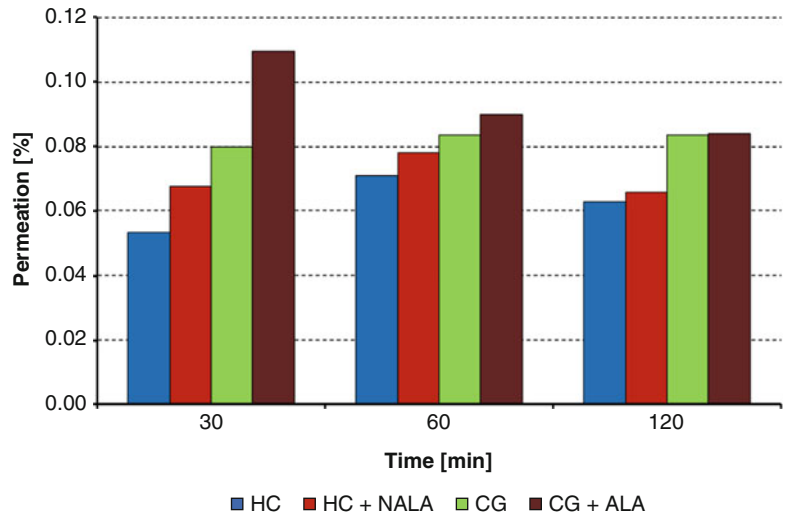


Fig. 8.24 Comparison of permeation of acyclovir through the skin from hydro-cream (*HC*) and carbomer gel (*CG*) without and with presence of micronized or nanonized alaptide (*ALA*, *NALA*) in time

Conclusion

As was discussed above, various APIs (bases, acids, salts, neutral molecules, small molecules, peptides, glycosides, steroid-like molecules) were tested on their transdermal permeation in the mixture with alaptide as a transdermal permeation modifier. In this chapter only a small number of structurally different compounds are mentioned. At present advanced experiments are carried out that are aimed at determination of the mechanism of alaptide influence on skin. Based on the fact that alaptide is able to influence the creation and function of keratinocytes, it can be supposed that the mechanism of action of alaptide as a transdermal permeation modifier will not be based strictly on physico-chemical interactions with the other components of the skin (especially in *stratum corneum* layer) as was described for a number of other skin CPEs. The influence of the type of formulation (ointment, cream, gel) on the effect of alaptide on skin is investigated intensively. All obtained results are correlated with the computer "in silico" modelling. It was observed that under specific conditions alaptide is able to suppress permeation/absorption of compounds through the skin, which can limit the site of action of potentially hazardous/toxic drugs to skin surface. The above-mentioned curative activity of alaptide can be helpful in reduction of possible skin irritant/injurious effects of permeating compounds.

It can be concluded that in transdermal application alaptide causes an increase or a decrease, in dependence on the used concentration, physical state and supporting medium (pharmaceutical formulation), in permeation/absorption of drugs into the skin and/or through the skin, so that the concentration of the used drug was increased at the place of administration, and/or the systemic concentration was increased, or it was ensured that drugs acted only on the skin surface/in the skin surface layer and did not penetrate into the deeper skin layers or did not have any systemic effects.

Alaptide unfortunately suffered the fate of many other promising molecules, the research and development of which have not been completed because of economic and property transformation problems. VUFB was privatized in the 1990s of the twentieth century, and although initially alaptide was in great demand on the part of large pharmaceutical companies, its clinical development has not been completed for several reasons. A big blow for its destiny was also the fact

that its creator and enthusiastic promoter, Dr. Evžen Kasafírek, tragically died. Although this compound was found in the 1980s of the twentieth century, even now it has great potential either as an active pharmaceutical ingredient or a permeation modifier.

Acknowledgement This study was supported by the Grant Agency of the Czech Republic (Czech Science Foundation) GAČR P304/11/2246, by the Technology Agency of the Czech Republic TA04010065 and by IGA VFU Brno 302/2015/FaF. Thanks to all our colleagues and numerous graduate and undergraduate students who participated in the evaluation of the discussed alaptide formulations.

References

Blažíčková S, Rovenský J, Kasafírek E, Buc M (1996) The immunomodulatory properties of low-molecular tri- and tetrapeptides. Folia Biol 42(1–2):57–59

Brychtová K, Jampílek J, Opatřilová R, Raich I, Farsa O, Csőllei J (2010a) Synthesis, physico-chemical properties and penetration activity of alkyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl) hexanoates as potential transdermal penetration enhancers. Bioorg Med Chem 18(1):73–79

Brychtová K, Opatřilová R, Raich I, Kalinowski D, Dvořáková L, Plaček L et al (2010b) Investigating the activity of 2-substituted alkyl-6-(2,5-dioxopyrrolidin-1-yl)hexanoates as skin penetration enhancers. Bioorg Med Chem 18(24):8556–8565

Brychtová K, Dvořáková L, Opatřilová R, Raich I, Káčerová S, Plaček L et al (2012) Investigation of substituted 6-aminohexanoates as skin penetration enhancers. Bioorg Med Chem 20(1):86–95

Celis ME, Taleisnik S, Walter R (1971) Regulation of formation and proposed structure of the factor inhibiting the release of melanocyte-stimulating hormone. Proc Natl Acad Sci U S A 68(7):1428–1433

Černíková A, Opatřilová R, Jampílek J (2014a) Rapid informative screening of nano-alaptide as potential transdermal permeation enhancer of acetylsalicylic acid and paracetamol. Mil Med Sci Lett 83(1):34–39

Černíková A, Opatřilová R, Bobál P, Jampílek J (2014b) Rapid screening of permeation of rutin through skin using alaptide enantiomers. ADMET 2(4):248–253

Cork MJ, Danby SG, Vasilopoulos Y, Hadgraft J, Lane ME, Moustafa M et al (2009) Epidermal barrier dysfunction in atopic dermatitis. J Invest Dermatol 129(8):1892–1908

Coufalová L, Mrózek L, Rárová L, Plaček L, Opatřilová R, Dohnal J et al (2013) New propanoyloxy derivatives of 5β-cholan-24-oic acid as drug absorption modifiers. Steroids 78(5):435–453

Douša M, Lemr K (2011) Liquid chromatographic method for enantiopurity control of alaptide using polysaccharide stationary phases. J Sep Sci 34(12):1402–1406

Evrard D, Touitou E, Kolusheva S, Fishov Y, Jelinek R (2001) A new colorimetric assay for studying and rapid screening of membrane penetration enhancers. Pharm Res 18(7):943–949

Fang JY, Tsai TH, Hung CF, Wong WW (2004) Development and evaluation of the essential oil from Magnolia fargesii for enhancing the transdermal absorption of theophylline and cianidanol. J Pharm Pharmacol 56(12):1493–1500

Franz TJ (1975) Percutaneous absorption on the relevance of in vitro data. J Invest Dermatol 64(3):190–195

Godwin DA, Michniak BB, Player MR, Sowell JW (1997) Transdermal and dermal enhancing activity of pyrrolidinones in hairless mouse skin. Int J Pharm 155(2): 241–250

Harding CR, Scott IR (2002) Stratum corneum moisturizing factors. In: Leyden JJ, Rawling AW (eds) Skin moisturization. Informa Health Care, London, pp 61–80

Herkenne C, Naik A, Kalia YN, Hadgraft J, Guy RH (2006) Pig ear skin ex vivo as a model for in vivo dermatopharmacokinetic studies in man. Pharm Res 23(8):1850–1856

Hliňák Z, Krejčí I, Hondlík J, Yamamoto A (1990) Behavioral consequences of sodium nitrite hypoxia in male rats: amelioration with alaptide treatment. Methods Find Exp Clin Pharmacol 12:385–393

Hliňák Z, Krejčí I (1991) Social recognition in male rats: age differences and modulation by MIF-1 and alaptide. Physiol Res 40(1):59–67

Hliňák Z, Krejčí I (1992) Prolonged social recognition in male rats treated with alaptide or oxiracetam. Behav Pharmacol 3(2):129–131

Hliňák Z, Vinšová J, Kasafírek E (1996) Effect of alaptide, its analogues and oxiracetam on memory for an elevated plus-maze in mice. Eur J Pharmacol 314(1–2):1–7

Hliňák Z, Krejčí I (2005) Oxiracetam pre- but not post-treatment prevented social recognition deficits produced with trimethyltin in rats. Behav Brain Res 161(2):213–219

Hliňák Z, Krejčí I, Hynie S, Klenerová V (2008) Dipeptide "alaptide" prevented impairments in spontaneous behavior produced with trimethyltin in male rats. Neuro Endocrinol Lett 29(6):917–923

Jacobi U, Kaiser M, Toll R, Mangelsdorf S, Audring H, Otberg N et al (2007) Porcine ear skin: an in vitro model for human skin. Skin Res Technol 13(1): 19–24

James W, Berger T, Elston D (2006) Andrews' diseases of the skin: clinical dermatology, 10th edn. Saunders-Elsevier, Philadelphia, pp 5–6

Jampílek J, Opatřilová R, Řezáčová A, Oktábec Z, Pávek P, Král V et al. inventors; University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Pharmacy, assignee (2011) Alaptide: methods of effecting its solubility, membrane penetration and pharmaceutical

compositions for human and veterinary applications. Czech Patent application PV 2011-232

Jampílek J, Brychtová K (2012) Azone analogues: classification, design, and transdermal penetration principles. Med Res Rev 32(5):907–947

Jampílek J, Opatřilová R, Dvořáková L. Dohnal, J, inventors; University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Pharmacy, assignee (2012a) Utilization of alaptide as transdermal penetration modifier in pharmaceutical compositions for human and veterinary applications containing glucocorticoids. Czech Patent application PV 2012-72

Jampílek J, Opatřilová R, Dvořáková L, Černíková A, Dohnal J, inventors; University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Pharmacy, assignee (2012b) Utilization of alaptide as transdermal penetration modifier in pharmaceutical compositions for human and veterinary applications containing antimicrobial chemotherapeutics. Czech Patent application PV 2012-511

Jampílek J, Opatřilová R. Coufalová L, Černíková A, Dohnal J, inventors; University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Pharmacy, assignee (2013a) Utilization of alaptide as transdermal penetration modifier in pharmaceutical compositions for human and veterinary applications containing anti-inflammatory drugs and/or antimicrobial chemotherapeutics. WO/2013/020527 A1

Jampílek J, Černíková A, Opatřilová R, Dohnal, J, inventors; University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Pharmacy, assignee (2013b) Utilization of Alaptide as Transdermal Penetration Modifier in Pharmaceutical Compositions for Human and Veterinary Applications Containing Drugs of Central and/or Vegetative Nervous System and/or Sex Hormones or Genital System Modulators. Czech Patent application PV 2013-1000

Jampílek J, Opatřilová R, Řezáčová A, Oktábec Z, Dohnal J, inventors; University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Pharmacy, assignee (2014a) Alaptide: Methods of effecting its solubility, membrane penetration and pharmaceutical compositions for human and veterinary applications. WO/2014/019556 A1

Jampílek J, Opatřilová R, Dvořáková L, Brychtová K, Dohnal J, inventors; University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Pharmacy, assignee (2014b) Utilization of alaptide as transdermal penetration modifier in pharmaceutical compositions for human and veterinary applications containing nonsteroidal anti-inflammatory and/or antipyretic-analgesic drugs. Czech Patent CZ 304915 B6

Julínek O, Setnička V, Řezáčová A, Dohnal J, Vosátka V, Urbanová M (2010) Product of alaptide synthesis: determination of the absolute configuration. J Pharm Biomed Anal 53(4):958–961

Kasafírek E, Vanžura J, Krejčí I, Křepelka J, Dlabač A, Valchář M, inventors; SPOFA – United Pharmaceutical Works & Research Institute for Pharmacy and Biochemistry, assignees (1984 & 1986) 2,5-Piperazinedione derivs. Belgian Patent Belg. 897843. Czechoslovakian Patent CS 231227

Kasafírek E, Šturc A, Roubalová A (1992a) Linear tri- and tetrapeptides acting as prodrugs. Collect Czech Chem Commun 57(1):179–187

Kasafírek E, Rybák M, Krejčí I, Šturs A, Křepela E, Šedo A (1992b) Two-step generation of spirocyclic dipeptides from linear peptide ethyl ester precursors. Life Sci 50(3):187–193

Kasafírek E, Korbová L, Kohout J, Jirásková M, Krejčí I, Galatík A, inventors; SPOFA – United Pharmaceutical Works & Research Institute for Pharmacy and Biochemistry, assignees (1992c) Preparation for local therapy of cutaneous and mucosal lesions. Czechoslovakian Patent CS 276270

Kasafírek E, Krejčí I, Hliňák Z, Valchář M, Dobrovský K, Šturc A et al., inventors; SPOFA – United Pharmaceutical Works & Research Institute for Pharmacy and Biochemistry, assignees (1994) Neuroprotective composition for preventing or treating of central nervous system impairment. United States Patent US 5318973

Katz M, Ben-Shlush I, Kolusheva S, Jelínek R (2006) Rapid colorimetric screening of drug interaction and penetration through lipid barriers. Pharm Res 23(3): 580–588

Kezic S, Kammeyer A, Calkoen F, Fluhr JW, Bos JD (2009) Natural moisturizing factor components in the stratum corneum as biomarkers of filaggrin genotype: evaluation of minimally invasive methods. Br J Dermatol 161(5):1098–1104

Korbová L, Čížková J, Kohout J, Kasafírek E, Krejčí I, Vanžura J (1988) Action of cyclo(L-alanyl-1-amino-1-cyclopentanecarbonyl)-alaptide – on experimental gastric ulcers in rats. Cas Lek Cesk 127(51):1574–1577

Korbová L, Kohout J, Kasafírek E (1994) Action of orally administered alaptide on experimental gastric lesions in rats. Ces Slov Gastroenterol 48(4):170–182

Kosař K, Vanžura J (1988) Embryotoxicity of L-prolyl-L-leucyl-glycinamide, cyclo(1-amino-cyclopentanecarbonyl-alanyl) and cyclo(glycyl-leucyl), new potential neuropeptides in chick embryos. Pharmazie 43(10):715–716

Krejčí I, Dlabač A, Vanžura J, Kosnař J (1986) Effect of a spirocyclic cyclodipeptide derivative of MIF on passive avoidance behavior and amnesia in rats. Act Nerv Super 28(4):241–246

Lapka R (1991) Pharmacokinetics and brain entry of alaptide, a novel nootropic agent, in mice, rats and rabbits. J Pharm Pharmacol 43(12):874–876

Maixner J, Rohlíček J, Kratochvíl B, Šturc A (2009) X-ray powder diffraction data for alaptide, 8(S)-methyl-6,9-diazaspiro/4,5/decane-7,10-dione or cyclo(L-Alanyl-1-amino-1-cyclopentan carbonyl), cyclo(L-Ala-Acp). Powder Diffr 24(1):32–34

McGrath JA, Eady RA, Pope FM (2004). Anatomy and Organization of Human Skin. In: Burns T, Breathnach S, Cox N, Griffiths C (eds) Rook's textbook of dermatology, 7th edn. Blackwell Publishing, Oxford. pp 3.1–3.84

McGrath JA (2012) Profilaggrin, dry skin, and atopic dermatitis risk: size matters. J Invest Dermatol 132(1): 10–11

Meyer W, Schwarz K, Neurand K (1978) The skin of domestic mammals as a model for the human skin, with special reference to the domestic pig. Curr Probl Dermatol 7(1):39–52

Minaskanian G, Peck JV, inventors; Whitby Research, Inc., assignee (1992) Penetration enhancers for transdermal delivery of systemic agents. United States Patent US 5142044

Mrózek L, Dvořáková L, Mandelová Z, Rárová L, Řezáčová A, Plaček L et al (2011) Investigation of new acyloxy derivatives of cholic acid and their esters as drug absorption modifiers. Steroids 76(10–11): 1082–1097

Mrózek L, Coufalová L, Rárová L, Plaček L, Opatřilová R, Dohnal J et al (2013) New polyfluorothiopropanoyloxy derivatives of 5β-cholan-24-oic acid designed as drug absorption modifiers. Steroids 78(9):832–844

Nedvídková J, Kasafírek E, Nedvídek J, Pacák K, Schreiber V (1994) An analog of MIF, alaptide: effect on serum prolactin, dopamine receptors and growth of rat adenohypophysis. Endocr Res 20(1):39–46

Nedvídková J, Kasafírek E, Nedvídek J, Pacák K, Schreiber V (1994) An analog of MIF, alaptide: effect on serum prolactin, dopamine receptors and growth of rat adenohypophysis. Endocr Res 20(1):39–46

Ni N, El-Sayed MM, Sanghvi T, Yalkowsky SH (2000) Estimation of the effect of NaCl on the solubility of organic compounds in aqueous solutions. J Pharm Sci 89(12):1620–1625

Opatřilová R, Černíková A, Coufalová L, Dohnal J, Jampílek J (2013) In vitro permeation of micronized and nanonized alaptide from semi-solid formulations. Sci World J 2013:Article ID 787283 (8 pages)

Opatřilová R, Jampílek J (2014) Rapid screening of mupirocin skin permeation modification by micronized and nanonized alaptide. ADMET 2(1):56–62

Petersson M, Uvnäs-Moberg K (2004) Prolyl-leucyl-glycinamide shares some effects with oxytocin but decreases oxytocin levels. Physiol Behav 83(3):475–481

Rachakonda VK, Yerramsetty KM, Madihally SV, Robinson RL, Gasem KAM (2008) Screening of chemical penetration enhancers for transdermal drug delivery using electrical resistance of skin. Pharm Res 25(11):2697–2704

Rádl S, Kasafírek E, Krejčí I (1990) Alaptide. Drugs Future 15(5):445–447

Sekkat N, Kalia YN, Guy RH (2002) Biophysical study of porcine ear skin in vitro and its comparison to human skin in vivo. J Pharm Sci 91(11):2376–2381

Sloan KB, Beall HD, Taylor HE, Getz JJ, Villaneuva R, Nipper R, Smith K (1998) Transdermal delivery of theophylline from alcohol vehicles. Int J Pharm 171(2):185–193

Spier HW, Pascher G (1956) Analytical and functional physiology of the skin surface. Hautarzt 7(2):55–60

Šturc A, Kacafírek E, inventors; SPOFA – United Pharmaceutical Works & Research Institute for Pharmacy and Biochemistry, assignees (1992) Process for preparing cyclo-(L-alanyl-1-amino-1-cyclopentanecarbonyl) [alaptide]. Czechoslovakian Patent CS 277132

Vanžura J, Kosař K, Kasafírek E (1986) Inhibition of proliferative activity by cyclic dipeptides: spirocyclic derivatives of 1-aminocyclopentane-carboxylic acid. Toxicol Lett 31(3):189–193

Vinšová J, Kosař K, Kasafírek E (1993) Synthesis and antiproliferative activity of spirocyclic cyclodipeptides, derivatives of 1-amino-1-cyclobutanecarboxylic acid. Collect Czech Chem Commun 58(12): 2987–2993

Vinšová J, Kosař K, Kasafírek E (1994) Spirocyclic dipeptides of 1-amino-1-cyclohexanecarboxylic acid. Collect Czech Chem Commun 59(1): 195–202

Williams AC, Barry BW (1989) Urea analogues in propylene glycol as penetration enhancers in human skin. Int J Pharm 56(1):43–50

Yamane MA, Williams AC, Barry BW (1995) Terpene penetration enhancers in propylene glycol/water cosolvent systems: effectiveness and mechanism of action. J Pharm Pharmacol 47(12A):978–989

9 Fatty Alcohols, Fatty Acids, and Fatty Acid Esters as Penetration Enhancers

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9.1 Introduction

Fatty alcohols, fatty acids, and fatty acid esters are used in a variety of skin and general healthcare products, to name a few: moisturizing creams, shaving creams, shampoos, bath oils, lipsticks, and perfumed products. The wide usage of these as topical ingredients indicates that these are nontoxic and considered as safe for topical use. Pimecrolimus 1 % cream (Elidel® cream, Valeant Pharmaceuticals International, USA),

N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_9

tretinoin 0.02 % cream (Renova® cream, Valeant Pharmaceuticals International, USA), and fluorouracil 1 % topical cream (Fluoroplex®, Aqua Pharmaceuticals LLC, USA) are some examples of topical pharmaceutical formulations using fatty alcohols and/or fatty acids as penetration enhancers or cream base ingredients. Vivelle-Dot® (Novartis, USA) estradiol transdermal patch uses oleic acid as a permeation enhancer. Isopropyl palmitate is a component of pluronic lecithin organogel (PLO) base, and this base has been widely used for transdermal delivery of several drugs belonging to antiemetics, antihistaminics, and anti-inflammatory agents (Willis-Goulet et al. 2003; Richards et al. 2006; Krotscheck et al. 2004). Alpha-lipoic acid, a ring-containing fatty acid, is an active pharmaceutical ingredient (API) for treating photodamages of the skin and is deemed safe as a topical agent (Matsugo et al. 2011; Segall et al. 2004; Beitner 2003). Fatty alcohols and fatty acids have been extensively investigated to characterize their interactions with the stratum corneum (SC) and to describe their effects in modulating the skin barrier function (Williams and Barry 2012). This chapter deals with the utility of fatty alcohols, fatty acids, and their esters as promising percutaneous penetration enhancers for topical and transdermal delivery of drugs. The role of vehicle or other ingredients from the formulation on the enhancement effects of the topical or transdermal formulation and their skin irritation potential has also been discussed.

9.2 General Overview

Most fatty acids are straight-chain compounds with carbon chain lengths between 2 and 24. Medium chain (C6–C10) and long chain (C12–C24) fatty acids are utilized as skin penetration enhancers. Fatty acids with unsaturated carbon atoms and branched carbon chains, as well as fatty acid esters reported as skin penetration enhancers, are listed in Table 9.1. These have been used as penetration enhancers mainly for lipophilic drugs and to a lesser extent for hydrophilic permeants

Table 9.1 Fatty acids reported in the literature as skin penetration enhancers

Chemical name	Carbon atoms	M. Wt.
(A) Saturated fatty acids		
Linear hydrocarbon chain		
Butyric acid	4	88.1
Pentanoic acid (valeric acid)	5	102.1
Hexanoic acid (caproic acid)	6	116.1
Octanoic acid (caprylic acid)	8	144.2
Nonanoic acid (pelargonic acid)	9	158.2
Decanoic acid (capric acid)	10	172.3
Dodecanoic acid (lauric acid)	12	200.3
Tetradecanoic acid (myristic acid)	14	228.4
Hexadecanoic acid (palmitic acid)	16	256.4
Octadecanoic acid (stearic acid)	18	284.4
Eicosanoic acid (arachidic acid)	20	412.5
Docosanoic acid (behenic acid)	22	340.5
Tetracosanoic acid (lignoceric acid)	24	368.6
Branched hydrocarbon chain		
Pentan-2-oic acid (isovaleric acid)	5	102.1
2,2-Dimethyl pentanoic acid (neoheptanoic acid)	7	130.2
2,2-Dimethyl heptanoic acid (neononanoic acid)	8	158.2
2,2-Dimethyl octanoic acid (neodecanoic acid)	10	172.3
2-Heptyl undecanoic acid (isostearic acid)	18	284.4
(B) Unsaturated fatty acids		
cis-9-Tetradecenoic acid (myristoleic acid)	14:1	226.4
cis-9-Hexadecenoic acid (palmitoleic acid)	16:1	254.4
All cis-9,12,15-octadecadienoic acids (α-linolenic acid)	18:3	278.4
All cis-6, 9 and 12-octadecatrienoic acids (γ-linolenic acid)	18:3	278.4
All cis-9, 12-octadecadienoic acids (α-linoleic acid)	18:2	280.4
cis-11-Octadecenoic acid (asclepic acid)	18:1	282.4
cis-6-Octadecenoic acid (petroselinic acid)	18:1	282.4
trans-9-Octadecenoic acid (elaidic acid)	18:1	282.4
cis-9-Octadecenoic acid (oleic acid)	18:1	282.4

(continued)

Table 9.1 (continued)

Chemical name	Carbon atoms	M. Wt.
All cis-5, 8, 11, and 14-eicosatetranoic acid (arachidonic acid)	20:4	304.5
cis-11-Eicosenoic acid (gondoic acid)	20:1	310.5
cis-13-Docosenoic acid (erucic acid)	22:1	338.6
(C) Fatty acid esters		
Ethyl ethanoate (ethyl acetate)	4	88.11
Methyl propionate (methyl propylate)	4	88.11
Butyl ethanoate (butyl acetate)	6	116.16
Methyl pentanoate (methyl valerate)	6	116.16
Diethyl decanedioate (diethyl sebacate)	14	258.36
Methyl laurate	15	214.34
Ethyl oleate	20	310.51
Isopropyl decanoate	13	214.35
Isopropyl myristate	17	270.45
Isopropyl palmitate	19	298.50
Sucrose laurate	24	524.6

and peptides (Williams and Barry 2012). Table 9.2 provides examples of drugs with various physicochemical properties utilizing fatty acids and their esters as penetration enhancers. In many cases, the vehicles, for example, propylene glycol (PG) and ethanol, have strong influence on the efficacy of fatty acids in enhancing the skin permeation of drugs. A number of patents describing the utility of fatty acids and their esters or alcohols as enhancers in transdermal formulations have been reported; some examples are given in Table 9.3. These patents describe topical formulations containing different therapeutic agents.

Fatty alcohols have generally lower melting points and are more polar than corresponding fatty acids. Table 9.4 presents some frequently reported fatty alcohols as skin penetration enhancers. It is generally believed that they increase the diffusivity and partitioning of drugs across the skin by disrupting the highly ordered structure of the lipids of the skin barrier. The penetration enhancement is a consequence of interactions of fatty alcohols with the polar head group region, which results in the increased fluidity of the alkyl chains (Brian and Walters Brain and Walters 1993). It is difficult to classify fatty alcohols and fatty acids in terms of their efficacy as penetration enhancers, as their enhancement effect is dependent on the physicochemical properties of both the drug molecule and the enhancer. The influence of n-alkanols, alkyl pyrrolidones, alkyl diols, and alkyl dimethylamides as skin permeation enhancers on the permeation of steroid molecules was studied by Higuchi and coworkers (Warner et al. 2001; Kim et al. 1992; Yoneto et al. 1995). They demonstrated that the enhancer potency of these four homologous series was the same when enhancers of the same alkyl chain length were compared, i.e., the contribution of the hydroxyl, pyrrolidone, diol, and dimethylamide groups to the enhancer's potency was the same. This implies that the enhancer potency of fatty alcohols depends on their alkyl chain, and the contribution of the polar head group may not be very significant (Warner et al. 2001; Kim et al. 1992; Yoneto et al. 1995). These observations are in contrast to what has been reported in the study on permeation enhancement effects of fatty acids and alcohols on melatonin penetration across porcine skin (Kandimalla et al. 1999; Andega et al. 2001). Clear differences between different fatty acids and corresponding alcohols were noted in the above studies. Studies on the skin permeation enhancement abilities of lauric acid and lauryl alcohol indicated substantial difference between these two compounds; lauric acid showed 30-fold higher flux of naloxone through human skin as compared with lauryl alcohol (Aungst et al. 1986). Similarly, oleic acid and lauric acid induced a 3.5 and tenfold higher permeation of ondansetron HCl as compared to oleyl alcohol and lauryl alcohol, respectively (Gwak et al. 2004). In another study, oleic acid provided a severalfold higher skin permeation of diclofenac sodium as compared to oleyl alcohol (Kim et al. 2008). All these studies indicate that fatty acids are more potent penetration enhancers than fatty alcohols. However, oleyl alcohol showed 1.5-fold higher skin permeation of zolmitriptan than oleic acid from a transdermal patch (Subedi et al. 2011).

Table 9.2 Fatty acids and vehicles as penetration enhancers for different drug molecules

Drug	Fatty acid/fatty acid ester	Vehicle	Skin species	Enhancement factor	Reference
Vinpocetine	Sucrose laurate ester (11 %)	Proniosomes made with lecithin	Mice skin	5	El-Laithy et al. (2011)
Lidocaine	Sucrose laurate ester (0.25 %)	Propylene glycol	Mice skin	2.2	Okamoto et al. (2011) Okamato et al. (2005)
Lornoxicam	Linoleic acid (3 %) Oleic acid (3 %) Lauric acid (3 %) Capric acid (3 %) Caprylic acid (3 %)	Dimethyl sulfoxide	Mice skin	37 29 25 19 2	Lee and Chun (2012)
Memantine	Oleic acid (5 %)	Ethanol	Pig skin	3.5	Rio-Sancho et al. (2012)
5-Fluorouracil	Isopropyl myristate (5 %)	pH 7 phosphate buffer	Human epidermis	24	Singh et al. (2005)
Flurbiprofen	Oleic acid (5 %) Linoleic acid (5 %) Linolenic acid (5 %)	Carboxymethyl cellulose (CMC) Hydrogel (5 %)	Rat skin	7.6 8.0 9.0	Fang et al. (2003a, b)
Caffeine	Oleic acid (20 %) Linolenic acid (20 %) Palmitoleic acid (20 %)	Benzyl alcohol	Human epidermis (pretreatment)	6 5 75	Nanayakkara et al. (2005)
Propofol	Isopropyl myristate (5 %)	–	Rat skin	2	Yamato et al. (2009)
Levosimendan	Oleic acid (5 %)	40 % ethanol	Human epidermis	22.7	Valjakka-koskela et al. (2000)
Arginine vasopressin	Oleic acid (5 %) Linoleic acid (5 %) Linolenic acid (5 %)	Ethanol:water (2:1)	Rat skin	19.5 19.7 19.5	Nair and Panchagnula (2003)
Ketoprofen	Sucrose laurate ester	Propylene glycol	Mice skin	30	Okamato et al. (2005)
Diclofenac Na	Oleic acid (20 %) Lauric acid (20 %)	Transcutol®:water (3:1)	Rat skin	16.7 18.9	Escribano et al. (2003)
Ondansetron	Oleic acid Lauryl alcohol	60 % ethanol	Snake skin (pretreatment)	45.5 28.0	Takahashi and Rytting (2001)
Tenoxicam	Oleic acid (3 %) Linoleic acid (3 %) Lauric acid (3 %) Capric acid (3 %) Caprylic acid (3 %)	Propylene glycol	Mice skin	72.8 237.5 9.8 6.0 4.4	Gwak and Chun (2002)

Table 9.3 Fatty alcohols/acids reported as skin penetration enhancers in the patent literature

Drug	Fatty acid/fatty acid ester	Vehicle	Important claims	Patent reference
Ondansetron	3 % Oleic acid	Propylene glycol; ethanol	31-fold high permeation across human cadaver skin	Cho et al. (2001)
Interferon α2b (IFNα2b)	Palmitic acid-IFNα2b Conjugate (1:20)	0.1 % methyl cellulose gel	Eightfold high cutaneous deposition	Foldwari et al. (2002)
Rasagiline	Oleic acid Linolenic acid	Polyacrylate-Eudragit E100 Patch	1000 μg/48 h through mice skin	Deng et al. (2012)
Norethindrone acetate	6 % Oleic acid + 12 % linolenic acid	Duro-Tek: ethyl cellulose matrix	Good adhesiveness and low skin irritation was claimed. The system acts by push-pull mechanism	Carrara (2001)
Buprenorphine	10 % oleic acid	Propylene glycol	Sevenfold higher permeation across human cadaver skin	Sharma et al. (1993)
Alprazolam	1 % Lauric acid	Propylene glycol	45-fold higher permeation across guinea pig skin	Carrara et al. (2010)
Testosterone	5 % Lauryl alcohol +5 % isopropyl myristate	Adhesive matrix patch	1.7-fold higher permeation through human epidermis	Anigbogu et al. (2006)
Rotigotine	10 % isopropyl myristate	Adhesive (BIO-PSA® 7–4302) (patch)	2.6-fold higher permeation through guinea pig skin	Wang et al. (2008)

Table 9.4 Fatty alcohols reported as skin penetration enhancers

Chemical name	M. Wt.
(A) n-alcohols	
1-Decanol (capric alcohol)	158.28
1-Dodecanol (lauryl alcohol)	186.34
1-Tetradecanol (myristyl alcohol)	214.39
1-Hexadecanol (cetyl alcohol)	242.44
1-Octadecanol (stearyl alcohol)	270.50
(B) Iso-alcohols	
10-Methyl-1-hendecanol (isolauryl alcohol)	186.34
12-Methyl-1-tridecanol (isomyristyl alcohol)	214.39
14-Methyl-1-pentadecanol (isopalmityl alcohol)	242.44
16-Methyl-1-heptadecanol (isostearyl alcohol)	270.50

9.3 Fatty Alcohols

Ethyl alcohol has been widely employed as a penetration enhancer in several marketed topical and transdermal formulations [e.g., Estraderm® patches (Novartis, USA), AndroGel® gel (AbbVie Inc., USA), Estrasorb® topical emulsion (Medicis Pharmaceutical Corp., USA), and Nimulid® gel (Panacea Biotec Ltd., India)] and is often the solvent of choice in transdermal patches. Ethyl alcohol can be used up to 74 % in transdermal gels such as testosterone gel (AndroGel®) and estradiol gel (Estrogel®) (Accessdata.fda.gov). The penetration enhancement effect increases with the increase in ethanol volume fraction up to 60 %

Table 9.5 Steady-state flux (μg/cm²/h ± SD) values of thalidomide and its *N*-alkyl analogs from a series of n-alcohols

	Steady-state flux (μg/cm²/h ± SD)	
Vehicle	Thalidomide	*N*-methylthalidomide
Methanol (C1)	0.147 ± 0.005	4.498 ± 0.220
Ethanol (C2)	0.066 ± 0.053	2.819 ± 0.391
Propanol (C3)	0.039 ± 0.002	0.822 ± 0.096
Butanol (C4)	0.037 ± 0.016	0.813 ± 0.196
Pentanol (C5)	0.039 ± 0.014	0.722 ± 0.034
Hexanol (C6)	0.028 ± 0.007	0.468 ± 0.037
Heptanol (C7)	0.029 ± 0.002	0.250 ± 0.016
Octanol (C8)	0.035 ± 0.010	0.185 ± 0.003
Nonanol (C9)	0.036 ± 0.018	0.174 ± 0.008
Decanol (C10)	0.018 ± 0.001	0.351 ± 0.220
Undecanol (C11)	0.010 ± 0.003	0.223 ± 0.040
Dodecanol (C12)	0.025 ± 0.004	0.172 ± 0.030

Goosen et al. (2002)

Table 9.6 Steady-state flux values of melatonin across rat and porcine skin using saturated fatty acids

Rat Melatonin flux (μg/cm²/h)		Pig Melatonin (μg/cm²/h)	
Control	5.29 ± 0.21	Control	5.34 ± 0.59
Nonanoic acid	20.31 ± 1.92	Nonanoic acid	7.77 ± 0.55
Decanoic acid	34.38 ± 2.43	Decanoic acid	18.79 ± 1.59
Undecanoic acid	45.33 ± 2.72	Undecanoic acid	23.70 ± 2.64
Lauric acid	32.30 ± 1.70	Lauric acid	24.98 ± 1.45
Myristic acid	18.75 ± 0.68	Myristic acid	17.29 ± 1.31

Kandimalla et al. (1999)

volume fraction for estradiol and 80 % volume fraction for testosterone, above which a reduction in the permeation rate was observed (Kim et al. 2000; Altenburger et al. 1998). Furthermore, lipid vesicles with high ethanol content are tailored for enhanced transdermal delivery of active agents (Godin and Touitou 2003).

Goosen et al. (2002) reported that short chain alkanols (C1 to C6) were better in enhancing the permeation of methyl thalidomide than medium chain alkanols (C7 to C12). The steady-state flux was highest with C1 (methanol), and the flux decreased linearly with the increase in the carbon chain length of alkanol up to C6 (hexanol). The medium chain alkanols (C7 to C12) showed a parabolic relationship (Tables 9.5 and 9.6) between the flux of methyl thalidomide and the carbon chain length of the alkanol with peak permeation rate at C10 (decanol). Sloan et al. (1998) reported that the flux of theophylline from a propylene glycol vehicle across hairless mouse skin was the least for methanol (C1); increased almost 480-fold by pentanol (C5); further increased by hexanol (C6), heptanol (C7), octanol (C8), and nonanol (C9) to a peak level to 656-fold; and then decreased to tenfold by undecanol (C11).

The enhancement effect of unsaturated fatty alcohols with one, two, or three double bonds (oleyl alcohol, linoleyl alcohol, linolenyl alcohol, respectively) and saturated fatty alcohols (C8 to C14) on melatonin permeation across porcine and human skin was studied (Andega et al. 2001). Linoleyl alcohol with two double bonds showed highest permeation-enhancing effect among the three unsaturated fatty alcohols, both in porcine and human skin. However, a decrease in the permeation was observed with the fatty alcohol with three double bonds (linolenyl alcohol). However, it has been found that the fatty alcohols which provided greater permeation of melatonin (decanol, undecanol, and dodecanol) also produced greater transepidermal water loss (TEWL), skin blood flow, and erythema in hairless rats (Kanikkannan and Singh 2002). Octanol and nonanol were found to be the most useful enhancers for the transdermal delivery of melatonin considering their lower skin irritation and a reasonably good permeation enhancement effect. The influence of 10 % saturated alcohols (C8 to C18) in PG on the flux of naloxone through the human skin was studied (Aungst et al. 1986). A parabolic effect of alkyl chain length with C12 being most effective was demonstrated. It was also shown in the same study that with an increase in the number of double bonds in the C18 fatty alcohol, the permeation of naloxone increased.

When alkanols were administered in isopropyl myristate as a vehicle, the enhancement potency for benztropine mesylate permeation increased linearly with increasing the carbon number of the alcohol present in the binary mixture (Gorukanti et al. 1999). In case of a polar, nonelectrolyte permeant, nicotinamide, penetration enhancement versus alkanol chain length

profile across hairless mouse skin was parabolic with a peak permeation rate at C6 (Kai et al. 1990). The authors suggested that the principal mechanism by which alkanols enhance percutaneous absorption of polar penetrants is by extraction of SC intercellular lipids. Medium chain aliphatic alcohols (C8–C12) enhanced the permeation of both hydrophilic and lipophilic model drugs (6-carboxyfluorescein and indomethacin) through excised rat skin (Seki and Morimoto 2003). The enhancing effects of the aliphatic alcohols for 6-carboxyfluorescein and indomethacin decreased with the increase in carbon chain length. This study indicates that the aliphatic alcohols can be used as permeation enhancers for both hydrophilic and lipophilic drugs. Biphasic fluxes (initial high flux followed by a lower flux) were noted for melatonin permeation across rat skin with various saturated and unsaturated fatty alcohols (Kandimalla et al. 2009).

The influence of hydrocarbon chain branching or positioning of polar head group (–OH) in the alkyl chain on the permeation enhancement effect was also examined. The effects of x-heptanol, x-octanol, and x-nonanol (where x is the position of the hydroxyl group ranging from 1 up to 5) on the transport of a probe permeant, corticosterone across hairless mouse skin was investigated. The enhancer potencies of 2-alkanol, 3-alkanol, 4-alkanol, and 5-alkanol were approximately 1.9-, 2.6-, 3.1-, and 3.9-fold lower, respectively, than those of 1-alkanols of the same molecular formula. This suggests that the branched chain alkanols have lower enhancer potency than 1-alkanols of the same molecular formula, i.e., the potency decreases as the hydroxyl group moves from the end of the chain toward the center of the enhancer alkyl chain. Branching of the alkyl chain reduces the ability of the enhancer to effect lipid fluidization in the SC lipid lamellae at the target site (Chantasart et al. 2004).

9.4 Fatty Acids

Fatty acids have been extensively studied as skin penetration enhancers for the development of successful topical and transdermal delivery systems of different classes of drugs. Structurally, fatty acids consist of an aliphatic hydrocarbon chain and a terminal carboxyl group. Generally C6 to C14 saturated fatty acids have been widely reported as skin penetration enhancers. Also, oleic acid, a C18 unsaturated fatty acid, is one of the most extensively studied penetration enhancers among the fatty acids (Yu et al. 2001; Fang et al. 2003a, b; Hathout et al. 2010; Zakir et al. 2010)

There are several reports on the effect of carbon chain length of fatty acids on the skin permeation enhancement of drugs. The enhancement effect of fatty acids on the permeation of piroxicam was studied in rat skin, and it decreases linearly with increasing carbon number of saturated fatty acid from 12 to 18 (Hsu et al. 1994). Similarly, the permeation-enhancing effect of saturated fatty acids for melatonin through excised hairless mouse skin decreased with the increase in the carbon chain length from C12 to C18 (Oh et al. 2001). In another study, skin permeation rate of melatonin across excised rat skin increased C11, and then the permeation rate decreased (Kandimalla et al. 1999). Among a series of saturated fatty acids investigated as permeation enhancers for propranolol in rabbit skin, C12 and 14 saturated fatty acids were the most potent agents in increasing the permeation of propranolol from gel formulations (Ogiso et al. 1990). Among C8, C12, and C14 saturated fatty acids, C12 acid was found to be the optimum permeation enhancer for flurbiprofen across rat skin (Chi et al. 1995). In another investigation, the skin permeation of albuterol across hairless mouse skin was studied using 0.5 % hydroxyl propyl cellulose gel (Klucel®, Hercules Incorporated, USA) containing C10, C12, and C14 acids as penetration enhancers. The formulation containing C12 acid and albuterol in 2:1 molar ratio enhanced the skin permeation by at least 3.0-fold albuterol as compared to other fatty acids (Chisty et al. 2002).

It has been proposed that acids with a certain chain length, i.e., around 12 carbons, possess an optimal balance between partition coefficient and affinity to the skin (Ogiso 1990). Shorter chain fatty acids would have insufficient lipophilicity

for the permeation into the skin, whereas longer chain fatty acids would have much higher affinity to lipids in the SC thereby retarding their own permeation and that of other permeants. This suggests that the mode of action of saturated fatty acids as enhancers is dependent on their own permeation across the stratum corneum/skin (Tanojo et al. 1997; Aungst et al. 1990).

It has been well established that unsaturated fatty acids are more potent permeation enhancers than the saturated species (Oh et al. 2001). Chi et al. (1995) compared the permeation enhancement effects of saturated and unsaturated fatty acids for flurbiprofen. Among saturated fatty acids (C10, C12, and C14 fatty acids), only C12 acid was effective as a penetration enhancer. The permeation rate of flurbiprofen increased by 18-fold with the addition of linolenic acid (C18:3). The other unsaturated acids (oleic, palmitoleic, linoleic, and arachidonic acid) showed less potent enhancing effect for flurbiprofen. Fang et al. (2003a, b) evaluated the efficacy of unsaturated fatty acids for the enhancement of flurbiprofen permeation through mouse skin. Unsaturated fatty acids (linolenic, linoleic, oleic) provided the greatest enhancement of flurbiprofen permeation compared to terpene class of enhancers. The flux of flurbiprofen increased with the increase in the number of double bonds of the unsaturated fatty acid (linolenic acid > linoleic acid > oleic acid). However, oleic acid produced higher skin retention of flurbiprofen than the other unsaturated fatty acids.

Gwak et al. (2004) investigated the effects of different penetration enhancers (fatty acids and fatty alcohols) on the in vitro permeation of ondansetron hydrochloride across hairless mouse skin. The greatest flux was attained by unsaturated fatty acids; the enhancement factors with the addition of 3 % oleic acid or linoleic acid to propylene glycol were about 1250 and 450, respectively. Saturated fatty acids failed to show a significant enhancing effect. In another study by Gwak et al. (2002), utilizing the same experimental design, reports that the enhancement factors for tenoxicam with the addition of oleic acid or linoleic acid to PG were 348 and 238, respectively, whereas the saturated fatty acids (lauric, capric, and caprylic acids) had no effect on the skin permeation of tenoxicam. Oleic acid has been shown in numerous studies to be an effective skin permeation enhancer (Dimas et al. 2004; Thomas and Panchagnula 2003; Valjakka-Koskela et al. 2000; Giannakou et al. 1998), while stearic acid usually showed no permeation-enhancing effect (Oh et al. 2001). Oleic acid in association with PG has been found to be a very potent enhancer for venlafaxine in a carbomer gel base (Baek et al. 2012). Furthermore, it has been reported that penetration of hydrophilic fluorescent nanoparticles into the skin can be greatly enhanced by the addition of oleic acid, which can be used for therapeutic purposes (Lo et al. 2012). Oleic acid modified chitosan nanoparticles increased the deposition of a peptide with 11 amino acids (known as Spantide II) and ketoprofen by severalfolds as compared to unmodified nanoparticles (Shah et al. 2011).

As the number of double bonds in fatty acids increased from one (oleic acid) to two (linoleic acid), there was a substantial increase in the flux of naloxone. However, an increase in the number of double bonds to three (linolenic acid) did not increase the flux further (Aungst et al. 1986). Golden et al. (1987) evaluated the effects of position and configuration of unsaturated (18:1) fatty acids using porcine SC and a vehicle containing 0.15 M fatty acid in ethanol. The *cis* isomers were effective permeation enhancers, whereas the corresponding *trans* isomers had less or no enhancing effect.

The effect of the number of double bonds (in cis-conformation) in straight chain polyunsaturated acids on the permeation of para-aminobenzoic acid was studied in human SC (Tanojo et al. 1997). Compared to a monounsaturated fatty acid, polyunsaturated fatty acids – linoleic, linolenic, and arachidonic acids with, respectively, 2, 3, and 4 double bonds – produced a significantly higher increase in the permeation of para-aminobenzoic acid. However, there was no significant difference in effects among the polyunsaturated fatty acids. Carelli et al. (1992) also reported that the enhancement of flux of alprazolam by linoleic acid (two double bonds) was greater than that of oleic acid (one double bond) through hairless mouse skin. In contrast, Morimoto et al. (1996) reported that the flux of indomethacin was unaffected by the number of double bonds.

An earlier investigation showed that among unsaturated fatty acids, oleic acid is an effective skin penetration enhancer for polar and nonpolar drugs (Barry 1987). Cis unsaturated fatty acids (viz., oleic acid, linoleic acid, and linolenic acid) have been reported to form separate domains within SC lipids, which effectively decrease either diffusional path length or the resistance (Ongpipattanakul et al. 1991).

The combination of fatty acids and iontophoretic delivery can result in enhanced permeation of many drugs. It has been suggested that skin appendages play important role in electro transportation for ionic drugs. Since fatty acids enhance permeation by selective perturbation in the intercellular lipids of the SC and ions are known to take the path with less resistance, combined use of iontophoresis and fatty acids is an alternative that researchers have used to increase the permeation of not only low molecular weight drugs but also high molecular weight proteins such as insulin and several small peptides (Bhatia and Singh 1998; Bhatia et al. 1997; Rastogi and Singh 2005).

9.5 Fatty Acid Esters

Methyl or propyl esters of medium chain fatty acids (C8–C12) enhanced the permeation of 6-carboxyfluorescein (hydrophilic) and indomethacin (lipophilic) through rat skin. Although the relationship between the structure and skin permeation-enhancing effect of the fatty acid esters used in this study is not yet fully understood, they are possible candidates for permeation enhancers for hydrophilic and lipophilic drugs (Seki and Morimoto 2003). Song et al. (2001) investigated the effects of oleic acid and of a group of chemically related cis- (ricinoleic acid) and trans- (ricinelaidic acid) 12-monohydroxylated derivatives and their corresponding ethyl and methyl esters on the skin permeation of a hydrophobic (hydrocortisone) and hydrophilic (5-fluorouracil) model drug using excised hairless mice skin. Whereas the addition of oleic acid markedly enhanced the transdermal flux of both drugs relative to PG alone (hydrocortisone approximately 1800-fold; 5-fluorouracil approximately 330-fold), the addition of a cis- or trans-12-monohydroxylated analog of oleic acid resulted in only a small flux increase (threefold for hydrocortisone and sevenfold for 5-fluorouracil). On the other hand, the methyl and ethyl esters of cis- and trans-12-hydroxy-9-octadecenoic acid exerted a much greater enhancing effect (up to 720-fold for hydrocortisone and up to 74-fold for 5-fluorouracil) than the corresponding parent fatty acids.

The effects of a series of polyol fatty acid esters (Sefsol®, Nikko Chemicals Co. Ltd., Japan) on diclofenac permeation through rat skin were investigated (Takahashi et al. 1996; 2001). Among four monoesters and one diester of sefsol, all monoesters except the glyceryl monoester enhanced the percutaneous permeation of diclofenac. The highest enhancement was observed with propylene glycol monocaprylate.

Different sucrose fatty acid esters were used as penetration enhancers in methacrylic polymer-based transdermal patches using metoprolol as a model drug. The release and permeation model studies indicated that shorter fatty acid chain length sucrose esters increased the drug release about tenfold than the patch with no enhancer (Csóka et al. 2007).

Isopropyl myristate is one of the widely studied esters among esters of fatty acids. This and other esters generally act by partitioning themselves in the ordered lipid domains of the SC (Karande and Mitragotri 2009). Binary combinations of isopropyl myristate and short chain alkanols show transdermal flux enhancement of estradiol when compared to alkanols alone (Goldberg-Cettina et al. 1995). A binary combination of isopropyl myristate and n-methyl pyrrolidone (25:75) significantly improved lidocaine flux across human skin showing an enhancement of 25-fold over isopropyl myristate alone (Lee et al. 2006).

9.6 Chemical Modification of Drugs with Fatty Acids and Fatty Acid Esters

Yamamoto et al. (2003) synthesized three novel lipophilic derivatives of phenylalanyl-glycine (Phe-Gly), C4-Phe-Gly (butyric acid conjugate),

C6-Phe-Gly (caproic acid conjugate), and C8-Phe-Gly(caprylic acid conjugate) by chemical modification. The effect of acylation on the stability, permeability, and accumulation of Phe-Gly in rat skin was investigated. The stability (in skin homogenates) and permeability of Phe-Gly was improved by chemical modification with fatty acids, and this enhanced permeability of Phe-Gly due to acylation may be attributed to the protection of Phe-Gly from enzymatic degradation in the skin and the increase in the partition of Phe-Gly into the SC. Of all the acyl-Phe-Gly derivatives, C6-Phe-Gly was the most permeable compound across the skin with twofold higher permeation than the parent peptide (Phe-Gly).

Setoh et al. (1995) studied in vitro permeation of tetragastrin and various fatty acid conjugates of tetragastrin through rat skin. The permeation of tetragastrin across intact skin was improved by chemical modification with acetic acid and butyric acid. However, tetragastrin and caproyl-tetragastrin did not permeate across intact skin. The stability of tetragastrin in skin homogenate was also significantly improved by chemical modification with fatty acids. Various fatty acid ester derivatives of cycloserine were synthesized to improve skin permeation of cycloserine (Thorsteinsson et al. 2002). The skin permeation of cycloserine across the hairless mouse skin increased 20-fold by the fatty acid esters compared to unmodified parent drug indicating their potential use in the treatment of various skin infections.

Yahalom et al. (1999) synthesized analogs of gonadotropin-releasing hormone (GnRH) with various aliphatic acids (C2, C6, C12, and C18 acids) to improve the transdermal delivery of GnRH. The transdermal penetration of the peptides was evaluated by in vivo functional experiments in rats. According to these studies, the efficiency of penetration is gradually lowered increasing the hydrocarbon chain length of the conjugate peptide, in the order of C2, C6, C12, and C18. The skin permeation of the peptide dramatically decreased by the attachment of a fatty acid to GnRH, either due to significantly increased molecular weight or due to substantial conformational changes.

Skin permeation and accumulation profiles of ketorolac stearate (C18:0), a model of a highly lipophilic fatty ester as local topical agent, were studied. No permeation and minimal skin accumulation of ketorolac stearate could indicate a possibility for designing safer topical agents without systemic absorption (Bhandari et al. 2008). Similarly, ketorolac linoleate (C18:2), oleate (C18:1), and stearate (C18:0) esters were found to be highly lipophilic, chemically stable, and enzymatically susceptible (to hydrolysis) in hairless mice skin and did not permeate into the receptor solution, indicating their safe use as local topical agents without their systemic absorption (Bhandari et al. 2007).

Morphine propionate and morphine enanthate prodrugs were more lipophilic than morphine, and these prodrugs enhanced transdermal drug delivery by two- and fivefold, respectively, as compared to morphine (Wang et al. 2010).

9.7 Penetration Enhancement Mechanisms

Fatty alcohols and fatty acids act as permeation enhancers due to their interaction with the skin barrier by one or more of the following mechanisms.

9.7.1 Lipid Disruption at the Intercellular Level: The Enhancer Disrupts Sc Lipid Organization, Making It Permeable to Drugs

The lipids of SC are mainly ceramides, cholesterol, and free fatty acids. The acyl chain length of ceramides and free fatty acids is between C22 and C26. Cholesterol is present in small amounts (typically 2–5 % w/w of total lipids). The intercellular lipids are arranged in an ordered structure of multiple bilayers of polar and nonpolar regions. Several studies have demonstrated that fatty acids increase the fluidity of SC lipids by disrupting the packing order of alkyl chains of lipids in SC (Chantasart et al. 2004; Aungst et al. 1990; Jasti and Abraham 1998).

Unsaturated fatty acids at 5 % w/v in ethanol (oleic acid, linoleic acid, linolenic acid) induced perturbation or increased bilayer fluidity of SC as observed by Fourier transform infrared spectroscopy (Jain et al. 2002). Oleic acid is readily absorbed into SC and increases rotational movement of the hydrocarbon chains and decreases the order of bilayer structure (Francocur et al. 1990; Gay et al. 1989). It was also proposed that oleic acid disrupts the packed structure of the intercellular lipids because of the incorporation of its *cis* double bond. In contrast, the corresponding C18 saturated compound, stearic acid, even though is a major component of SC is not efficient enough to increase the fluidity of ceramides (Neubert et al. 1997). This explains why oleic acid is a significantly more effective permeation enhancer than stearic acid.

9.7.2 Fatty Acids Form Solvated Complexes or Molar Addition Compounds (Ionic Compounds) and Permeate Through the Skin Simultaneously with Drugs

The transdermal delivery of tamoxifen and linolenic acid from borage oil across human skin was monitored. Initial uptake of vehicle into the SC would be a consequence of the miscibility of the vehicle and the intercellular lipids of the SC. The dermis is polar in nature compared to the SC which is reach in lipids, and it is generally perceived as a barrier to the ingress of highly lipophilic molecules. From the relatively high amounts of both tamoxifen and linolenic acid that permeated the skin, it appears that the driving force of the vehicle was sufficient to overcome the dermis as a barrier. From this, it appears that the rate of permeation of linolenic acid and tamoxifen from the oil was linked to that of solute via a fixed solvation cage, rather than discrete molecules (Karia et al. 2004). When nonsteroidal anti-inflammatory drugs (NSAIDs), ibuprofen or ketoprofen, were applied to pig ear skin in a fish oil vehicle, similar high fluxes of ibuprofen or ketoprofen concomitant with eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids were obtained (Heard et al. 2003). In the same study, it was found that the rate of permeation of NSAID was linked to the rate of permeation of EPA and DHA in the fish oil, suggesting permeation involved fixed NSAID/triacylglycerol complexes. Consequently, the permeation rates observed pertain not only the permeant but also the *overall* solvated complex.

Stott et al. (2001) demonstrated the formation of 1:1 molar addition compounds of propranolol with lauric or capric acid from their binary mixtures (as determined by FT-IR). The addition compounds are formed by interaction between the carbonyl group of the fatty acid and the amino group of the β-blocker, to form a salt. The oppositely charged species of the salt have been shown to permeate the human epidermal membrane by an ion-pair mechanism. Green and Hadgraft (1987) reported similar findings suggesting the formation of ion pairs between metoprolol or oxprenolol and lauric and oleic acids.

9.7.3 Fatty Acids Increase the Diffusivity and Partitioning of Drugs and Vehicles in/Through Sc

The permeation of a drug into/through the skin is influenced by: (1) permeation of vehicle into SC and (2) affinity of drug to the vehicle. If the vehicle permeates the skin readily and the drug has high affinity to the solvent, the permeation through the skin can be increased by "solvent drag mechanism" (drug-vehicle permeating together) (Aungst et al. 1990). Propylene glycol (PG) is able to permeate the skin better than mineral oil. The PG-fatty acid mixtures are able to permeate the skin better than MO-fatty acid mixtures. Also, the permeation of PG-long-chain fatty acid mixture is less than the PG-short-chain fatty acid (Wang et al. 2004). While PG-palmitic acid localized in the SC lipids, PG-myristic acid was able to penetrate the deeper epidermal layers of the skin (Cotte et al. 2004). This indicates a mutual increase in the permeation of fatty acids and propylene glycol under the influence of each

other, and this explains "the solvent drag mechanism." In a recent study, it has been determined that fatty acids act as permeation enhancers mainly through the enhancement of permeant partitioning into the intercellular lipid domain of the SC (Ibrahim and Li 2010).

A "push-pull mechanism" of enhancement by fatty acids (combination of lauric acid and oleic acid) was proposed for steroidal hormones, like estradiol, testosterone, and norethindrone (Carrara 2001). It is possible that fatty acids are mainly distributed to the SC because of their lipophilicity, where they interact with the SC lipids causing a "pull effect" for the drug. Further, fatty acids that remain within the formulation alter the thermodynamic activity by affecting the solubility of the active agent within the formulation causing a "push effect." The use of combination of penetration enhancers of the same chemical family resulted in sustained and controlled percutaneous absorption of the drugs from an adhesive matrix formulation. Various enhancers, such as fatty acids (saturated, unsaturated), glycerides, and nonionic surfactants, were incorporated in the loratadine-EVA matrix to increase the rate of skin permeation of loratadine from an EVA matrix. The drug and enhancer concentration of the matrix were not revealed. Fatty acids generally provided higher flux values as compared to other classes of enhancers. Myristic and linolenic acids enhanced flux by 3.3- and 3.9-fold higher flux values as compared to the matrix patch with no enhancer (Cho et al. 2009).

9.8 Formulation Considerations

Many studies in the past have assessed the permeation-enhancing activity of compounds as a result of placing the pure enhancer or its solutions onto the skin surface. This may not be relevant to the incorporation of an enhancer into a transdermal system, and that must be designed such that both the active drug and enhancer are released into the skin. During the pre-formulation stage of transdermal product development, the concentration of the enhancer, its chemical constitution, and its compatibility with polymer or gel components must be evaluated by the development scientist. The enhancers to be incorporated into the final transdermal devices should possess the following attributes: (1) compatible with formulation ingredients and device components, (2) chemically stable in the system, (3) promote drug release from the system and be also released to act on the skin to reduce its barrier function, and (4) non-irritating, non-sensitizing, non-phototoxic, and pharmacologically inert.

Fatty acids, fatty alcohols, and fatty acid esters attracted a lot of attention as some of these compounds are classified as generally recognized as safe (GRAS) by Food and Drug Administration (FDA). Few examples of GRAS status compounds include oleic acid, stearic acid, oleyl alcohol, cetyl alcohol, stearyl alcohol, isopropyl palmitate, isopropyl myristate, ethyl oleate, etc.

Despite the knowledge that fatty acids are generally irritant, there are several patents which propose their use in transdermal drug delivery and ways to minimize the skin irritation (Table 9.3). The fatty acids may be skin irritants in their undiluted form, but the skin irritation can be suppressed in the diluted form in the formulation and by means delivering the enhancer in a controlled release dosage form such as transdermal patch (Hille et al. 1993). One of the requirements for transdermal patches is the stability of the drug as well as enhancer during the shelf life of the patch. Transdermal systems with a multiple layer design may contain nonhomogeneous distribution of the enhancer. In some cases, the enhancer will migrate into different layers in the entire matrix until equilibrium is established.

The technological aspects of fatty acids and alcohols in transdermal formulations have not been systematically investigated. There are not many papers dealing with the incorporation of fatty acids and alcohols into transdermal patches. A polyacrylate or polyisobutylene adhesive patch containing saturated or unsaturated fatty acids (C6–C18) and estradiol or estradiol/progestin mixture as active drugs was described (Gonella 1997). This patent claims a non-irritating patch for a three-day delivery of hormones in the hormone replacement therapy. A novel formulation of a monolithic transdermal patch comprising a combination of fatty acids and/or fatty alcohols as penetration enhancers was described (Carrara 2001). This patent claims that a combination of

oleic and lauric acid acts as the most adequate composition for enhancing the skin permeation for many active agents. It was also claimed in the patent that a combination of fatty acid(s) and/or fatty alcohol(s) with different chain lengths as a penetration enhancer provides controlled drug permeation rates at all application times.

Fatty alcohols (5 % oleyl alcohol and lauryl alcohol) were incorporated as penetration enhancers into monolithic adhesive matrix type patches containing 20 % captopril. These enhancers provided at least twofold higher permeation rates as compared to oleic acid, dimethyl sulfoxide, N-methyl pyrrolidone, and Transcutol (Park et al. 2001). Oleic acid and propionic acid were incorporated into a transdermal patch formulation containing physostigmine (Jenner et al. 1995; Kochinke et al. 1994). It was shown that inclusion of oleic acid allowed the amount of physostigmine and the size of the transdermal patch to be substantially reduced, while maintaining effective drug delivery rates. The formulation containing oleic acid was nonirritant to guinea pigs when applied to the skin for 48 h.

Different fatty alcohols and fatty acids were incorporated at 5 % w/w into acrylic adhesive transdermal patches of melatonin. Decanol, myristyl alcohol, and undecanoic acid provided significantly higher drug flux values through hairless rat skin than patches without enhancers (Kanikkannan et al. 2004).

A meloxicam transdermal patch comprising fatty acid derivatives (sorbitan fatty acid derivative, a polyglyceryl fatty acid derivative, a polyethylene glycol vegetable oil ester) in an acrylic polymer coated on a backing layer has been described (Choi 2007). Transdermal delivery system comprising glycopyrrolate and a combination of fatty acids or esters (such as oleic and lauric acid esters) in an adhesive polymer matrix to treat sialorrhea has been described (Dillaha 2008).

9.9 Skin Irritation Potential

Many fatty acids and their esters are generally recognized as safe (GRAS) as mentioned in section 8 and have been used in personal care products, topical and transdermal products. However, many fatty acids and alcohols are reported to cause skin irritation (Kozema et al. 1998). Studies on a series of saturated and unsaturated fatty acids of different chain lengths under occlusive patch test revealed that the saturated fatty acids of carbon chain length C8 to C12 and C18 dienoic unsaturated fatty acid (linoleic) were most harmful irritants to human skin (Stillman et al. 1975). In general, unsaturated fatty acids cause more skin irritation than saturated fatty acids (Tanojo et al. 1998; Boelsma et al. 1997). A recent study demonstrated that unsaturated fatty acids at an extremely low concentration (0.0015 % in propylene glycol) induced the production of prostaglandin-E2 (Fang et al. 2003a, b). Furthermore, unsaturated fatty acids increased interleukin-1 alpha, interleukin-8, and interleukin-8 mRNA levels in cultured epidermis (human skin equivalent), whereas saturated fatty acids were not effective (Boelsma et al 1996). It was also shown that a low level of oleic acid (0.01–0.03 %) is capable of elevating interleukin-1α and interleukin-lα mRNA levels in the living epidermal cell layers in submerged keratinocyte cultures (Boelsma et al. 1996). Oleic acid has been reported to cause changes in the morphology of the Langerhans cells that are located in the super basal layer of the epidermis and play a key role in the initiation and coordination of the T-cell-mediated immune response. Their depletion from the epidermis can cause skin immunosuppression (Touitou et al. 2002).

Application of a 5 % oleic acid/propylene glycol vehicle to the skin of six human subjects for 6 h resulted in a minor irritation; however, severe irritation occurred with a 20 % oleic acid/propylene glycol vehicle (Loftsson et al. 1987). A 5 % oleic acid in 66.6 % ethanol (gelled with hydroxyl propyl methyl cellulose) induced significant histopathological changes (collagen fiber swelling, inflammatory cell infiltration, and subepidermal edema) in rat skin (Narisetty and Panchagnula 2004). An aqueous vehicle containing 10 % oleic acid was applied to the skin of nude mice for 24 h under occlusion and resulted in ulcerative eruptions, hyperplasia, and edema of the epidermis and inflammation of the dermis. While 10 % oleic acid was severely irritating to nude mice skin, 10 % oleyl alcohol induced no discernible change in the histological appearance of the skin (Lashmar et al. 1989).

Aungst (1995) stated three approaches to separate the skin irritation of fatty acids from their permeation-enhancing effects: (1) to control the concentration and delivery of fatty acids; (2) selection of less irritating fatty acids, e.g., myristic acid; and (3) inclusion of other ingredients in the vehicle to overcome the skin irritation induced by fatty acids, e.g., glycerin, vitamin E, and squalene. As described in the previous section, there are several patents utilizing fatty acids as skin penetration enhancers in the transdermal formulations, and these were claimed to be effective and non-irritating to the skin.

Conclusion

Fatty alcohols, fatty acids, and their esters are effective in enhancing the skin permeation of several classes of drugs. Many compounds belonging to these classes are approved by FDA for the use in topical and transdermal products. Saturated fatty acids and alcohols of medium chain length (in particular, capric lauric and myristic) and unsaturated fatty acids or alcohols (oleic, linoleic, and linolenic) are frequently reported to be more effective. However, formulations that showed maximum permeation also showed skin irritation. Therefore, the selection of a compound, its concentration, and controlled release dosage form are of crucial importance in overcoming skin irritation. Fatty acid esters and fatty acid ester prodrugs also have been frequently reported for enhancing transdermal delivery of drugs. The effects of fatty acids and fatty alcohols are highly dependent on the vehicle, with propylene glycol generally providing maximum permeation enhancement.

References

Altenburger R, Rohr UD, Kissel T (1998) Rate control in transdermal beta-estradiol reservoir membrane systems: the role of membrane and adhesive layer. Pharm Res 8:1238–1243

Andega S, Kanikkannan N, Singh M (2001) Comparison of the effect of fatty alcohols on the permeation of melatonin between porcine and human skin. J Control Release 77(1):17–25

Anigbogu A, Roy S, Anteljevic V (2006) Formulations and methods for enhancing the transdermal penetration of a drug.US Patent 0,065,494 A1, 31 Jul 2006

Aungst BJ (1995) Fatty acids as skin permeation enhancers. In: Smith E, Maibach HI (eds) Percutaneous penetration enhancers. CRC Press, New York, pp 277–287

Aungst BJ, Rogers NJ, Shefter E (1986) Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactants, sulfoxides and amides. Int J Pharm 33(1):225–234

Aungst BJ, Blake JA, Rogers NJ, Hussain MA (1990) Transdermal oxymorphone formulation development and methods for evaluating flux and lag times for two skin permeation-enhancing vehicles. J Pharm Sci 79(12):1072–1076

Baek JS, Lim JH, So JW, Kim JI, Lee TW, Hwang SJ et al (2012) The feasibility study of transdermal drug delivery systems for antidepressants possessing hydrophilicity or hydrophobicity. J Pharm Inves 42(1):109–114

Barry BW (1987) Mode of action of penetration enhancers in human skin. J Control Release 6:85–97

Beitner H (2003) Randomized, placebo-controlled, double blind study on the clinical efficacy of a cream containing 5% a-lipoic acid related to photoageing of facial skin. Br J Dermatol 149(4):841–849

Bhandari KH, Newa M, Yoon SI, Kim JS, Jang KY, Kim JA et al (2007) Evaluation of physicochemical properties, skin permeation and accumulation profiles of ketorolac fatty ester prodrugs. Biol Pharma Bull 30(11):2211–2216

Bhandari KH, Lee DX, Newa M, Yoon SI, Kim JS, Kim DD et al (2008) Evaluation of skin permeation and accumulation profiles of a highly lipophilic fatty ester. Arch Pharm Res 31(2):242–249

Bhatia KS, Singh J (1998) Synergistic effect of iontophoresis and a series of fatty acids on LHRH permeability through porcine skin. J Pharm Sci 87(4):462–469

Bhatia KS, Gao S, Freeman TP, Singh J (1997) Effect of penetration enhancers and iontophoresis on the ultrastructure and cholecystokinin-8 permeability through porcine skin. J Pharm Sci 86(9):1011–1015

Boelsma E, Tanojo H, Bodde HE, Ponec M (1996) Assessment of the potential irritancy of oleic acid on human skin: evaluation in vitro and in vivo. Toxicol In Vitro 10:729–742

Boelsma E, Tanojo H, Bodde HE, Ponec M (1997) An in vitro-in vivo study of the use of a human skin equivalent for irritancy screening of fatty acids. Toxicol In Vitro 11:365–376

Brain K, Walters K (1993) Molecular modeling of skin permeation enhancement by chemical agents. In: Walters K, Hadgraft J (eds) Pharmaceutical skin penetration enhancement. Marcel Dekker Inc, New York, pp 389–416

Carelli V, Di Colo G, Nannipieri E, Serafini MF (1992) Enhancement effects in the permeation of alprazolam through hairless mouse skin. Int J Pharm 88(1):89–97

Carrara D (2001) Composition for controlled and sustained transdermal administration, US Patent 6,231,885 B1, 15 May 2001

Carrara D, Porto G, Rodriguez J (2010) Composition for transdermal and/or transmucosal administration of active compounds that ensures adequate therapeutic levels. European Patent, 1,323,431 B1, 27 Oct 2010

Chantasart D, Li SK, He N, Warner KS, Prakongpan S, Higuchi WI (2004) Mechanistic studies of branched-chain alkanols as skin permeation enhancers. J Pharm Sci 93(3):762–779

Chi SC, Park ES, Kim H (1995) Effect of penetration enhancers on flurbiprofen permeation through rat skin. Int J Pharm 126(1):267–274

Chisty MN, Bellantone RA, Taft DR, Plakogiannis FM (2002) In vitro evaluation of the release of albuterol sulfate from polymer gels: effect of fatty acids on drug transport across biological membranes. Drug Dev Ind Pharm 28(10):1221–1229

Cho CW, Choi JS, Kim SJ, Shin SC (2009) Enhanced transdermal delivery of loratadine from the EVA matrix. Drug Deliv 16(4):230–235

Choi HK (2007) Preparation and composition of meloxicam transdermal drug delivery system, US Patent 0,246,266 A1, 20 Sept 2007

Cotte M, Dumas P, Besnard M, Tchoreloff P, Walter P (2004) Synchrotron FT-IR microscopic study of chemical enhancers in transdermal drug delivery: example of fatty acids. J Control Release 97:269–281

Csóka G, Marton S, Zelko R, Otomo N, Antal I (2007) Application of sucrose fatty acid esters in transdermal therapeutic systems. Eur J Pharm Biopharm 65(2):233–237

Del Rio-Sancho S, Serna-Jiménez CE, Calatayud-Pascual MA, Balaguer-Fernández C, Femenía-Font A, Merino V et al (2012) Transdermal absorption of memantine. Effect of chemical enhancers, iontophoresis and role of enhancer lipophilicity. Eur J Pharm Biopharm 82(1):164–170

Deng J, Lin J, Xiao J (2012) Transdermal patch containing rasagiline for treatment or prophylaxis of nervous system disease and its preparation process, European Patent, 2,011,488 B1, 22 Aug. 2012

Dillaha L (2008) Transdermal delivery system comprising glycopyrrolate to treat sialorrhea, US Patent 144,285, 23 Jun 2008

Dimas DA, Dallas PP, Rekkas DM (2004) Use of an 8132 asymmetrical factorial design for the in vitro evaluation of ondansetron permeation through human epidermis. Pharm Dev Technol 9(1):39–48

El-Laithy HM, Shoukry O, Mahran LG (2011) Novel sugar esters proniosomes for transdermal delivery of vinpocetine: preclinical and clinical studies. Eur J Pharm Biopharm 77(1):43–55

Escribano E, Calpena AC, Queralt J, Obach R, Doménech J (2003) Assessment of diclofenac permeation with different formulations: anti-inflammatory study of a selected formula. Eur J Pharm Sci 19:203–210

Fang JY, Hwang TL, Fang CL, Chiu HC (2003a) In vitro and in vivo evaluations of the efficacy and safety of skin permeation enhancers using flurbiprofen as a model drug. Int J Pharm 255:153–166

Fang JY, Hwang TL, Leu YL (2003b) Effect of enhancers and retarders on percutaneous absorption of flurbiprofen from hydrogels. Int J Pharm 250:313–325

Foldwari M, Kwadwo S, Poku A (2002) Composition and method for dermal and transdermal administration of a cytokine, US Patent, 6,444,200 B2, 3 Sep 2002

Francoeur ML, Golden GM, Potts RO (1990) Oleic acid: its effects on stratum corneum in relation to transdermal drug delivery. Pharm Res 7:621–627

Gay CL, Murphy TM, Hadgraft J, Kellaway IW, Evans JC, Rowlands CC (1989) An electron spin resonance study of skin penetration enhancers. Int J Pharm 49:39–45

Giannakou SA, Dallas PP, Rekkas DM, Choulis NH (1998) Development and in vitro evaluation of nimodipine transdermal formulations using factorial design. Pharm Dev Technol 3(4):517–525

Godin B, Touitou E (2003) Ethosomes: new prospects in transdermal delivery. Crit Rev Ther Drug Carrier Syst 20:63–102

Goldberg-Cettina M, Liu P, Nightingale J, Kurihara-Bergstrom T (1995) Enhanced transdermal delivery of estradiol in vitro using binary vehicles of isopropyl myristate and short-chain alkanols. Int J Pharm 114(2):237–245

Golden GM, Mckie JE, Potts RO (1987) Role of stratum corneum lipid fluidity in transdermal drug flux. J Pharm Sci 76(1):25–28

Gonella J (1997) Administration system for estradiol, US Patent 5,665,377, 9 Sep 1997

Goosen C, Laing TJ, Du Plessis J, Goosen TC, Lu GW, Flynn GL (2002) Percutaneous delivery of thalidomide and its N-alkyl analogs. Pharm Res 19:434–439

Gorukanti SR, Li L, Kim KH (1999) Transdermal delivery of antiparkinsonian agent, benztropine. I. Effect of vehicles on skin permeation. Int J Pharm 192:159–172

Green PG, Hadgraft J (1987) Facilitated transfer of cationic drugs across a lipoidal membrane by oleic acid and lauric acid. Int J Pharm 37:251–255

Gwak HS, Chun IK (2002) Effect of vehicles and penetration enhancers on the in vitro percutaneous absorption of tenoxicam through hairless mouse skin. Int J Pharm 236(1–2):57

Gwak HS, Oh IS, Chun IK (2004) Transdermal delivery of ondansetron hydrochloride: effects of vehicles and penetration enhancers. Drug Dev Ind Pharm 30(2):187–194

Hathout RM, Woodman TJ, Mansour S, Mortada ND, Geneidi AS, Guy RH (2010) Microemulsion formulations for the transdermal delivery of testosterone. Eur J Pharm Sci 40:188–196

Heard CM, Gallagher SJ, Harwood J, Maguire PB (2003) The in vitro delivery of NSAIDs across skin was in proportion to the delivery of essential fatty acids in the vehicle: evidence that solutes permeate skin associated with their solvation cages? Int J Pharm 261: 165–169

Hille T (1993) Technological aspects of penetration enhancers in transdermal systems. In: Walters K,

Hadgraft J (eds) Pharmaceutical skin penetration enhancement. Marcel Dekker, New York, pp 335–344
Hsu LR, Huang YB, Wu PC, Tsai YH (1994) Percutaneous absorption of piroxicah from fapg base through rat skin: effects of oleic acid and saturated fatty acid added to fapg base. Drug Dev Ind Pharm 20(8):1425–1437
Ibrahim SA, Li SK (2010) Efficiency of fatty acids as chemical penetration enhancers: mechanisms and structure enhancement relationship. Pharm Res 27(1): 115–125
Jain AK, Thomas NS, Panchagnula R (2002) Transdermal drug delivery of imipramine hydrochloride. I. Effect of terpenes. J Control Release 79:93–101
Jasti BR, Abraham W (1998) Fluorescence spectroscopic investigation of effect of excipients on epidermal barrier and transdermal systems. J Investig Dermatol Symp Proc 3(2):128–130
Jenner J, Saleem A, Swanston D (1995) Transdermal delivery of physostigmine: a pretreatment against organophosphate poisoning. J Pharm Pharmacol 47: 206–212
Kai T, Mak VH, Potts RO, Guy RH (1990) Mechanism of percutaneous penetration enhancement: effect of n-alkanols on the permeability barrier of hairless mouse skin. J Control Release 12:103–112
Kandimalla K, Kanikkannan N, Andega S, Singh M (1999) Effect of fatty acids on the permeation of melatonin across rat and pig skin in vitro and on the transepidermal water Loss in rats in-vivo. J Pharm Pharmacol 51(7):783–790
Kandimalla KK, Babu RJ, Singh M (2009) Biphasic flux profiles of melatonin: the Yin–Yang of transdermal permeation enhancement mediated by fatty alcohol enhancers. J Pharm Sci 99(1):209–218
Kanikkannan N, Singh M (2002) Skin permeation enhancement effect and skin irritation of saturated fatty alcohols. Int J Pharm 248:219–228
Kanikkannan N, Andega S, Burton S, Babu RJ, Singh M (2004) Formulation and in vitro evaluation of transdermal patches of melatonin. Drug Dev Ind Pharm 30:205–212
Karande P, Mitragotri S (2009) Enhancement of transdermal drug delivery via synergistic action of chemicals. Biochim Biophys Acta 1788(11):2362–2373
Karia C, Harwood JL, Morris AP, Heard CM (2004) Simultaneous permeation of tamoxifen and γ linolenic acid across excised human skin. Further evidence of the permeation of solvated complexes. Int J Pharm 271:305–309
Kim YH, Ghanem AH, Mahmoud H, Higuchi WI (1992) Short chain alkanols as transport enhancers for lipophilic and polar/ionic permeants in hairless mouse skin: mechanism (s) of action. Int J Pharm 80(1): 17–31
Kim MK, Lee CH, Kim DD (2000) Skin permeation of testosterone and its ester derivatives in rats. J Pharm Pharmacol 52:369–375
Kim MJ, Doh HJ, Choi MK, Chung SJ, Shim CK, Kim DD et al (2008) Skin permeation enhancement of diclofenac by fatty acids. Drug Deliv 15(6):373–379
Kochinke F, Baker RW (1994) Device and method for enhanced administration of physostigmine, US Patent, 5,364,629, 15 Nov 1994
Kojima H, Sato A, Hanamura A, Katada T, Konishi H (1998) Evaluation of skin irritation in a reconstituted human dermal model (3-D model) using water insoluble fatty acids, fatty alcohols and hydrocarbons. Altern Animal Test Exp 5:201–210
Krotscheck U, Boothe DM, Boothe HW (2004) Evaluation of transdermal morphine and fentanyl pluronic lecithin organogel administration in dogs. Vet Ther 5:202–211
Lashmar UT, Hadgraft J, Thomas N (1989) Topical application of penetration enhancers to the skin of nude mice: a histopathological study. J Pharm Pharmacol 41:118–121
Lee JH, Chun IK (2012) Effects of various vehicles and fatty acids on the skin permeation of lornoxicam. J Pharm Invest 42(5):235–241
Lee PJ, Ahmad N, Langer R, Mitragotri S, Prasad Shastri V (2006) Evaluation of chemical enhancers in the transdermal delivery of lidocaine. Int J Pharm 308(1): 33–39
Lo W, Ghazaryan A, Tso CH, Hu PS, Chen WL, Kuo TR, Dong CY (2012) Oleic acid-enhanced transdermal delivery pathways of fluorescent nanoparticles. Appl Phys Lett 100(21):213701
Loftsson T, Gildersleeve N, Bodor N (1987) The effect of vehicle additives on the transdermal delivery of nitroglycerin. Pharm Res 4:436–437
Matsugo S, Bito T, Konishi T (2011) Photochemical stability of lipoic acid and its impact on skin ageing. Free Radic Res 45(8):918–924
Morimoto K, Tojima H, Haruta T, Suzuki M, Kakemi M (1996) Enhancing effects of unsaturated fatty acids with various structures on the permeation of indomethacin through rat skin. J Pharm Pharmacol 48(11):1133–1137
Nair VB, Panchagnula R (2003) Effect of iontophoresis and fatty acids on permeation of Arginine Vasopressin through rat skin. Pharmacol Res 47:563–569
Nanayakkara GR, Bartlett A, Forbes B, Marriott C, Whitfield PJ, Brown MB (2005) The effect of unsaturated fatty acids in benzyl alcohol on the percutaneous permeation of three model penetrants. Int J Pharm 301(1):129–139
Narishetty ST, Panchagnula R (2004) Transdermal delivery of zidovudine: effect of terpenes and their mechanism of action. J Control Release 95:367–379
Neubert R, Rettig W, Wartewig S, Wegener M, Wienhold A (1997) Structure of stratum corneum lipids characterized by FT-Raman spectroscopy and DSC. II. Mixtures of ceramides and saturated fatty acids. Chem Phys Lipids 89:3–14
Ogiso T, Shintani M (1990) Mechanism for the enhancement effect of fatty acids on the percutaneous absorption of propranolol. J Pharm Sci 79(12):1065–1071
Oh HJ, Oh YK, Kim CK (2001) Effects of vehicles and enhancers on transdermal delivery of melatonin. Int J Pharm 212(1):63–71

Okamoto H, Sakai T, Danjo K (2005) Effect of sucrose fatty acid esters on transdermal permeation of lidocaine and ketoprofen. Biol Pharm Bull 28(9): 1689–1694

Okamoto H, Sakai T, Tokuyama C, Danjo K (2011) Sugar ester J-1216 enhances percutaneous permeation of ionized lidocaine. J Pharm Sci 100(10):4482–4490

Ongpipattanakul B, Burnette RR, Potts RO, Francoeur ML (1991) Evidence that oleic acid exists in a separate phase within stratum corneum lipids. Pharm Res 8(3):350–354

Rastogi SK, Singh J (2005) Effect of chemical penetration enhancer and iontophoresis on the in vitro percutaneous absorption enhancement of insulin through porcine epidermis. Pharm Dev Technol 10(1):97–104

Richards H, Thomas CP, Bowen JL, Heard CM (2006) In-vitro transcutaneous delivery of ketoprofen and polyunsaturated fatty acids from a pluronic lecithin organogel vehicle containing fish oil. J Pharm Pharmacol 58(7):903–908

Segall A, Sosa M, Alami A, Enero C, Hormaechea F, Pizzorno MT et al (2004) Stability study of lipoic acid in the presence of vitamins A and E in o/w emulsions for cosmetic application. J Cosmet Sci 55(5):449–462

Seki T, Morimoto K (2003) Enhancing effects of medium chain aliphatic alcohols and esters on the permeation of 6-carboxyfluorescein and indomethacin through rat skin. Drug Deliv 10(4):289–293

Setoh K, Murakami M, Araki N, Fujita T, Yamamota A, Muranishi S (1995) Improvement of transdermal delivery of tetragastrin by lipophilic modification with fatty acids. J Pharm Pharmacol 47(10):808–811

Shah P, Desai P, Singh M (2011) Effect of oleic acid modified polymeric bilayered nanoparticles on percutaneous delivery of spantide II and ketoprofen. J Control Release 158(2):336–345

Sharma K, Roy DS, Roos JE (1993) Transdermal administration of buprenorphine, European Patent 0,535,111 A1, 7 Apr 1993

Singh BN, Singh RB, Singh J (2005) Effects of ionization and penetration enhancers on the transdermal delivery of 5-fluorouracil through excised human stratum corneum. Int J Pharm 298(1):98–107

Sloan KB, Beall HD, Taylor HE, Getz JJ, Villaneuva R, Nipper R et al (1998) Transdermal delivery of theophylline from alcohol vehicles. Int J Pharm 171: 185–193

Song JF, Lau-Cam CA, Kim KH (2001) Monohydroxylation and esterification as determinants of the effects of cis- and trans-9-octadecenoic acids on the permeation of hydrocortisone and 5-fluorouracil across hairless mouse skin in vitro. Int J Pharm 212:153–160

Stillman MA, Maibach HI, Shalita AR (1975) Relative irritancy of free fatty acids of different chain length. Contact Dermatitis 1:65–69

Stott PW, Williams AC, Barry BW (2001) Mechanistic study into the enhanced transdermal permeation of a model β-blocker, propranolol, by fatty acids: a melting point depression effect. Int J Pharm 219:161–176

Subedi RK, Ryoo JP, Moon C, Choi HK (2011) Influence of formulation variables in transdermal drug delivery system containing zolmitriptan. Int J Pharm 419(1):209–214

Takahashi K, Rytting JH (2001) Novel approach to improve permeation of ondansetron across shed snake skin as a model membrane. J Pharm Pharmacol 53:789–794

Takahashi K, Matsumoto T, Kimura T, Sakano H, Mizuno N, Yata N (1996) Effect of polyol fatty acid esters on diclofenac permeation through rat skin. Biol Pharm Bull 19(6):893

Takahashi K, Sakano H, Yoshida M, Numata N, Mizuno N (2001) Characterization of the influence of polyol fatty acid esters on the permeation of diclofenac through rat skin. J Control Release 73(2):351–358

Tanojo H, Bouwstra JA, Junginger HE, Boddé HE (1997) In vitro human skin barrier modulation by fatty acids: skin permeation and thermal analysis studies. Pharm Res 14(1):42–49

Tanojo HT, Boelsma E, Junginger HE, Ponec M, Bodde HE (1998) In vivo human skin barrier modulation by topical application of fatty acids. Skin Pharmacol Physiol 11:87–97

Thomas NS, Panchagnula R (2003) Combination strategies to enhance transdermal permeation of zidovudine (AZT). Pharmazie 58(12):895–898

Thorsteinsson T, Másson M, Jarvinen T, Nevalainen T, Loftsson T (2002) Cycloserine fatty acid derivatives as prodrugs: synthesis, degradation and in vitro skin permeability. Chem Pharm Bull 50:554–557

Touitou E, Godin B, Karl Y, Bujanover S, Becker Y (2002) Oleic acid, a skin penetration enhancer, affects Langerhans cells and corneocytes. J Control Release 80(1):1–7

Valjakka-Koskela R, Hirvonen J, Mönkkönen J, Kiesvaara J, Antila S, Lehtonen L et al (2000) Transdermal delivery of levosimendan. Eur J Pharm Sci 11(4):343–350

Wang MY, Yang YY, Heng PW (2004) Role of solvent in interactions between fatty acids-based formulations and lipids in porcine stratum corneum. J Control Release 94:207–216

Wang S, Xue H, Wang L (2008) Composition containing rotigotine and use thereof and transdermal patch containing the composition, US Patent 0,027,345 A1, 3 Jul 2008

Wang JJ, Sung KC, Huang JF, Yeh CH, Fang JY (2010) Ester prodrugs of morphine improve transdermal drug delivery: a mechanistic study. J Pharm Pharmacol 59(7):917–925

Warner KS, Li SK, Higuchi WI (2001) Influences of alkyl group chain length and polar head group on chemical skin permeation enhancement. J Pharm Sci 90:1143–1153

Williams AC, Barry BW (2012) Penetration enhancers. Adv Drug Deliv Rev 64:128–137

Willis-Goulet HS, Schmidt BA, Nicklin CF, Marsella R, Kunkle GA (2003) Comparison of serum dexamethasone concentrations in cats after oral or transdermal administration using pluronic lecithin organogel (PLO): a pilot study. Vet Dermatol 14(2):83–89

Yahalom D, Koch Y, Ben-Aroya N, Fridkin M (1999) Synthesis and bioactivity of fatty acid-conjugated GnRH derivatives. Life Sci 64:1543

Yamamoto A, Setoh K, Murakami M, Shironoshita M, Kobayashi T, Fujimoto K, Muranishi S (2003) Enhanced transdermal delivery of phenylalanylglycine by chemical modification with various fatty acids. Int J Pharm 250(1):119–128

Yamato K, Takahashi Y, Akiyama H, Tsuji K, Onishi H, Machida Y (2009) Effect of penetration enhancers on transdermal delivery of propofol. Biol Pharm Bull 32(4):677–683

Yoneto K, Ghanem AH, Higuchi WI, Peck KD, Li SK (1995) Mechanistic studies of the 1-Alkyl-2-pyrrolidones as skin permeation enhancers. J Pharm Sci 84(3):312–317

Yu B, Dong CY, So PT, Blankschtein D, Langer R (2001) In vitro visualization and quantification of oleic acid induced changes in transdermal transport using two-photon fluorescence microscopy. J Invest Dermatol 117(1):16–25

Zakir F, Vaidya B, Goyal AK, Malik B, Vyas SP (2010) Development and characterization of oleic acid vesicles for the topical delivery of fluconazole. Drug Deliv 17:238–248

Ethanol and Other Alcohols: Old Enhancers, Alternative Perspectives

10

Charles M. Heard

Contents

10.1 Introduction

Topical drug delivery remains very important in the methodologies available for the administration of drugs, whether it be for dermatological, transdermal or transcutaneous use. Much has been written over the last 30 years about the penetration enhancer approach to increasing the delivery of therapeutic agents across the skin. The driving force is simple – mammalian skin has evolved to be remarkably efficient at presenting a biological, chemical and physical barrier between our bodies and the external environment. This means that for the majority of otherwise useful therapeutic substances, the amounts deliverable across the skin are insufficient to attain therapeutic responses. Although recent research has tended to focus upon physical methods, such as iontophoresis and microneedles, passive or chemical penetration enhancement remains the preferred option on grounds of simplicity and the lack of trauma inflicted to the skin.

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N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_10

Many articles have been published that describe the delivery of xenobiotics into and across the skin as facilitated by alkanols (sometimes simply referred to as alcohols), and many others have provided us with mechanistic processes that have become widely accepted and form the basis of current wisdom in the area. The distinction between a penetration enhancer and an enhancing vehicle is unclear, although a vehicle is generally taken to mean the solvent or solvents in which the drug is initially dissolved in the formulation process. A penetration enhancer can be defined simply as a chemical additive formulated in a system that provides elevated delivery of a drug relative to the same system lacking the additive. Whereas many chemical penetration enhancers tend to be long chain non-volatile substances, e.g. 1-dodecylazacycloheptan-2-one (Azone®) and oleic acid, most vehicles enhancing drug penetration tend to be small, low boiling point fluids. Ethanol comes into this category. The subject of skin penetration and penetration enhancement has been documented in numerous review articles and books (Williams and Barry 2004; Benson 2005).

This chapter seeks firstly to review the major findings on the use of ethanol and alcohols as chemical penetration enhancers. Secondly, current wisdom regarding mechanisms is considered. Finally, alternative mechanistic perspectives are proposed based on the often overlooked fact that it is not only the active drug that can penetrate skin, but also solvents – a reality that is seldom discussed within the field.

10.2 Ethanol and Other Alcohols in Topical Delivery

Solvents such as ethanol and propylene glycol are frequently used as enhancers or enhancing vehicles – they are not only penetration enhancers, but also have good solvating power and are thus frequently used as co-solvents, typically to increase the concentration of a lipophilic drug in an applied formulation. However, polar/ionic actives have also been used (Kurihara-Bergstrom et al. 1990). A great deal has been reported on the use of these enhancers, which are usually added in low concentration (e.g. <10 %) to the drug formulation (Table 10.1); other times they are applied to the skin as pretreatments (Tanojo et al. 1999; Santoyo and Ygartua 2000; Ibrahim and Li 2009).

The skin penetration-enhancing capability of ethanol has been recognised for a long time. The ability to properly compare between published articles is hampered by different groups using different skin penetration models; however, the capability of ethanol to transform drug delivery potential can be exemplified in the paper by Heard and Screen (2008). It was found that the permeation of mefenamic acid across porcine skin was enhanced from 'below limit of detection' from polyethylene glycol (PEG) 400–0.83 μg cm^{-2} h^{-1} by the addition of 10 % ethanol, even though both solutions were saturated; hence, equal fluxes would have been anticipated. A saturated solution of mefenamic acid in hexadecyltrimethylammonium bromide (cetrimide) provided a value of only 0.1 μg cm^{-2} h^{-1} indicating no penetration enhancement arising from the use of this compound.

Ethanol and other alkanols have been successfully used as penetration enhancers to enhance the flux of levonorgestrel, estradiol, hydrocortisone and 5-fluorouracil across excised skin (Friend et al. 1988). Ethanol was also used to study the penetration of estradiol using a human skin in vivo flap model (Pershing et al. 1990) and nitroglycerine across human epidermis in vitro (Berner et al. 1989). Fang et al. (2008) studied the synergistically enhanced percutaneous penetration of tetracaine from a carbomer gel with menthol and ethanol – interestingly, enhanced permeation correlated with enhanced analgesia in human volunteers, indicating successful delivery across the epidermis to the dermis. The enhanced transdermal delivery of tamoxifen and bioactive polyunsaturated fatty acids, e.g. γ-linolenic acid, has been achieved from liquid formulations containing borage oil with ethanol and 1,8 cineole (Ho et al. 2004). The enhancing properties of ethanol when used alongside other excipients have been studied, for example, Verma and Fahr (2004), who found synergism involving ethanol

Table 10.1 Reports of ethanol and related compounds on skin penetration, in chronological order

Reference	Permeant under study	Membrane	Formulation	Alcohol (and other enhancers)	Alcohol permeation determined?
Flynn et al. (1981)	Methanol, ethanol, n-propanol, n-butanol, n-pentanol, n-hexanol, n-heptanol, n-octanol, n-nonanol, n-decanol	Hairless mouse skin – full thickness, heat-separated epidermis, heat-separated dermis	Liquid	Methanol, ethanol, n-propanol, n-butanol, n-pentanol, n-hexanol, n-heptanol, n-octanol, n-nonanol, n-decanol	Yes
Hoelgaard and Møllgaard (1985)	Metronidazole	Human skin in vitro	Liquid	Propylene glycol	Yes
Twist and Zatz (1986)	Parabens	Polydimethylsiloxane	Liquid	Propylene glycol, polyethylene glycol 400, glycerin	No
Friend et al. (1988)	Levonorgestrel, estradiol, hydrocortisone and 5-fluorouracil	Human skin, rat skin	Liquid	Ethanol, propanol, butanol, pentanol, hexanol, octanol	No
Berner et al. (1989)	Nitroglycerin	Human epidermis	Liquid	Ethanol	Yes
Pershing et al. (1990)	Estradiol	Human, in vivo	Liquid	Ethanol	Yes
Kurihara-Bergstrom et al. (1990)	Salicylate (ion)	Human epidermal membranes	Liquid	Ethanol	No
Liu et al. (1991)	Estradiol	Human in vitro	Liquid	Ethanol	No
Pardo et al. (1991)	Physostigmine	Excised human skin	Liquid	Isopropanol (isopropyl myristate)	No
Bendas et al. (1995)	Betamethasone 17-valerate, hydrocortisone -17 butyrate, hydrocortisone	Excised human skin	Hydrogels	Propylene glycol (water)	Yes
Megrab et al. (1995)	Estradiol	Human stratum corneum, silastic and snake skin	Liquid	Ethanol (water)	No
Squillante et al. (1998)	Propylene glycol and dimethyl isosorbide	Hairless mouse skin	liquid	Propylene glycol (oleic acid, dimethyl isosorbide)	Yes
Tanojo et al. (1999)	p-Aminobenzoic acid	Human stratum corneum	Liquid	Propylene glycol	No
Levang et al. (1999)	Acetylsalicylic acid	Porcine skin	Liquid	Ethanol, propylene glycol	No

(continued)

Table 10.1 (continued)

Reference	Permeant under study	Membrane	Formulation	Alcohol (and other enhancers)	Alcohol permeation determined?
Pendlington et al. (2001)	Ethanol	Human, in vivo	Liquid	Ethanol	Yes
Andega et al. (2001)	Melatonin	Dermatomed porcine skin, human skin	Liquid	Saturated fatty alcohols, octanol, nonanol, decanol, undecanol, lauryl alcohol, tridecanol, myristyl alcohol; unsaturated fatty alcohols, oleyl alcohol, linoleyl alcohol, linolenyl alcohol	No
Funke et al. (2002)	Lipophilic antiestrogens: AE1 and AE2	Hairless mouse	Liquid	Propylene glycol (lauric acid)	No
Morimoto et al. (2002)	Morphine hydrochloride	Hairless rat skin	Liquid (finite)	Ethanol (L- menthol)	No
Ho et al. (2004)	Tamoxifen, γ-linolenic acid	Porcine ear skin	Liquid	Ethanol (+cineole)	No
Chantasart et al. (2004)	Corticosterone	Hairless mouse skin	Liquid	Branched-chain alkanols: x-hexanol, x-heptanol, x-octanol, and x-nonanol (where x is the position of the hydroxyl group ranging from 1 up to 5)	No
Verma and Fahr (2004)	Cyclosporin A	Human abdominal skin	Vesicles	Ethanol	No
Van der Merwe and Riviere (2005)	Phenol, 4-nitrophenol, pentachlorophenol, dimethyl parathion, parathion, chlorpyrifos, fenthion, triazine, atrazine, simazine and propazine.	Porcine stratum corneum and Silastic	Liquid	Ethanol	No
Leichtnam et al. (2006)	Testosterone	Hairless rat skin	Liquid (spray)	Ethanol, propylene glycol (water)	No
Heard et al. (2006)	Mefenamic acid	Porcine	Liquid	Ethanol	Yes
Wang et al. (2008)	Aminophylline	Human skin	Cream	Ethanol	No
Kramer et al. (2007)	Ethanol	Human in vivo	Hand rub gel	Ethanol	Yes

Fang et al. (2008)	Tetracaine	Mouse skin in vitro, human volunteers in vivo	Gel (carbomer)	Ethanol, menthol	No
Schaefer-Korting et al. (2008)	Hydrocortisone, testosterone	Full-thickness skin and reconstructed epidermis	Liquid	Ethanol	No
Heard and Screen (2008)	Mefenamic acid	Porcine ear skin, full thickness, heat-separated epidermis, heat-separated dermis	Liquid	Ethanol	Yes
Liu et al. (2009)	Estradiol	Human epidermis	Liquid	Isopropanol (and isopropyl myristate)	No
Andanson et al. (2009)	Benzyl nicotinate	Human stratum corneum	Liquid	Ethanol	No
Ibrahim and Li (2009)	Corticosterone	Human epidermal membrane	Liquid	Ethanol	No
Duracher et al.(2009)	Caffeine	Porcine	Liquid	Propylene glycol, ethanol, 1,2-pentanediol (and water)	No
Patel et al. (2009)	Fluconazole	Rat skin, abdominal	Microemulsion	Ethanol, lauryl alcohol	No
Geusens et al. (2010)	siRNA	Excised intact human skin	Liquid (nanosome)	Ethanol	No
Jaimes-Lizcano et al. (2011)	Fluorescein isothiocyanate-labelled bovine serum albumin	Porcine	Double emulsions	Ethanol	No
Kim and Oh (2011)	Calcitonin	Human epidermal membrane	Liquid	Ethanol (plus iontophoresis)	No
Fasano et al. (2011)	Propylene glycols	Human abdominal skin	Liquid	Monopropylene glycol, dipropylene glycol	Yes
Ahmed-Lecheheb et al. (2012)	Ethanol	Human volunteers	Alcohol-based hand rubs	Ethanol	Yes
Santos et al. (2012)	Fentanyl	Human skin in vitro	Liquid (spray)	Propylene glycol, ethanol	No
Ikeda et al. (2012)	Piroxicam, pranoprofen	Porcine skin	Nanoparticles in liquid	Propylene glycol (water)	No

and phospholipids when used for the topical delivery of cyclosporin A, and Wang et al. (2008), who found that the skin penetration enhancement of aminophylline from cream formulations containing a range of essential (rosemary, ylang-ylang, lilacin and peppermint oils) and plant oils (jojoba oil, corn germ oil and olive oil) was less than that when ethanol only was used, although the enhancement level was not stated.

In recent years, the skin has been examined as a portal of the delivery of macromolecules – molecules that are far larger than those usually considered deliverable by this route. It seems that enhancement by ethanol is important in such studies, which tend to use larger volumes of ethanol than typically seen in skin penetration experiments. Nanosomes (nanoscale liposomes) have been used to encapsulate small interfering RNA (siRNA), a large biomolecule with high cutaneous therapy potential. The overall nanosome–siRNA complex was found to penetrate freshly excised human skin and enter keratinocytes when ethanol was used, at a concentration of 30 % (Geusens et al. 2010). Ethanol, at 40 %, has been used recently to enhance the penetration of isothiocyanate-labelled bovine serum albumin (FITC-BSA) up to 86 μm into porcine skin from a cream formulation (Jaimes-Lizcano et al. 2011). Kim and Oh (2011) investigated the iontophoretic delivery of calcitonin (32-amino acid linear polypeptide hormone) from microemulsions containing isopropyl myristate or oleic acid across full-thickness hairless mouse skin, with a 70 % ethanol level proving superior.

It has also been suggested that the consumption of ethanol via alcoholic beverages can render the consumer with skin that is more permeable to the ingress of xenobiotics (Brand et al. 2007). Male Wistar rats were gavaged with ethanol, and the skin permeation of paraquat, dimethyl formamide (DMF), 2,4-dichlorophenoxyacetic acid (2,4-D) and N,N-diethyl-m-toluamide (DEET) was determined. Increases in permeation rate were attributed to ethanol-induced changes in peroxidation of lipids and transepidermal water loss.

Propylene glycol has also been widely used as a penetration enhancer or a component of an enhancing vehicle; it also has a long history of use in cosmetics by virtue of its humectant properties, and the safety of this and other glycols in cosmetics has recently been supported (Fiume et al. 2012). In topical drug delivery, propylene glycol generally tends to be used in relatively low levels, e.g. <20 %, although a concentration of 40 % aqueous propylene glycol was found by Priborsky et al. (1987) to be optimum for the in vitro transdermal delivery of insulin. However, another early paper claimed that the enhancement of levonorgestrel using neat (100 %) propylene glycol was about the same as water (Friend et al. 1988). Propylene glycol has been used to formulate hydroxypropyl methylcellulose (HPMC) nanoparticles, with pranoprofen permeation across hairless mouse skin increasing with propylene glycol content, although that of piroxicam did not (Ikeda et al. 2012). Santos et al. (2012) found that propylene glycol increased the permeation of fentanyl by a factor of 3 in comparison with other solvents despite having the same thermodynamic activity – a result which was attributed to a mechanism of permeation enhancement for propylene glycol that was different from octyl salicylate and isopropyl myristate.

Mixtures of ethanol and propylene glycol 80:20 have also been used as enhancing vehicles. Levang et al. (1999) found that the flux of acetylsalicylic acid through porcine skin increased with increasing concentrations of ethanol in the solvent systems, with a maximum flux obtained with 80 % ethanol in combination with 20 % propylene glycol, beyond which (i.e. 100 % ethanol) there was no further increase in the flux. However, at such high concentrations delipidisation also occurs as noted in the same article.

Although ethanol and propylene glycol are the most frequently reported alcohols used as penetration enhancers, others have also been used. Like ethanol, methanol also has excellent solubilising properties; however, it rarely features as a vehicle/enhancer in topical delivery due to its toxicity. There is a small number of papers where methanol has been used. One example is Hostynek et al. (2001) who used methanol as a vehicle to characterise the penetration of nickel salts in the human stratum corneum, by tape

stripping. Isopropanol (IPA) and isopropyl myristate (IPM) were used as vehicles to study the penetration of physostigmine through excised human skin (Pardo et al. 1991). The highest permeability coefficient was seen from the IPA–IPM mixture exhibiting the highest solvation effect (see Sect. 10.6). The same solvent system was later used by Liu et al. (2009) who found that the solubility of estradiol in the stratum corneum was enhanced 35 times by increasing the concentration of isopropyl alcohol, from neat 0 to 100 %; estradiol diffusivity in the stratum corneum also increased by 8 times from 100 % isopropyl myristate to 50 % isopropyl alcohol. A relatively unusual glycol, 1,2-pentanediol, was claimed to be a more effective enhancer than water, ethanol or propylene glycol (or mixtures thereof) in the study of caffeine permeation across full-thickness pig ear skin (Duracher et al. 2009). However, at a level of 5 % 1,2-pentanediol, a flux of 15.49 ± 2.58 μg cm^{-2} h^{-1} was in fact statistically the same as the flux of 14.20 ± 4.27 mg cm^{-2} h^{-1} obtained by propylene glycol–ethanol (25:25 % in water).

Alcohol chain length and branched-chain alcohols have also been investigated. In the paper by Friend et al. (1988), a range of linear alkanols were used to examine the flux of levonorgestrel across excised rat skin, which increased from ethanol to butanol, then diminished in the order: pentanol, hexanol and octanol. A similar trend was seen with secondary alkanols, where the steady-state flux was highest for 2-butanol, with 2-propanol and 2-pentanol giving lower values. Generally, the flux of levonorgestrel was lower for secondary alkanol relative to the corresponding primary alcohol. Propylene glycol performed similar to water, which was low relative to all the alcohols. The paper also compared the permeation of levonorgestrel in pure ethanol across rat skin with that across human cadaver skin, finding that the latter was approximately lower by a factor of 4. Chantasart et al. (2004) used hairless mouse skin to determine that branched-chain alkanols have lower enhancer potency for corticosterone than the 1-alkanols of the same molecular formula; the potency decreases as the hydroxyl group moves from the end of the chain towards the centre of the enhancer alkyl chain. The authors introduced the 'isoenhancement' parameter, being the aqueous concentrations for which different enhancers induce the same extent of permeant transport enhancement (E) across the stratum corneum. The isoenhancement concentrations of 2-alkanol, 3-alkanol, 4-alkanol and 5-alkanol to induce $E = 10$ were 1.9-, 2.6-, 3.1- and 3.9-fold higher, respectively, than those of the 1-alkanols of the same molecular formula.

To conclude, alcohols have a long track record of providing substantial enhancements in the delivery of drugs across the skin. However, it is difficult to define an overarching value for the level of this enhancement due to the great variability in different experimental set-ups reported in the literature (Table 10.1). The extent to which enhancement occurs depends on many factors, including the type of alcohol used, amount of alcohol in the formulation, drug concentration in the donor, chemical potential, amount dosed onto the skin, occlusion/non-occlusion and the in vitro model membrane used – there are reports using the human stratum corneum, human epidermis, dermatomed human skin, full-thickness human skin, pig skin and rat skin.

10.3 Current Rationalisation of Skin Penetration and Enhancement

To begin to rationalise the capability of ethanol and other alcohols to produce skin penetration enhancement, we need to consider the major approaches currently expounded to rationalise skin penetration. These are briefly considered below, followed with a brief commentary on how such theories relate vehicle/enhancer absorption.

10.3.1 Fickian Diffusion

Classical considerations of skin permeation have their roots in the laws of diffusion as described by Fick's first law

$$dm / dt = DC_0 P / h \qquad (10.1)$$

where the flux (dm/dt) is governed by the diffusion coefficient of the drug in the stratum corneum (D), the dissolved effective concentration of the drug in the vehicle, (C_0), the partition coefficient between the formulation and the stratum corneum (P) and the membrane thickness (h). The molecules move in response to a thermodynamic force arising from a concentration gradient. Penetration enhancers may increase the D of the drug in the stratum corneum (modulate the barrier nature of the stratum corneum), may act to increase the effective concentration of the drug in the vehicle (e.g. acting as an anti-solvent) and could improve partitioning between the formulation and the stratum corneum (e.g. by altering the solvent nature of the skin membrane to improve partitioning into the tissue) or by decreasing the skin route/thickness (e.g. by delipidisation). Solvent effects have long been known to be of importance. Fick's second law (where x is the length) illustrates that diffusion causes the concentration of the permeant to change with time.

$$\delta C / \delta t = D\left(\delta^2 C / \delta x^2\right) \qquad (10.2)$$

10.3.2 Chemical Potential

It has been suggested that skin penetration rates are maximal when the drug is at its highest thermodynamic activity (Higuchi 1962). The theory suggests that if two or more formulations are compared for delivering drug X across the skin, as long as X is at same thermodynamic activity (e.g. both at saturation), the two formulations are directly comparable in terms of the delivery of the solute. Kadir et al. (1987) referred to a 'push' mechanism for the delivery of theophylline from vehicles containing alkanoic acids, based on the consideration of solubility parameters and the tendency of a solute to exit from a high chemical potential environment. Essentially, the vehicle cannot accommodate any more solute and is at the very point of expelling drug molecules, or 'pushing' them into an adjacent receiving domain, i.e. the stratum corneum. Such a scenario is generally short lived, but may be sustained by the incorporation of excess drug particulates. Santos et al. (2012) found that propylene glycol significantly increased the permeation of fentanyl in comparison to octyl salicylate and isopropyl myristate despite having the same thermodynamic activity. In supersaturated systems, greater flux levels may be achieved because the amount of drug per unit volume is above that ordinarily obtainable due to the presence of an additive that prevents the formation of crystalline drug – an antinucleant (Moser et al. 2001). This essentially increases donor drug concentration in the applied drug formulation as per Fickian transport. However, in such cases there is the implicit assumption that the solute dissociates entirely from the formulation and penetrates the skin once applied onto the skin, i.e. the vehicle remains confined to the point of application.

10.3.3 Mathematical Prediction

Perhaps the most commonly used mathematical model to estimate drug flux is the semi-empirical relationship developed by Potts and Guy (1991). This equation formulates an empirical relationship between permeability coefficient (K_p) and two simple measurable characteristics of the permeant: the octanol–water partition coefficient (K_{oct}) and the molecular weight (MW).

$$\text{Log}\, K_p = 0.71 \log K_{oct} - 0.0061\text{MW} - 2.72 \qquad (10.3)$$

The equation teaches us that as log k_{oct} increases, the permeability also increases, whereas the greater the molecular weight, the smaller the K_p.

This equation was the first to use a wide-ranging database of permeability coefficients. The model works reasonably well, and the K_p can be calculated from the equation very easily, particularly if one considers that the octanol–water partition coefficient K_{oct} can be readily measured in vitro or computed from the structure of the compound. To avoid confusion and to simplify interpretation, permeation data from aqueous solutions alone are used.

10.3.4 Drug–Skin Interaction

The influence of hydrogen bond interactions has been investigated, based on the premise that a diffusing drug molecule encounters and interacts with skin components (Pugh 1999). Typically, the intercellular space which drugs pass through contains a complex arrangement of lipids, the main components being long chain fatty acids, ceramides and cholesterol. Such processes look beyond simplistic partitioning events and consider potential reciprocal interactions, such as the functionality of the permeant and the –COOH groups of the fatty acids and the –OH groups and amides on the ceramides. Increasing the number of H-bonding groups on a permeant is believed to decrease flux by causing dermal retardation due to the interactions with skin components. The lipophilicity of a solute is recognised as a major determinant for partitioning into the stratum corneum from aqueous solutions, although it is proposed that H-bonding is a major determinant of solute diffusion across SC. It is claimed that diffusion is related to the number of H-bonding groups on the permeant, and the presence of 1, 2 or 3 H-bonding groups successively reduces the diffusion coefficient. Du Plessis et al. (2001) determined that the stratum corneum was predominantly an H-bond donor environment with donor–acceptor effects in the ratio 0.6:0.4, which indicated propensity to retard the diffusion of H-bond-donating permeants.

10.3.5 Non-accounting for the Alcohol Enhancer/ Vehicle

A commonality among each of the above approaches is that permeation of ethanol or other solvent in the vehicle cannot easily be accounted for and is therefore essentially ignored. Fickian diffusion theory was developed on the assumption that the membrane was an isotropic media; however, the skin is a complex heterogeneous tissue and is also prone to modulation by applied substances. Furthermore, it cannot account for changes in donor concentration that occur as a consequence of the concurrent permeation of the vehicle. Similarly, thermodynamically equal formulations of a drug rarely result in the anticipated equal skin permeation fluxes – if the absorption of the solvent from one formulation differs substantially to that of the other, then thermodynamic activity becomes of questionable relevance. Mathematical predictors, such as the Potts–Guy equation, are limited to aqueous vehicles, even though water can also exert a drag effect (Sect. 10.5.2), although this is not covered in this chapter. Finally, drug–skin component theories, such as those involving hydrogen bonding, do not account for other types of interactions, such as π-π (Heard et al. 2005), and ignore the fact that the solute is already engaged in intermolecular interactions with molecules of the solvent.

10.3.6 Current Mechanistic Theories Concerning Alcohols

As water-miscible co-solvents, ethanol and other glycols can markedly increase the solubility of lipophilic compounds in aqueous vehicles and thus provide increased flux in line with Fickian diffusion. Friend et al. (1988) proposed a mechanism for permeability enhancement of levonorgestrel by alcohols based upon alcohol structure and solubility. Co-solvency effects are well known and exemplified in Fig. 10.1. Even in the absence of other processes, it is clear from Fick's first law that significant increases in donor phase concentrations would be expected to result in elevated fluxes across the skin.

However, numerous papers have attempted to elucidate the mechanisms of action of ethanol and propylene glycol in terms of modulations to skin lipid domains. Tanojo et al. (1999) used differential scanning calorimetry (DSC) analysis to examine the human stratum corneum, where propylene glycol (as a pretreatment) interacted with lipids, especially those with low melting points, causing a depression in their phase transition temperatures. Levang et al. (1999) used Fourier transform infrared (FTIR) spectroscopy to rationalise the permeation of acetylsalicylic acid across

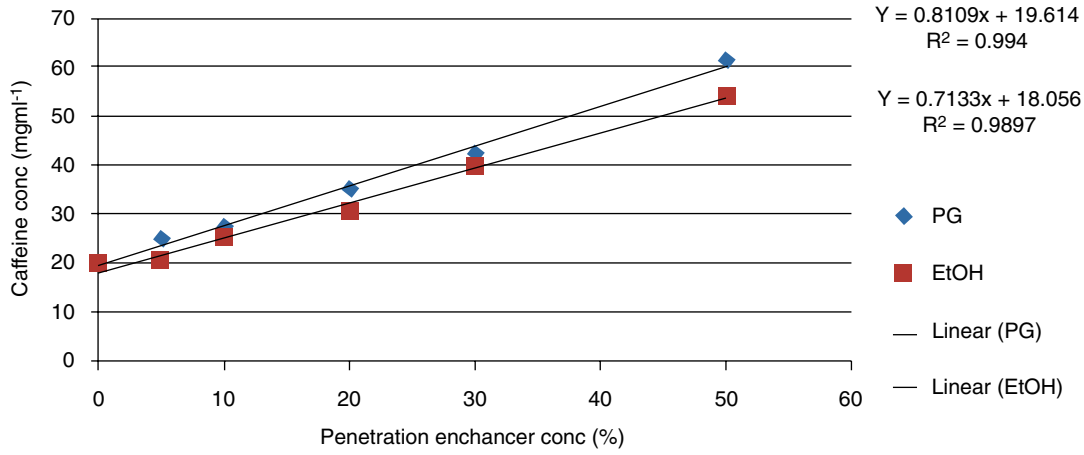

Fig. 10.1 Co-solvency as a means to increase the solubility, thereby donor phase concentration, of caffeine in aqueous propylene glycol and aqueous ethanol vehicles ($n = 3 \pm SD$) (Houston D et al. 2011)

porcine ear epidermal membranes from a range of alcohol-based vehicles. It was determined that the flux of acetylsalicylic acid increased with increasing concentrations of ethanol in the solvent system. The maximum flux of acetylsalicylic acid was obtained with 80 % ethanol/20 % propylene glycol, with no further enhancement of flux at a level of 100 % ethanol. FTIR analysis revealed that increasing concentrations of ethanol in the solvent systems decreased peak height and peak area under both asymmetric and symmetric C-H absorbances until 80 % ethanol/20 % propylene glycol. Further increases in the concentration of ethanol (i.e. 100 % ethanol) did not correspondingly decrease the peak heights and areas under asymmetric and symmetric absorbances. The overall conclusion was that 80 % ethanol/20 % propylene glycol enhanced the percutaneous absorption of acetylsalicylic acid by perturbing (or fluidising) the macroscopic barrier integrity of the stratum corneum and through a loss of stratum corneum lipids. Dias et al. (2008) also used FTIR to determine that the uptake of solvent (i.e. the higher alkanols hexanol, octanol, decanol) was proportional to C-H shift and that lipid disorder was induced by all alkanols examined and proportional to the amount of vehicle present in the skin. Anandson et al. (2009) later used attenuated total reflectance FTIR (ATR-FTIR) to show a strong correlation between the distribution of lipids and drugs within the stratum corneum, whilst noting the homogenous distribution of ethanol in the stratum corneum. Vaddi et al. (2002) used data from a combination of FTIR and DSC to propose a mechanism for the enhanced permeation of haloperidol across the human stratum corneum in vitro. They used vehicles containing three different terpenes (5 %) in 50 % ethanol. It was concluded that the terpenes disrupted the lipid bilayer and extracted the lipids and that ethanol also extracted lipids without fluidising the lipids, and its effect on the stratum corneum remained almost similar in spite of changes in the treatment time and the amount of ethanol used. Raman spectroscopy has been used to study molecular interactions between ethanol (also limonene, dimethyl sulfoxide) and skin ceramide organisation (Tfayli et al. 2012). The effects of the three penetration enhancers were varied, with ethanol presenting no effect on ceramide III and 9, although it decreased the strength of the polar interactions for ceramide 5 and VI and increased the presence of strong H-bonds for CER 1, 2 and IIIb. However, the earlier paper by Bommanan et al. (1991) found no evidence by ATR-FTIR of lipid disorder caused by the application of neat ethanol to the skin and instead suggested that lipid leaching was the dominant mechanism.

In studies on the bioelectrical potential measurement of ions across the skin, ethanol and propylene glycol were found to reduce the permeation barrier of the stratum corneum so that ions in an electrolyte gel penetrate more readily into the skin (Ng et al. 2009). It was further found

that for the gel with higher percentage of ethanol, lower minimum skin electrode impedance value was obtained. Propylene glycol did not demonstrate this effect. van der Merwe and Riviere (2005) found that ethanol and ethanol/water mixtures altered the stratum corneum through lipid extraction, rather than through disruption of lipid order. The application of finite doses of an ethanolic solution to the skin is believed to result in the evaporation of the majority of ethanol molecules, a scenario that has been associated with drug supersaturation and thus higher chemical potential (Sect. 10.3.2) or simply drug precipitation at the skin surface.

Observations of lipid leaching/delipidisation do not sit well with other claims suggesting that lipid and barrier modulations caused by topically applied alcohols are reversible (Tfayli et al. 2012) and within a relatively short timescale, e.g. within 24 h as reported by Bommanan et al. (1991). Part of the problem here relates to the time it takes for new stratum corneum to be regenerated from stratum basale, which is approximately 4 weeks. Generally, it is clear that the use of very high concentrations of alcohol should be avoided. Using porcine skin homogenates, Van Hulst et al. (1998) demonstrated that ethanol and 2-phenoxyethanol affected skin metabolic processes. Delipidisation of the skin by neat alcohol, as exemplified in the work of Bommanan et al. (1991), will render the barrier in a compromised state in an in vivo situation, leading to excess water loss. Indeed, treatment with ethanol is an important and routine step in the dehydration of skin for immunostaining purposes (Thomas et al. 2007). In this context, the assertion that alcoholic vehicles do not result in skin trauma may not be entirely true.

To conclude, numerous papers have proposed mechanisms for the penetration enhancement action of ethanol and other alcohols – typically this involves increasing the donor permeant concentration and some element of modulating the lipid domains of the stratum corneum bilayers. However, what is absent from the aforementioned mechanistic propositions is the fact that alcohols and other solvents have the propensity to permeate skin and therefore behave as permeants in their own right.

10.4 Penetration of Alcohols Across the Skin

For reasons that are self-evident, the delivery of the formulated active agent is the main concern in investigations into topical drug delivery. However, a question posed in a number of papers has concerned the ‘fate’ of the vehicle and/or enhancer, although in most articles this is limited to the deposition of solvent within (and not beyond) the stratum corneum. Full answers to such questions can only be found by determining their delivery, preferably as part of a mass-balance exercise, to include analysis of receptor phases. There can only be 3 possibilities: evaporation, absorption or remaining at the region of application, as illustrated by Pendlington et al. (2001). In this review we focus on absorption, although in reality a combination of each of these processes is likely to occur, which will vary depending on the amount of formulation dosed onto the skin and the volatility of the vehicle components.

10.4.1 Evidence for Skin Penetration of Alcohols

The issue of absorption of solvent into the skin was recognised at least as far back as 1964, where dimethyl sulfoxide was found to penetrate biological membranes including skin (Horita and Weber 1964). However, the first major publication on the use of alcohols in topical delivery was by Flynn et al. (1981), who used a range of alkanol homologs to determine the permeability of mouse skin strata. A number of studies contain statements indirectly alluding to the skin penetration of alcohols, such as ethanol ‘appeared to be homogeneously distributed in the SC’ (Andanson et al. 2009).

Dose-dependent permeation of ethanol was reported across full-thickness porcine skin from PEG400 gels (Heard et al. 2006) (Fig. 10.2) and demonstrated that even at ~5 % level, commonly used in skin penetration evaluations (Verma and Fahr 2004), a steady state is established for the permeation of ethanol across the skin, confirming that although employed as an excipient, the substance in fact behaves like any other permeant.

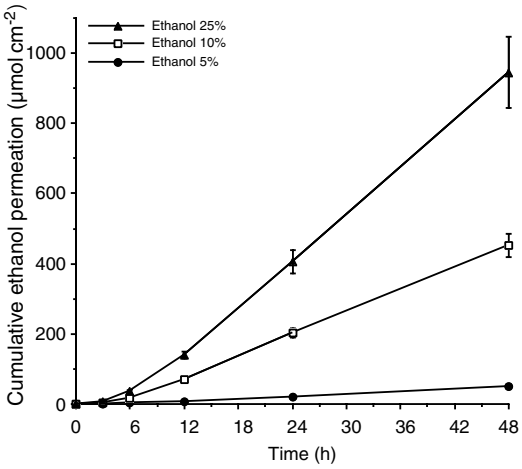

Fig. 10.2 Concentration-dependent steady-state flux of ethanol across porcine ear skin (Heard et al. 2006)

Interestingly, porcine skin was found to be 5–13 x more permeable to ethanol than to 1,8-cineole, even though both were used at the same levels. In other work, Kramer et al. (2007) studied the amount of ethanol absorption from *infinite* doses of hand rubs in human volunteers: 12 volunteers applied three different hand rubs, containing 95 % (hand rub A), 85 % (hand rub B) and 55 % ethanol (hand rub C; all w/w). For hygienic hand disinfection, 4 mL was applied 20 times for 30 s, with 1 min break between applications. For surgical hand disinfection, 20 mL of each hand rub was applied to the hands and arms up to the level of the elbow 10 times for 3 min, with a break of 5 min between applications. The median of absorbed ethanol after hygienic hand disinfection was 1365 mg (A), 630 mg (B) and 358 mg (C). The ethanol absorbed was 2.3 % (A), 1.1 % (B) and 0.9 % (C). After surgical hand disinfection, the median of absorbed ethanol was 1067 mg (A), 1542 mg (B) and 477 mg (C). The proportion of absorbed ethanol was 0.7 % (A), 1.1 % (B) and 0.5 % (C). The highest median acetaldehyde concentration after 20 hygienic hand disinfections was 0.57 mg/L (hand rub C, after 30 min) and after 10 surgical hand disinfections 3.99 mg/L (hand rub A, after 20 min). It was concluded that the absorption of ethanol was below toxic levels. However, Pendlington et al. (2001) found that most of the ethanol from a *finite*, 5 µL, applied dose of alcohol-based deodorant spray evaporated from the skin, with a half-life of ~12 s. However, the level of ethanol penetrating the skin through porcine skin in vitro was greater in diffusion cells that had been occluded than cells that had not been occluded, highlighting the effect of evaporation on determining ethanol flux.

Propylene glycol was reported by Hoelgaard and Møllgaard (1985) to 'easily' permeate the human skin in vitro. A decade later, Bendas et al. (1995) studied the skin penetration of glucocorticoids from binary propylene glycol/water mixtures and noted that propylene glycol penetrates rapidly into the artificial acceptor and into excised human skin. The amount of propylene glycol to penetrate the skin from an applied finite dose was found to vary as a function of film drying time (Bowen and Heard 2006). Fasano et al. (2011) determined the dermal penetration of undiluted monopropylene glycol and dipropylene glycol in vitro using human abdominal skin under conditions of infinite dose application – the steady-state fluxes were 97.6 and 39.3 µg cm^{-2} h^{-1}, respectively. Guo et al. (2010) used the biophysical technique of optical coherence tomography to quantify the amount of propylene glycol in human skin.

In summary, there is much evidence that ethanol and other alcohols are readily absorbed into and through the skin. It is therefore pertinent to ask how the permeation of an alcohol across the skin is related to the permeation of a co-formulated drug.

10.5 Simultaneous Skin Permeation of Solute and Alcohol in the Vehicle

Although reports of the simultaneous skin permeation of solvent and drug solute are relatively uncommon, the notion is far from novel.

10.5.1 Reported Observations

In one of the earliest paper to consider simultaneous permeant and vehicle permeation across the

skin, Hoelgaard and Møllgaard (1985) ascribed the skin penetration-enhancing effect of metronidazole by propylene glycol to a 'carrier–solvent' effect, having noted the rapid permeation of propylene glycol across the skin. Interestingly, the addition of Azone® had the effect of reducing permeation lag time for both the metronidazole and propylene glycol. Kadir et al. (1987) later observed that when theophylline was applied to the skin in 1.5 % solutions in various alkane carboxylic acids and their mixtures, propionic acid enhanced penetration of theophylline due to 'promoting its solubility in the skin-propionic acid medium through the pull effect'. It was proposed that the push/pull effects could operate jointly, although no empirical evidence was provided for the localisation of alkane carboxylic acids within the skin. Berner et al. (1989) used aqueous ethanol vehicles to increase nitroglycerine flux across the skin, also noting high ethanol skin permeation that was far greater than reported previously. 'Symmetric' ethanol (in both donor and receptor phases) and 'asymmetric' (ethanol in donor phase only) models were used by Liu et al. (1991) to demonstrate that ethanol enhances the stratum corneum transport of estradiol and of itself by increasing the respective diffusion coefficients when used at lower concentrations (less than 50 %) and by both increasing the diffusion coefficients and decreasing a 'membrane activity coefficient' when used at concentrations of 50–75 %.

Bendas et al. (1995) studied the skin penetration of glucocorticoids from binary propylene glycol/water mixtures. It was found that hydrocortisone penetration increased with increasing propylene glycol content in the formulation. In the knowledge that propylene glycol penetrates rapidly into the artificial acceptor and into the excised human skin, it was proposed that a drag effect was responsible. In the case of the more lipophilic hydrocortisone, 17-butyrate penetration was thermodynamically driven, up to 40 % propylene glycol, but at higher levels the drag transport mechanism dominated. Squillante et al. (1998) studied the co-diffusion of propylene glycol and dimethyl isosorbide (DMI) in relation to nifedipine penetration across hairless mouse skin. After 24 h 57 % of propylene glycol and 40 % of DMI had permeated across the skin with nearly linear permeation between 4 and 18 h, and the relative order of permeation was propylene glycol > DMI > nifedipine. It was concluded that 'nifedipine flux was dependent on concomitant solvent permeation'. Having determined that high solvent (including polyethylene glycols 200 and 400) uptake promoted the partitioning of methylparaben into human stratum corneum enabling the solute to exist within the solvent fraction/solvent-rich areas inside the skin in a concentration equivalent to that in the bulk solvent/vehicle, Oliveira et al. (2012) further acknowledged that this may also ultimately impact on the diffusion coefficient of the solute across the skin.

In vitro experimentation was borne out with in vivo clinical effects in a paper by Fang et al. (2008), who studied the synergistically enhanced percutaneous penetration of tetracaine and skin analgesia upon the application of a carbomer gel containing 4 % tetracaine together with menthol and ethanol. Tests in mice showed a synergistically increased penetration-enhancing effect of menthol and ethanol on the anaesthetic effect of the tetracaine gel in volunteers. The gel with 5 % menthol and 70 % ethanol possessed the shortest anaesthesia onset time, the longest anaesthesia duration and the strongest anaesthesia efficacy. This last paper is important as it establishes that clinical effects reflect in vitro studies.

10.5.2 Terminology

The articles mentioned in Sect. 10.5.1 thus provide compelling evidence for the concomitant skin penetration of alcohol enhancers alongside the main active solute. Various terms have been coined to account for such observations. *Co-permeation* involves the diffusion of both solute (solvated) and solvent molecules. In a saturated solution, the solute and its solvated solvent molecules penetrate only, as all the solvent molecules are engaged in maintaining the state of saturation. However, if a subsaturated solution is applied, then the solvated solute AND solvent

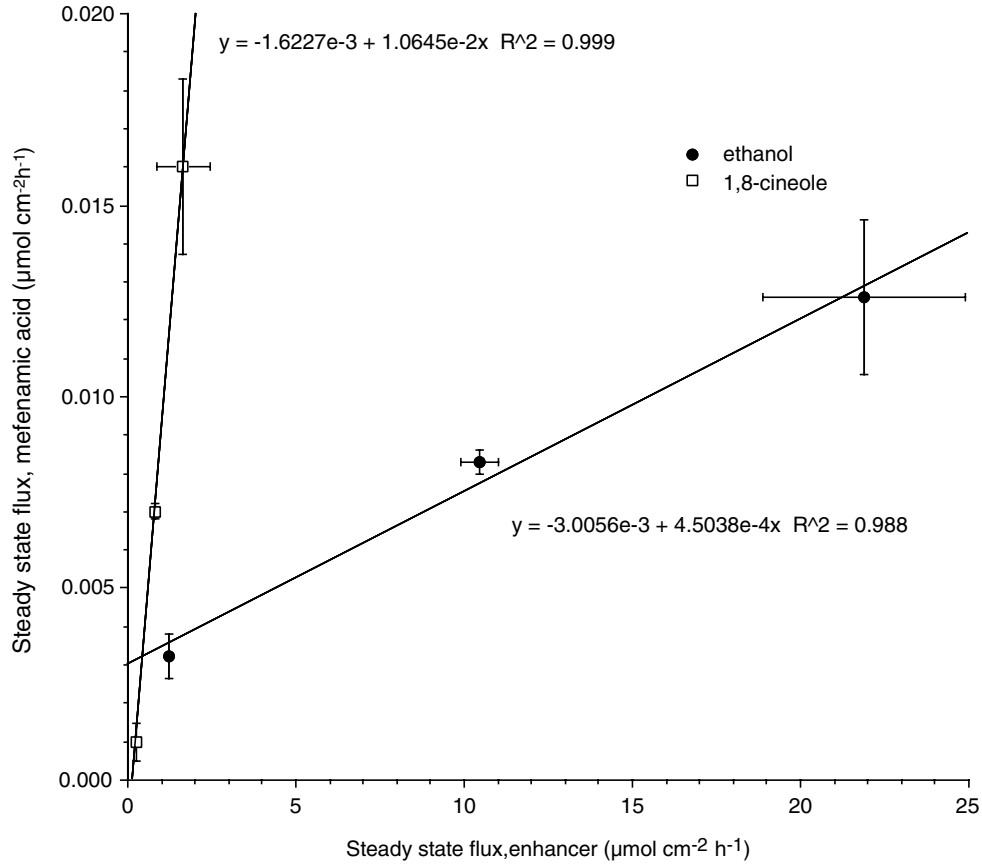

Fig. 10.3 Steady-state flux correlations: mefenamic acid vs. 1,8-cineole (□); mefenamic acid vs. ethanol (●) (*left to right*: 5, 10, 25 % enhancer; $n = 8 \pm$ SEM) (Heard et al. 2006)

molecules may permeate. The lower the degree of saturation is, the greater is the amount of solvent that is available to permeate independently. However, it is not easy to deconvolute the ethanol that permeates the skin as discrete or free molecules from those which are associated with solute molecules.

The solvated solute will be a larger entity, and so it is likely to be the smaller solvent that permeates first. This would have the effect of establishing a more favourable partitioning environment thus permitting increased diffusion of the solvated complex both into the skin and beyond – this can be considered as the *Pull* or *Drag* effect (the term 'carrier–solvent' effect was used by Hoelgaard and Møllgaard 1985). The drag effect can be seen as an extension of co-permeation, where the diffusion of the solvent is facilitating the free solvent by, in effect, washing it through the tissue. Whereas both ethanol and propylene glycol provide a drag effect, it is clear that the former is more effective than the latter (Fig. 10.3). The permeation of theophylline correlated negatively with the permeability coefficients of the donor carboxylic acids (Kadir et al. 1987), whereas the undetectable permeation of mefenamic acid from a saturated solution in PEG400 was indicative of the minimal permeation enhancing/drag effect associated with this excipient (Heard and Screen 2008). The enhancement trend was thus: ethanol > propylene glycol > PEG400 – interestingly this corresponded with boiling point, 78.4, 188.2 > 250 °C.

In addition to ethanol and propylene glycol, it is important to emphasise that the same considerations may apply to other vehicles and enhancers, including water that may be present in an applied dose.

10.6 Rationalising Pull/Drag/ Co-permeation Mechanisms

Given that drug and vehicular alcohol can permeate the skin simultaneously, it is pertinent to consider the relationship between the two.

10.6.1 Drug Dissolution and Solvation Shells

It is useful at this point to remind ourselves that the formulation of drugs into solutions, creams, gels and patches invariably involves the dissolution of solid drug in a solvent as an initial step of the preparation process. This occurs because there is thermodynamic favourability for drug molecules to associate with molecules of a solvent rather than other drug molecules – alcohols, in particular ethanol and methanol, are very effective at this. Solvent–solute interactions involved in alcohol solvation can involve hydrogen bonding, van der Waals forces and ion–dipole and dipole–dipole forces. Solvent molecules can solvate charges/partial charges of a solute because they can orient the polar moiety (e.g. hydroxyl groups in alcohols) towards corresponding moieties on the solute (Fig. 10.4), and different solvents may do so in different manners in a process generally referred to as solvatochromism. However, solvation will be thermodynamically favoured only if the overall Gibbs energy of solvation is decreased, compared to the Gibbs energy of the separated solvent and solid drug; overall the free energy must be negative for net dissolution to occur and establish solvation shells. The group of Perlovich has published a number of papers on the dissolution characteristics of drugs in various solvents including alcohols (Perlovich et al. 2003; 2004). Using solution calorimetry, the Gibbs free energy of the solvation of a range of nonsteroidal anti-inflammatory drugs in a range of alcohols has been determined (Perlovich and Bauer-Brandl 2004). For example, the dissolution of naproxen in ethanol, this has been determined to require 47.3 kJ mol^{-1}.

The state of saturation (i.e. solubility) can be considered as the amount of solvent molecules required to solvate a given mass of the solute. Above this (and in the absence of other factors, e.g. an anti-nucleating agent), no more molecules of solvent are available to dissolve further molecules of the solute. This can be more readily conceptualised in a single solvent – however, the scenario becomes far more complicated when binary, tertiary or more co-solvent systems are

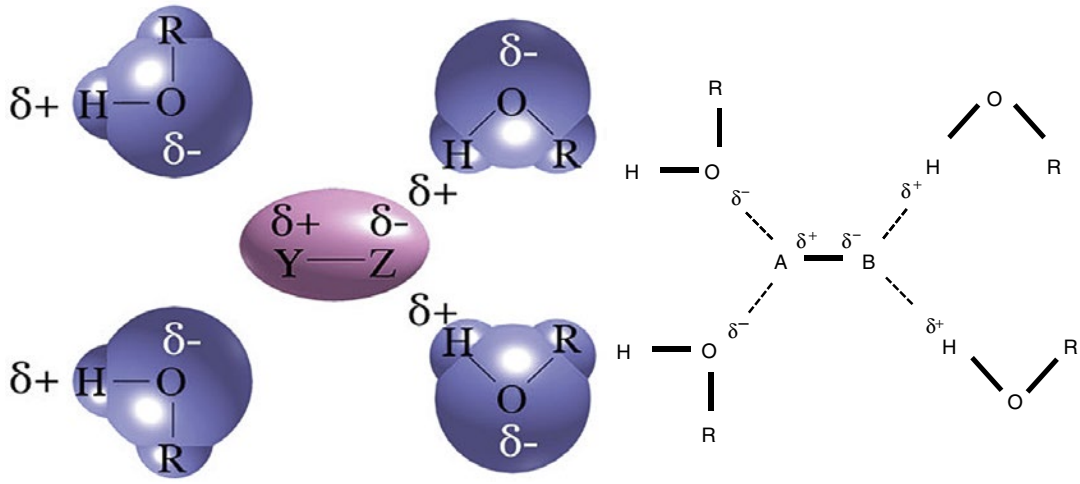

Fig. 10.4 Representation of the dipolar moiety of a permeant molecule solvated with alcohol molecules, based on the attraction of reciprocal partial charges δ^- on oxygen atoms and δ^+ on hydrogen atoms

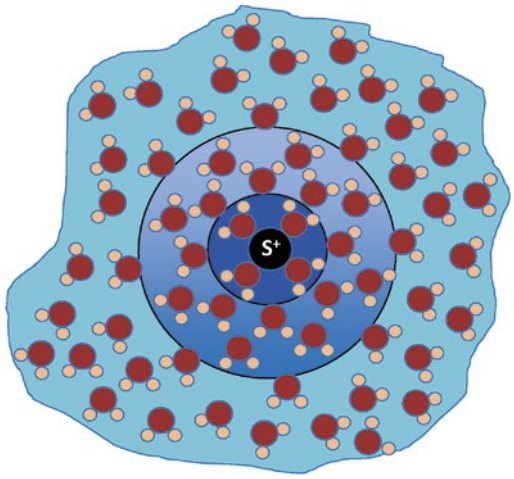

Fig. 10.5 Representation of solvation shell arrangements for a permeant in a saturated solution (S^+ is a solute or permeant molecule bearing a positive charge)

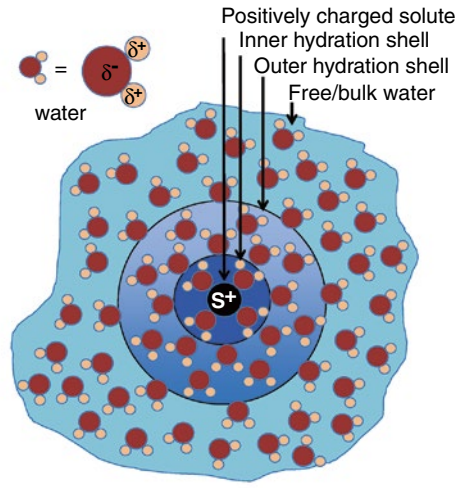

Fig. 10.6 Representation of the solvation shell arrangement of a permeant dissolved at subsaturation (S^+ is a solute or permeant molecule bearing a positive charge)

used due to preferential solvation/solvatochromic effects (Farajtabar et al. 2011). A solvation shell is a complex of a solute plus the solvent molecules interacting with it to maintain its state of solution; solvent molecules in an inner shell are more tightly bound than those of an outer shell, which can be considered metastable. Such complexes have been studied by IR spectroscopy, where shifts in bond absorbances have been found to correspond with hydrogen bonding (Kumar and Keyes 2012). In a saturated solution such complexes are adjacent to each other (Fig. 10.5); at subsaturation there will be free solvent in between, the amount of which depending on the degree of saturation (Fig. 10.6).

Pardo et al. (1991) noted that the highest permeability coefficient of the delivery of physostigmine through excised human skin from isopropyl alcohol–isopropyl myristate mixtures was from the mixture exhibiting the highest solvation effect, giving additional evidence that physostigmine penetrates through the skin, possibly in combination with isopropyl alcohol.

10.6.2 Uptake of Solvation Shells and Free Solvent into Skin

When applied to the skin, a formulation is confronted by the diverse elements that constitute the stratum corneum. These include the relatively vast areas of proteinaceous corneocytes, the relatively limited domains of intercellular lipids and water (either resident within normally hydrated skin or appendageal efflux). Generally, the solvated drug complex must remain intact unless interactions between the drug molecule and other species present are more favourable; perhaps the most notable possibility is water that exists at the skin surface or within the stratum corneum. However, this is dependent upon the energies of solvation involved, the stoichiometric availability of water molecules and the tendency of the water to approach the complex, e.g. it would be unfavourable for polar water to approach a drug solvated in an oily excipient. The events occurring when a molecule solvated in a shell of alcohol (solvent) molecules partitions from one medium (e.g. topical formulation) into a second medium (e.g. skin) were described by Perlovich and Bauer-Brandl (2004). Solute-partitioning scenarios, in terms of solvation shell transit, from the vehicle into the skin are depicted in Fig. 10.7: (i) partitioning of the solute within the complete solvation (alcohol) shell – most likely where the skin is saturated with solvent; (ii) partitioning of the solute with weekly bound outer solvation shell stripped, e.g. by interaction with other compounds; (iii) partial replacement of

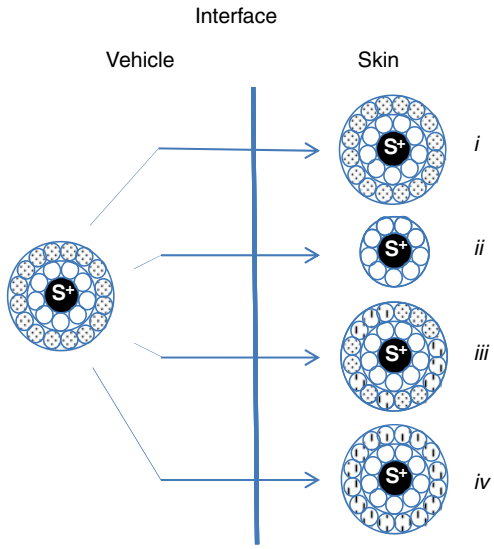

Fig. 10.7 Representations of solute (S^+) partitioning scenarios, in terms of solvation shell transit, from vehicle into the skin: (i) partitioning of the solute within the complete solvation shell; (ii) partitioning of the solute with weekly bound outer solvation shell stripped; (iii) partial replacement of weekly bound outer shell with skin electrolytes or other components; (iv) complete replacement of weekly bound outer shell with skin electrolytes or other components (Perlovich and Bauer-Brandl 2004)

weekly bound outer shell with skin compounds or other components; and (iv) complete replacement of weekly bound outer shell with skin compounds or cellular components. Thus, a drug/solute in solution will be engaged in interactions with molecules of the solvent. Any further interaction between solvated solute and other materials (e.g. skin components) is therefore unlikely unless thermodynamically favourable. The reader is referred to the work of Perlovich and Bauer-Brandl (2004), who published an in-depth study of drug solvation in relation to partitioning and passive transport.

10.6.3 Linear Relationships Between Permeation of Alcohol and Drug

The following papers have generally reported linear relationships between the permeation of the active permeant and the enhancer, i.e. as the rate of permeation of active increases, so does that of the enhancer and vice versa (Heard et al. 2003). Berner et al. (1989) used aqueous ethanol vehicles to increase nitroglycerin flux across the skin, also noting high ethanol skin permeation, and that the flux of nitroglycerin across skin was linear with the ethanol flux. In a study involving simple formulations of mefenamic acid in PEG400 with either ethanol or 1,8-cineole, it was demonstrated that the steady-state flux of each enhancer was linearly linked to that of the drug (Heard et al. 2006). Figure 10.3 shows the correlation between the fluxes of mefenamic acid versus ethanol in PEG400 and mefenamic acid versus 1,8-cineole in PEG400. The correlation coefficients were 0.988 and 0.999, respectively, whilst the permeation from PEG400 alone (control) was below the detection limit. The plot for 1,8-cineole is generally in the expected direction, with the Y-axis intercept almost at 0. However, for ethanol the intercept does not pass through 0, and at low ethanol contents, a process other than drag-facilitated diffusion occurs, possibly leaching of non-covalently bound amphiphilic stratum corneum lipids. In recent work the permeation of caffeine from saturated solutions in ethanol/water mixtures has been examined (Houston D et al. 2011), where the permeation rates of caffeine and ethanol were clearly linked as evidenced by the high correlation (Fig. 10.8) and similar to previous observations for mefenamic acid and ethanol (Heard et al. 2006) (Fig. 10.8). However, whereas the bulk solvent used in the earlier work was PEG400 (Heard et al. 2006) with poor permeation enhancing properties, the latest work used water which permeates the skin relatively readily; furthermore, linear penetration enhancement of both caffeine and water was shown from the aqueous solution (0 % ethanol). Based on the previous discussion, it is likely that a pull effect occurs with a simple water vehicle (not considered in this chapter).

The observed linearity implies a fixed relationship between the amount of alcohol and drug permeating the skin. Thus, the issue of quantitating the relationship becomes relevant, although there are very few examples in the literature where this has been attempted. The simultaneous permeation of ketoprofen and

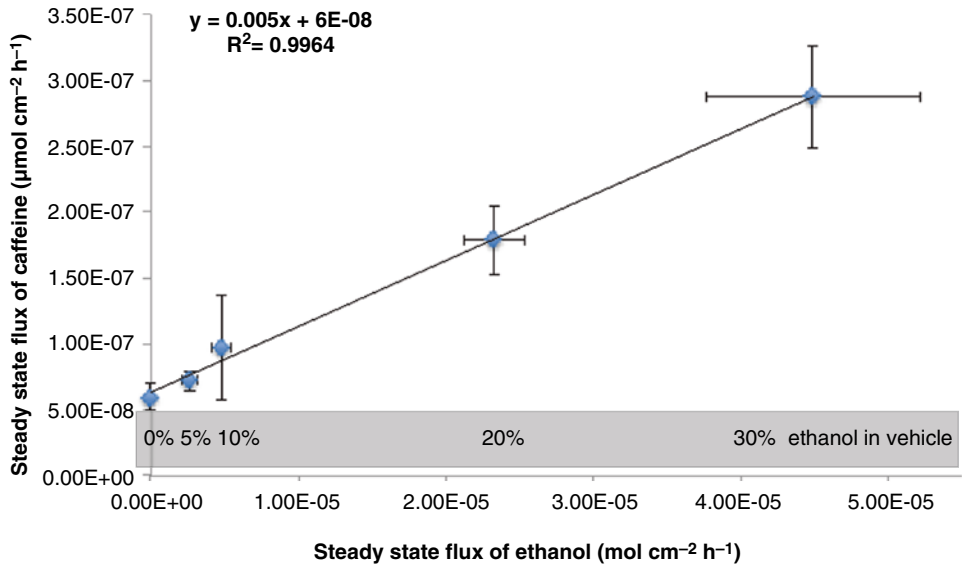

Fig. 10.8 Correlation of caffeine flux vs. ethanol flux in relation to vehicle ethanol content: 0, 5, 10, 20 and 30 % ethanol/water v/v across porcine skin in vitro ($n = 8$, ±SEM) (Houston D et al. 2011)

propylene glycol across pig ear skin from hydroxypropyl cellulose gel formulations under simulated finite/in-use conditions was investigated in an attempt to model a simple topical formulation in the process of drying (Bowen and Heard 2006). Figure 10.9 shows relationships between the cumulative moles of ketoprofen and propylene glycol permeated from the tested gels at 24 h and 48 h timepoints. The decrease in the delivery of propylene glycol relative to the increase in delivery of ketoprofen was linear across the gel formulations. Although a simple gel system, it was nevertheless a challenge to effectively mimic the processes taking placing in the drying film. For example, Reid et al. (2009) later found evidence of transient supersaturation of the active (beclomethasone dipropionate) following the drying of ethanol from applied films, although the paper did not account for any ethanol that might permeate skin and drag some drug with it. Clearly, much work remains to be done to further establish the permeation of solvated complexes completely through the skin.

Seemingly in contrast to the above, Liu et al. (1991) demonstrated that ethanol enhances the stratum corneum transport of estradiol and of itself, although they concluded that permeant flux is not simply linear with the co-transported enhancer in general. Again, it is worth making the distinction between those ethanol molecules permeating as part of a solvated complex and those that are permeating independently under their own concentration gradient. Such factors would result in lack linearity, with an excess in terms of the ethanol permeated.

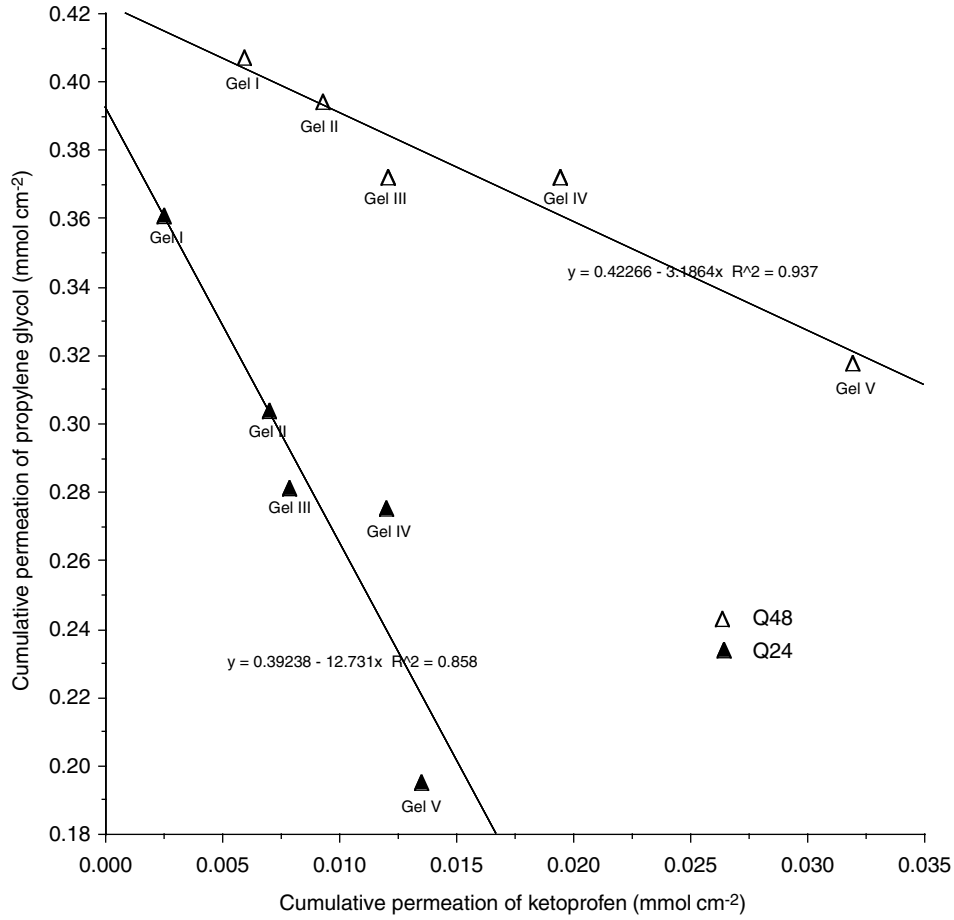

Fig. 10.9 Correlation between permeated propylene glycol (mmol cm^{-2}) and permeated ketoprofen (mmol cm^{-2}) at 24 and 48 h. Gel I – V: 90.9, 88.5, 84.7, 75.4, 38.1 % propylene glycol in polyethylene glycol 400

Conclusions

Based on a summation of the preceding discussions, we are now in a position to define a new composite model to account for the skin penetration enhancement arising from the use of alcohols:

1. Dissolution of solute in vehicle and enhancer, establishment of solvation shells and elevated donor phase concentration of drug.
2. Upon application to the skin, interaction with and modulation of skin lipids, possible leaching of lipids if alcohol content is high.
3. Permeation of free solvent which is not engaged in solvating the permeant, but is being delivered across the skin independently.
4. Co-permeation of solvated drug solute and free alcohol molecules, along the concentration gradient, in the skin that is

enriched or even saturated with free alcohol providing higher partitioning environment (pull effect).

5. Interaction of solvated complex with skin components and diffusional barriers within the skin tissue which acts to retard permeation.

Finally, pick up a book or review article on the subject of skin penetration enhancement, and a statement to the effect 'penetration enhancement is caused by modulations in the lipids of the stratum corneum' will invariably be found. It is hoped that having read this article, the reader will be inspired to consider that such processes may not provide a complete picture, as the mechanism of enhancement, by in particular ethanol and other alcohols, must be extended to include the permeation of the enhancer itself. Moreover, the importance of alcohol vehicle uptake into the skin has been seriously overlooked, and there are clearly issues relating to intoxicating issues of topically applied formulations that contain appreciable quantities of ethanol, e.g. alcohol hand rubs. Studying the skin permeability of vehicles and enhancers in tandem with the active compound, although simplistic, sheds additional valuable light on the complex processes involved in skin permeation enhancement mechanisms. However, unless one takes the trouble to assay diffusion cell receptor fluids for all permeants, the presence and potential significance of all species may be missed. It is hoped that such practice will become more widespread.

References

Ahmed-Lecheheb D, Cunat L, Hartemann P, Hautemanière A (2012) Dermal and pulmonary absorption of ethanol from alcohol-based hand rub. J Hosp Infect 81:31–35

Andanson JM, Hadgraft J, Kazarian SG (2009) In situ permeation study of drug through the stratum corneum using attenuated total reflection Fourier transform infrared spectroscopic imaging. J Biomed Opt 14:034011

Andega S, Kanikkannan N, Singh M (2001) Comparison of the effect of fatty alcohols on the permeation of melatonin between porcine and human skin. J Control Release 77:17–25

Bendas B, Schmalfuβ U, Neubert R (1995) Influence of propylene glycol as cosolvent on mechanisms of drug transport from hydrogels. Int J Pharm 116:19–30

Benson H (2005) Transdermal drug delivery: penetration enhancement techniques. Curr Drug Deliv 2:23–33

Berner B, Mazzenga GC, Otte JH, Steffens RJ, Juang RH, Ebert CD (1989) Ethanol: water mutually enhanced transdermal therapeutic system II: skin permeation of ethanol and nitroglycerin. J Pharm Sci 78:402–407

Bommanan D, Potts R, Guy RH (1991) Examination of the effect of ethanol on human stratum corneum in vivo using infrared spectroscopy. J Control Release 16:299–304

Bowen JL, Heard CM (2006) Film drying and complexation effects in the simultaneous skin permeation of ketoprofen and propylene glycol from simple gel formulations. Int J Pharm 307:251–257

Brand RM, Jendrzejewski JL, Charron AR (2007) Potential mechanisms by which a single drink of alcohol can increase transdermal absorption of topically applied chemicals. Toxicology 235:141–149

Chantasart D, Li SK, He N, Warner KS, Prakongpan S, Higuchi WI (2004) Mechanistic studies of branched-chain alkanols as skin permeation enhancers. J Pharm Sci 93:762–779

Dias M, Naik A, Guy RH et al (2008) In vivo infrared spectroscopy studies of alkanol effects on human skin. Eur J Pharm Biopharm 69:1171–1175

Du Plessis J, Pugh WJ, Judefeind A, Hadgraft J (2001) The effect of hydrogen bonding on diffusion across model membranes: consideration of the number of H-bonding groups. Eur J Pharm Sci 13:135–141

Duracher L, Blasco L, Hubaud J-C et al (2009) The influence of alcohol, propylene glycol and 1,2-pentanediol on the permeability of hydrophilic model drug through excised pig skin. Int J Pharm 374:39–45

Fang C, Liu Y, Ye X et al (2008) Synergistically enhanced transdermal permeation and topical analgesia of tetracaine gel containing menthol and ethanol in experimental and clinical studies. Eur J Pharm Biopharm 68:735–740

Farajtabar A, Jaberi F, Gharib F (2011) Preferential solvation and solvation shell composition of free base and protonated 5, 10, 15, 20-tetrakis(4-sulfonatophenyl) porphyrin in aqueous organic mixed solvents. Spectrochim Acta A 83:213–220

Fasano WJ, ten Berge WF, Banton MI et al (2011) Dermal penetration of propylene glycols: measured absorption across human abdominal skin in vitro and comparison with a QSAR model. Toxicol In Vitro 25:1664–1670

Fiume MM, Bergfeld WF, Belsito DV et al (2012) Safety assessment of propylene glycol, tripropylene glycol, and propylene glycols as used in cosmetics. Int J Toxicol 31:245S–260S

Flynn GL, Durrheim H, Higuchi WI (1981) Permeation of hairless mouse skin II: membrane sectioning techniques and influence on alkanol permeabilities. J Pharm Sci 70:52–56

Friend D, Catz P, Heller J, Reid J, Baker R (1988) Transdermal delivery of levonorgestrel I. Alkanols as permeation enhancers. J Control Release 7:243–250

Funke AP, Schiller R, Motzkus HW, Gunther C, Muller RH, Lipp R (2002) Transdermal delivery of highly lipophilic drugs: in vitro fluxes of antiestrogens, permeation enhancers, and solvents from liquid formulations. Pharm Res 19:661–668

Geusens B, Van Gele M, Braat S (2010) Flexible nanosomes (secosomes) enable efficient siRNA delivery in cultured primary skin cells and in the viable epidermis of ex vivo human skin. Adv Funct Mater 20:4077–4090

Guo X, Guo ZY, Wei HJ et al (2010) In vivo quantification of propylene glycol, glucose and glycerol diffusion in human skin with optical coherence tomography. Laser Phys 20:1849–1855

Heard CM, Screen C (2008) Probing the permeation enhancement of mefenamic acid by ethanol across full thickness skin, heat-separated epidermal membrane and heat-separated dermal membrane. Int J Pharm 349:323–325

Heard CM, Gallagher SJ, Harwood JL, Maguire PB (2003) The in vitro delivery of NSAIDs across skin was in proportion to the delivery of essential fatty acids in the vehicle – evidence that solutes permeate skin associated with their solvation cages? Int J Pharm 261:165–169

Heard CM, Congiatu C, Gallagher SJ, Harwood JL, Karia C, McGuigan C, Nemcova M, Nouskova T, Thomas CP (2005) Preferential π-π complexation between tamoxifen and borage oil/γ linolenic acid: transcutaneous delivery and NMR spectral modulation. Int J Pharm 302:47–55

Heard CM, Kung D, Thomas CP (2006) Skin penetration enhancement of mefenamic acid by ethanol and 1,8-cineole can be explained by the pull effect. Int J Pharm 321:167–170

Higuchi WI (1962) Analysis of data on the medicament release from ointments. J Pharm Sci 51:802–804

Ho S, Calder RJ, Heard CM, Thomas CP (2004) In vitro transcutaneous delivery of tamoxifen and γ linolenic acid from a borage oil formulation containing ethanol and 1,8-cineole. J Pharm Pharmacol 56:1–8

Hoelgaard A, Møllgaard B (1985) Dermal drug delivery. Improvement by choice of vehicle or drug derivative. J Control Release 2:111–120

Horita A, Weber LJ (1964) Skin penetrating property of drugs dissolved in dimethylsulfoxide (DMSO) and other vehicles. Life Sci 3:1389–1395

Hostýnek JJ, Dreher F, Nakada T, Schwindt D, Anigbogu A, Maibach HI (2001) Human stratum corneum adsorption of nickel salts. Investigation of depth profiles by tape stripping in vivo. Acta Derm Venereol Suppl 212:11–18

Houston DM, Rogers S, Heard CM. Skin penetration enhancement of caffeine by ethanol and propylene glycol: probing the co-permeation effect. Unpublished date

Ibrahim SA, Li SK (2009) Effects of solvent deposited enhancers on transdermal permeation and their relationship with Emax. J Control Release 136: 117–124

Ikeda Y, Higashi K, Moribe K, Yamamoto K (2012) Enhanced skin permeation of piroxicam and pranoprofen induced from nanoparticles dispersed in propylene glycol aqueous solution. J Drug Deliver Sci Technol 22:131–137

Jaimes-Lizcano YA, Lawson LB, Papadopoulos KD (2011) Oil-Frozen W-1/O/W-2 double emulsions for dermal biomacromolecular delivery containing ethanol as chemical penetration enhancer. J Pharm Sci 100:1398–1406

Kadir R, Stempler D, Liron Z, Cohen S (1987) Delivery of theophylline into excised human-skin from alkanoic acid-solutions - a push-pull mechanism. J Pharm Sci 76:774–779

Kim K, Oh S (2011) Calcitonin transport through skin using iontophoresis. J Pharm Invest 41:9–17

Kramer A, Below H, Bieber N et al (2007) Quantity of ethanol absorption after excessive hand disinfection using three commercially available hand rubs is minimal and below toxic levels for humans. BMC Infect Dis 7:117

Kumar R, Keyes T (2012) The relation between the structure of the first solvation shell and the IR spectra of aqueous solutions. J Biol Phys 38:75–83

Kurihara-Bergstrom T, Knutson K, DeNoble LJ, Goates CY (1990) Percutaneous absorption enhancement of an ionic molecule by ethanol-water systems in human skin. Pharm Res 7:762–766

Leichtnam ML, Rolland H, Wüthrich P, Guy RH (2006) Formulation and evaluation of a testosterone transdermal spray. J Pharm Sci 95:1693–1702

Levang AK, Zhao K, Singh J (1999) Effect of ethanol/propylene glycol on the in vitro percutaneous absorption of aspirin, biophysical changes and macroscopic barrier properties of the skin. Int J Pharm 30: 255–263

Liu P, Kurighara-Bergstrom T, Good WR (1991) Cotransport of estradiol and ethanol through human skin in vitro: understanding the permeant/enhancer flux relationship. Pharm Res 8:938–944

Liu P, Cettina M, Wong J (2009) Effects of isopropanol-isopropyl myristate binary enhancers on in vitro transport of estradiol in human epidermis: a mechanistic evaluation. J Pharm Sci 98:565–572

Megrab NA, Williams AC, Barry BW (1995) Oestradiol permeation across human skin, silastic and snake skin membranes: the effects of ethanol/water co-solvent systems. Int J Pharm 116:101–112

Morimoto H, Wada Y, Seki T, Sugibayashi K (2002) In vitro skin permeation of morphine hydrochloride during the finite application of penetration-enhancing system containing water, ethanol and L-menthol. Biol Pharm Bull 25:134–136

Moser K, Kriwet K, Froehlich C, Kalia YN, Guy RH (2001) Supersaturation: enhancement of skin penetration and permeation of a lipophilic drug. Pharm Res 18:1006–1011

Ng WC, Ng MH, Lee KS et al (2009) Investigation on ethanol and propylene glycol as enhancers for skin-electrode conductivity in bioelectrical potential measurement. Funct Mater Lett 2:175–177

Oliveira G, Hadgraft J, Lane ME (2012) The role of vehicle interactions on permeation of an active through model membranes and human skin. Int J Cosmetic Sci 34:536–545

Pardo A, Shiri Y, Cohen S (1991) Partial molal volumes and solubilities of physostigmine in isopropanol-isopropyl myristate solvents in relation to skin penetrability. J Pharm Sci 80:567–572

Patel MR, Patel RB, Parikh JR, Solanki AB, Patel BG (2009) Effect of formulation components on the in vitro permeation of microemulsion drug delivery system of fluconazole. AAPS PharmSciTech 10: 917–923

Pendlington RU, Whittle E, Robinson JA, Howes D (2001) Fate of ethanol topically applied to skin. Food Chem Toxicol 39:169–174

Perlovich GL, Bauer-Brandl A (2004) Solvation of drugs as a key for understanding partition and passive transport as exemplified by NSAIDs. Curr Drug Deliv 1:213–226

Perlovich P, Kurkov AN, Kinchin A, Bauer-Brandl A (2003) Thermodynamics of solutions IV: solvation of ketoprofen in comparison with other NSAIDS. J Pharm Sci 92:2502–2511

Perlovich GL, Kurkov SV, Kinchin AN, Bauer-Brand A (2004) Solvation and hydration characteristics of ibuprofen and acetyl salicylic acid. AAPS PharmSci 6:3

Pershing LK, Lambert LD, Knutson K (1990) Mechanism of ethanol-enhanced estradiol permeation across human skin in vivo. Pharm Res 7:170–175

Potts RO, Guy RH (1991) Predicting skin permeability. Pharm Res 9:663–669

Priborsky J, Takayama K, Nagai T et al (1987) Combination effect of penetration enhancers and propylene glycol on in vitro transdermal absorption of insulin. Drug Des Deliv 2:91–97

Pugh WJ (1999) Relationship between H-bonding of penetrants to stratum corneum lipids and diffusion. In: Bronaugh RL, Maibach HI (eds) Percutaneous Absorption: drugs, Cosmetics, Mechanisms, Methods, 3rd edn. Marcel Dekker, New York, Chapter 9

Reid ML, Jones SA, Brown M (2009) Transient drug supersaturation kinetics of beclomethasone dipropionate in rapidly drying films. Int J Pharm 371:114–119

Santos P, Watkinson AC, Hadgraft J, Lane ME (2012) Influence of penetration enhancer on drug permeation from volatile formulations. Int J Pharm 439:260–268

Santoyo S, Ygartua P (2000) Effect of skin pre-treatment with fatty acids on percutaneous absorption and skin retention of piroxicam after its topical application. Eur J Pharm Biopharm 50:245–250

Schaefer-Korting M, Mahmoud A, Borgia SL et al (2008) Reconstructed epidermis and full-thickness skin for absorption testing: influence of the vehicles used on steroid permeation. Altern Lab Anim 36: 441–452

Squillante E, Needham T, Maniar A, Kislaliogu S, Zia H (1998) Co-diffusion of propylene glycol and dimethyl isosorbide in hairless mouse skin. Eur J Pharm Biopharm 46:265–271

Tanojo H, Bouwstra JA, Junginger HE, Bodde HE (1999) Thermal analysis studies on human skin and skin barrier modulation by fatty acids and propylene glycol. J Therm Anal Calorim 57:313–322

Tfayli A, Emmanuelle G, Michel M, Baillet-Guffroy A (2012) Molecular interactions of penetration enhancers within ceramides organization: a Raman spectroscopy approach. Analyst 137:5002–5010

Thomas CP, Davison Z, Heard CM (2007) Probing the skin permeation of fish oil/EPA and ketoprofen 3. Influence of fish oil/ketoprofen on epidermal COX-2 and LOX. Prostaglandins Leukot Essent Fatty Acids 76:357–362

Twist JN, Zatz JL (1986) Influence of solvents on paraben permeation through idealized skin model membranes. J Soc Cosmet Chem 37:429–444

Vaddi HK, Ho PC, Chan YW, Chan SY (2002) Terpenes in ethanol: haloperidol permeation and partition through human skin and stratum corneum changes. J Control Release 81:121–133

Van der Merwe D, Riviere JE (2005) Comparative studies on the effects of water, ethanol and water/ethanol mixtures on chemical partitioning into porcine stratum corneum and silastic membrane. Toxicol In Vitro 19: 69–77

Van Hulst M, van Gompel AHP, Cornwell PA (1998) Percutaneous absorption and skin metabolism: the effects of formulation excipients on metabolism. In: Brain KR, James VJ, Walters KA (eds) Perspectives in percutaneous penetration, vol 5b. STS Publishing, Cardiff, pp 45–49

Verma DD, Fahr A (2004) Synergistic penetration enhancement effect of ethanol and phospholipids on the topical delivery of cyclosporin A. J Control Release 97:55–66

Wang LH, Wang CC, Kuo SC (2008) Vehicle and enhancer effects on human skin penetration of aminophylline from cream formulations: Evaluation in vivo. Int J Cosmet Sci 30:310

Williams AC, Barry BW (2004) Penetration enhancers. Adv Drug Deliv Rev 56:603–618

Terpenes and Essential Oils as Skin Penetration Enhancers

11

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11.1 Introduction

Dermal and transdermal delivery of drugs is limited by poor drug permeability which in turn is attributed to the stratum corneum which serves as a rate-limiting lipophilic barrier against the uptake of chemical and biological agents and the loss of water (Fang et al. 2003; Shah 1994).

Use of penetration enhancers (or sorption promoters) is the most common approach to mitigate stratum corneum permeability (Williams and Barry 1989; Benson 2005; Vávrová et al. 2005; Barry 2004). In recent years, efforts have been directed at identifying safe and effective penetration enhancers from natural and synthetic sources (Williams and Barry 1991). Terpenes are one of the most extensively studied classes of chemical penetration enhancers. Terpenes of natural origin have a GRAS (generally regarded as safe) status with the Federal Drug Administration of the USA, which offer advantages over other traditional enhancers such as alcohols, fatty acids, 1-dodecylazacycloheptan-2-one (Azone®),

N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_11

sulfoxides, and pyrrolidones (Thakur et al. 2006; Fox et al. 2011). In general, they have less systemic toxicity and skin irritancy in addition to having good penetration-enhancing abilities (Cornwell et al. 1996). Terpenes have reversible effects on the lipids of the stratum corneum. A number of terpenes have been shown to enhance percutaneous absorption of both lipophilic and hydrophilic drugs. However, proper selection of terpenes based on the functional groups, values of the logarithm of partition coefficient (log *P*), and metabolic disposition is important for the optimum sorption-promoting effect (Sapra et al. 2008).

In this chapter we present the classification of terpenes, factors affecting the activity of terpenes as penetration enhancers, and discussion on the utility of terpenes and essential oils as penetration enhancers.

11.2 Terpenes

Terpenes are described as compounds that are basically constituents of an essential oil and contain carbon and hydrogen atoms with or without oxygen (Pinder 1960). Terpenes are one of the most extensively studied classes of chemical penetration enhancers. They have received considerable interest in dermal and transdermal delivery of drugs with a wide range of physicochemical properties (Thakur et al. 2006).

11.2.1 Classification of Terpenes

The carbon skeletons of most terpenes are built up by the union of two or more isoprene (C_5H_8) units. Figure 11.1 shows the chemical structure of an isoprene unit (Pinder 1960; Torssell 1983). Terpenes can be classified according to the number of isoprene units in the molecule as illustrated in Table 11.1.

On the basis of a number of carbon rings present in the structure of the terpene, it can be subdivided into "acyclic," "monocyclic," and "bicyclic" categories (Thakur et al. 2006; Pinder 1960). Acyclic monoterpenes can be regarded as derivatives of 2,6-dimethyloctane, while

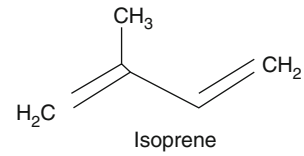

Fig. 11.1 Isoprene unit

Table 11.1 Classification of terpenes according to the number of isoprene units (Thakur et al. 2006; Aqil et al. 2007; Torssell 1983)

Number of isoprene units (*n*)	Number of carbon atoms	Type of terpene
2	C10	Monoterpenes ($C_{10}H_{16}$)
3	C15	Sesquiterpenes ($C_{15}H_{24}$)
4	C20	Diterpenes ($C_{20}H_{32}$)
5	C25	Sesterterpenes ($C_{25}H_{40}$)
6	C30	Triterpenes ($C_{30}H_{48}$)
8	C40	Tetraterpenes ($C_{40}H_{64}$)
>8	>C40	Polyterpenes $(C_5H_8)_n$

monocyclic monoterpenes are derivatives of cyclohexane containing isopropyl substituents. Bicyclic monoterpenes are arranged in more than one aromatic ring, which contains the same number of carbon atoms. Sesquiterpenes are present as acyclic, monocyclic, bicyclic, tricyclic, and tetracyclic structures and are mostly found in higher plants. The subdivision of each category is given in Figs. 11.2, 11.3, 11.4, and 11.5 (Thakur et al. 2006; Pinder 1960; Torssell 1983).

11.2.2 Factors Influencing the Activity of Terpenes as Penetration Enhancers

The activity of terpenes as penetration enhancers is primarily associated to their chemical structure as well as the physicochemical properties of the permeant drug (Aqil et al. 2007), as discussed below.

11.2.2.1 Size and Chirality

The size of a terpene is an important factor in determining its enhancing capacity. Smaller

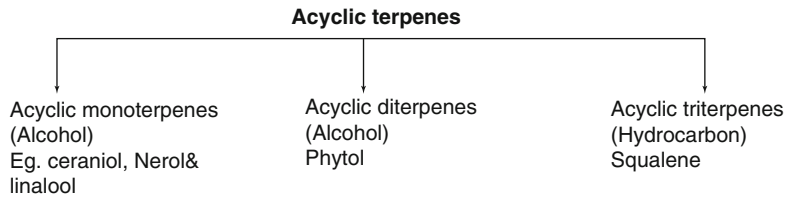

Fig. 11.2 Types of acyclic terpenes

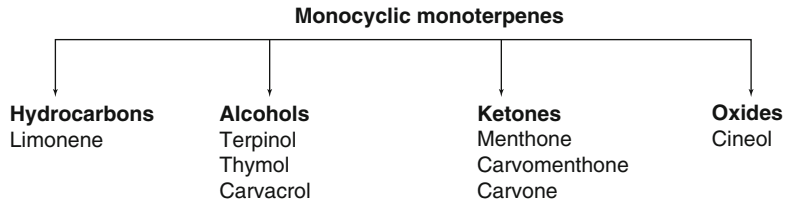

Fig. 11.3 Types of monocyclic monoterpenes

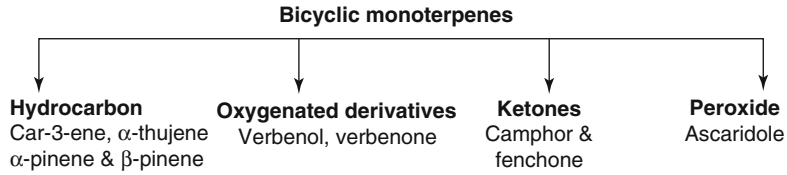

Fig. 11.4 Types of bicyclic monoterpenes

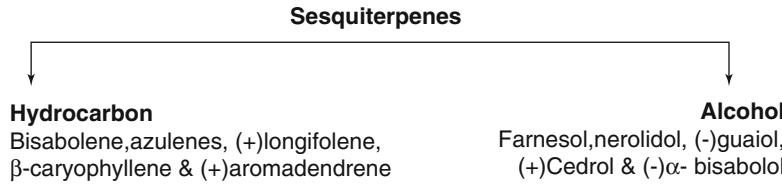

Fig. 11.5 Types of sesquiterpenes

terpenes are more active sorption enhancers than larger terpenes (Williams and Barry 2004). The stereoisomers of terpene also affect the enhancement potential of particular molecules. The (−) enantiomer of a terpene has been observed as a more effective penetration enhancer than the corresponding (±) racemate or the (+) isomer (Monti et al. 1995).

11.2.2.2 Degree of Unsaturation

It has been reported that smaller alcoholic terpenes with a higher degree of unsaturation are good candidates to enhance the permeation of hydrophobic drugs (Ghafourian et al. 2004). The terpenes with a minimal degree of unsaturation, like l-menthol and 1,8-cineole, are good sorption promoters for polar and water-soluble drugs (Jain et al. 2002).

11.2.2.3 Lipophilicity

High lipophilicity is an important structural feature for terpenes as sorption promoters for lipophilic drugs (Ghafourian et al. 2004). The hydrocarbon and nonpolar terpenes (e.g., limonene) are reported as more potent enhancers for lipophilic drugs (indomethacin) than oxygen-containing polar terpenes (1,8-cineole, carvone) (Nagai et al. 1989; Okabe et al. 1989) and vice versa (Narishetty and Panchagnula 2004).

11.2.2.4 Boiling Point and Energy of Vaporization

The skin permeation-enhancing capacity of a terpene is inversely related to its boiling point, for example, cineole, with boiling point of 173 °C, is reported to be a more effective enhancer compared to terpenes with higher boiling points (carvone,

230 °C; pulegone, 224 °C; menthone, 210 °C; α-terpineol, 217 °C; and menthol, 215 °C) for skin permeation of zidovudine (Narishetty and Panchagnula 2004).

The energy of vaporization of terpenes also shows an inverse relationship with respect to their permeation-enhancing ability. Terpenes with lower energies of vaporization show greater permeation-enhancing properties than those with higher energies of vaporization toward hydrophilic drugs, for example, cyclic ether terpenes, such as anethole and cineole, are good enhancer candidates for the hydrophilic drug, 5-fluorouracil (Ghafourian et al. 2004).

11.3 Application of Terpenes as Penetration Enhancers

The application of various individual terpenes and essential oils in the area of transdermal drug delivery is discussed below, and a summary is given in Table 11.2.

11.3.1 Geraniol

Geraniol is a monoterpenoid alcohol and also known as rhodinol. The oil of rose and palmarosa oil are rich sources of geraniol. It also occurs in small quantities in geranium, lemon, citronella, and many other essential oils. It is commonly used in perfumes for its roselike odor. Godwin and Michniak have investigated the effect of 11 monoterpenes (including geraniol, limonene, menthone, terpinen-4-ol, α-terpineol, 1,8-cineole, carvone, verberone, fenchone, p-cymene, neomenthol) on percutaneous absorption of three different model drugs with varying lipophilicities (caffeine, hydrocortisone, and triamcinolone acetonide) (Godwin and Michniak 1999). Terpenes were applied in propylene glycol to mouse skin. Geraniol provided a 16-fold increase in the permeation of caffeine compared to caffeine without permeation enhancer, and no significant increase in permeation of hydrocortisone and triamcinolone acetonide was observed, though the other terpenes were not as effective for the delivery of hydrocortisone. The addition of tetra hydrogeraniol (THG), which is a chief chemical constituent of rose oil, in a gel containing 5-fluorouracil has been investigated (Hanif et al. 1998). It has shown a markedly enhanced permeability of 5-fluorouracil with a maximum flux obtained at a concentration of 8 %.

11.3.2 Linalool

It is a naturally occurring alcohol terpene obtained from the fruits of *Coriandrum sativum* with many commercial applications. The majority of applications are based on its pleasant scent. It is found in many flowers and spice plants, such as coriander seeds. Vaddi et al. compared the penetration enhancement capacity of linalool with carvacrol and terpineol using haloperidol (HP) as model drug in neat propylene glycol, a widely used vehicle for dermatological preparations. The solubility and flux of HP were increased by all terpenes over control. Out of three terpenes they claimed linalool as the best possible enhancer for transdermal delivery of HP followed by carvacrol and terpineol. Fourier transform Infra red spectroscopy (FT-IR) and differential scanning calorimetry (DSC) techniques suggested that the interaction of terpenes with stratum corneum lipids may be the reason for permeation enhancement (Vaddi et al. 2002a, b).

11.3.3 Limonene

Limonene is a lipophilic hydrocarbon terpene, obtained from the lemon peel of *Citrus limon*. It is a chiral molecule, and biological sources produce one specific enantiomer: D-limonene ((+)-limonene). Racemic form is known as dipentene.

Limonene was found to be the best penetration enhancer when compared with bisabolol, 1,8-cineole, ethanol, polyethylene glycol 600, sorbitan monolaurate (Span® 20, Sigma Aldrich, USA), and oleic acid, using sumatriptan succinate as the model drug (Femenía-Font et al. 2005). The transdermal therapeutic system containing nicardipine hydrochloride gives not only increased bioavailability but also prolonged steady-state concentra-

Table 11.2 Applications of various terpenes in transdermal drug delivery

Type	Terpene	Chemical formula	Permeant	Description
Acyclic monoterpenes				
Alcohol	Geraniol	$C_{10}H_{18}O$	Caffeine	A 16-fold increase in permeation of caffeine is reported as compared to steroid (Godwin and Michniak 1999)
	Linalool	$C_{10}H_{18}O$	Haloperidol	It is reported as the best enhancer for transdermal delivery of HP followed by carvacrol and terpineol (Vaddi et al. 2002a, b)
			Labetalol	Basil oil containing alcoholic terpenes is reported as a promising penetration enhancer for TDD of labetalol (Monti et al. 2009)
Monocyclic monoterpenes				
Hydrocarbon	Limonene	$C_{10}H_{16}$	Sumatriptan succinate	Limonene was reported as a better enhancer than α-bisabolol and 1,8-cineole for sumatriptan succinate (El-Kattan et al. 2011)
			Nicardipine hydrochloride	4 %(w/w) limonene in nicardipine therapeutic system was found to increase bioavailability and prolonged steady-state concentration (Nokhodchi et al. 2007)
			Butylparaben, mannitol	It effectively enhances the permeation of lipophilic but ineffective for hydrophilic drug (Vaddi et al. 2002a, b)
			Ondansetron hydrochloride	The optimum transdermal permeation was observed with 3 % w/w of limonene followed by 3 % w/w of nerolidol and 8 % w/w of carvone (Krishnaiah et al. 2008)
Alcohol	Terpinolene	$C_{10}H_{16}$	Dapiprazole	Terpinolene acts as an effective enhancer for the transdermal delivery of dapiprazole through mouse skin (Monti et al. 1995)
	Menthol	$C_{10}H_{20}O$	Buspirone hydrochloride	Menthol yielded the highest flux in comparison to cineole and terpineol (Ghafourian et al. 2004)
			Imipramine hydrochloride	It showed better penetration enhancer than terpineol menthone, pulegone, carvone, etc. (Jain et al. 2002)
			Methotrexate	It has been reported that the combination of menthol with methotrexate in vesicular gel base improved the penetration into the skin and drug in the skin (Nagle et al. 2011)
Ketones	Carvone	$C_{10}H_{14}O$	Nicardipine hydrochloride	A threefold increase in the bioavailability of NHCL across rat skin was observed (Hanif et al. 1998)
			Tamoxifen	Carvone significantly enhances the permeation of tamoxifen followed by cineole, thymol, and menthol (Krishnaiah et al. 2003)
			Lysozyme	Smaller terpene containing ketone (carvone, fenchone, and verbenone) have showed maximum preserving conformational stability and biological activity of lysozyme in a transdermal formulation (Liu et al. 2011)

(continued)

Table 11.2 (continued)

Type	Terpene	Chemical formula	Permeant	Description
Oxide	Cineole	$C_{10}H_{18}O$	Propranolol hydrochloride	Cineole was found as the best penetration enhancer than menthol and propylene glycol for the permeation of propranolol hydrochloride across rat skin (Amnuaikit et al. 2005)
			Chlorhexidine digluconate	Eucalyptus oil (10 %.v/v) in combination with 2 % (w/v) CHG in 70 % (v/v) isopropyl alcohol would significantly increase the amount of CHG which penetrate into the skin (Ahad et al. 2011)
			Synthetic peptide	Menthone, carveol, and cineole along with ethanol were tested for permeation of synthetic peptides.
				Cineol in 50 % ethanol solution has given maximum enhancement (Cornwell and Barry 1994)
			Valsartan	It is reported as safe and potential penetration enhancer for enhancement of skin penetration of lipophilic drug (Ahad et al. 2011)
			Ofloxacin, lidocaine	Cineol is found to be more effective and safer enhancer than menthol and D-limonene for moisture-activated patches containing lidocaine and ofloxacin (Song et al. 2009)
Bicyclic monoterpene				
Peroxide	Ascaridole	$C_{10}H_{16}O_2$	5-Fluorouracil	Ascaridole reported as comparable to 1,8-cineole and more effective than ylang-ylang oil and anethole (Williams and Barry 1989)
Sesquiterpenes				
Alcohol	Nerolidol	$C_{15}H_{26}O$	Nicardipine hydrochloride, hydrocortisone, carbamazepine, tamoxifen	It is reported as good candidate for the enhancement of hydrophilic drugs than of lipophilic drugs (Cornwell and Barry 1994)
	Farnesol	$C_{15}H_{26}O$	Diclofenac sodium	A 78-fold increase in permeation of DS was provided by farnesol (0.25 %,v/v) followed by carvone, nerolidol, menthone, limonenoxide (Krishnaiah et al. 2006)

tion when 4 % (w/w) limonene was incorporated in a 2 % (w/w) hydroxypropyl cellulose gel (Krishnaiah et al. 2002). Limonene enhances the permeation of lipophilic (butylparaben) and amphiphilic (6-mercaptopurine) compounds, but is ineffective for hydrophilic compounds, such as mannitol (Koyama et al. 1994). Another gel formulation was developed using various terpenes, like limonene, linalool, and cineole at (5 % v/v), in propylene glycol (Lim et al. 2006). This has improved permeation of haloperidol by 26.5-fold when used with limonene and reduced the lag time.

The combination of 5 % w/v of D-limonene in 30 % ethanol and 20 % propylene glycol is reported as the more effective enhancer system for permeation of midazolam (a lipophilic drug) compared with control (ethanol and propylene glycol only) (Ota et al. 2003). Elsewhere, limonene (2 % v/v in propylene glycol) is claimed to

be a better skin penetration enhancer than oleic acid (10 % v/v in PG or transcutol) for the percutaneous delivery of dihydrotestosterone, another lipophilic drug through hairless mouse skin (Clarys et al. 1998). Zhao and Singh have studied the effect of three terpenes (eugenol, limonene, and menthone) at 5 % concentration in combination with 50 % propylene glycol (PG) in enhancing permeation of tamoxifen through porcine epidermis. Of the above terpenes, limonene showed the greatest improvement in the permeation of tamoxifen. The extraction of lipid from stratum corneum was the suggested mechanism of action (Zhao and Singh 2000).

In one study, terpenes from four different chemical classes, namely, hydrocarbons (D-limonene), alcohols (geraniol), epoxides (α-pinene oxide), and cyclic ethers (1,8-cineole) were studied to pretreat third-degree burn eschar from abdominal and lower external burns before the application of silver sulfadiazine. The permeation flux of the antimicrobial drug, silver sulfadiazine, was increased, and the highest enhancement ratio was observed for limonene (about 9 times the normal flux), followed by geraniol (5.5 times), eucalyptus oil (4.7 times), and α-pinene oxide (4.3 times). The lag time was significantly (20 %) decreased in case of limonene, whereas the other terpenes showed a negligible increase in permeation lag times. The increased permeation of silver sulfadiazine can be attributed to the increased partition of the drug in the presence of terpenes into the eschar. When terpenes are applied on intact skin, both the diffusion coefficient and partitioning are increased (Moghimi et al. 2009).

Liu et al. investigated microemulsion systems composed of terpenes, polysorbate 80, cosurfactants, and water as transdermal delivery vehicles for curcumin. Limonene, 1,8-cineole, and α-terpineol were used in the study. The result showed that curcumin permeation rates in the limonene microemulsion were 30- and 44-fold higher than those of 1,8-cineole and α-terpineol microemulsions, respectively (Liu et al. 2011). Okyar et al. have investigated the influence of different terpenes (D-limonene, menthol, and nerolidol) on the percutaneous penetration of tiaprofenic acid from 1 % carbomer (Carbopol® 940, BF Goodrich Chemical Co. Cleveland, OH, USA)-based gel formulations in an ex vivo experiment in rat skin. D-limonene was reported as the most outstanding penetration enhancer among the other terpenes. It was further corroborated by in vivo study in rats. The result showed that the $AUC_{0–48}$(h) was increased by about tenfold by the addition of 5 % D-limonene to the formulation (Okyar et al. 2010).

In another study, Krishnaiah et al. investigated the in vitro transdermal permeation of ondansetron hydrochloride from a hydroxyl propyl cellulose (HPC) gel using nerolidol, carvone, and limonene as penetration enhancers. The transdermal permeation of ondansetron hydrochloride was increased in the presence of terpenes, and the optimal permeation was obtained with 3 % w/w of nerolidol (175.3 ± 3.1 μg/cm^{2}h), 8 % w/w of carvone (87.4 ± 1.6 μg/cm^{2}h), or 3 % w/w of limonene (181.9 ± 0.9 μg/cm^{2}h). It was concluded that HPC gel drug reservoir systems with 3 % w/w nerodilol or 3 % w/w limonene act as optimal formulations in fabrication of membrane-controlled transdermal therapeutic system (TTS) of ondansetron hydrochloride (Krishnaiah et al. 2008).

11.3.4 Menthol

Menthol is obtained from flowering tops of *Mentha piperita*. It occurs in nature as (−)-menthol. Its melting point is 42–45 °C, and it is frequently used in antipruritic creams and as an upper respiratory tract decongestant (Bruneton 1999). Menthol along with limonene has been reported as the most effective penetration enhancer. It has been used as an enhancer for transdermal delivery of variety of drugs including imipramine hydrochloride (Jain et al. 2002), caffeine, hydrocortisone, triamcinolone (Godwin and Michniak 1999), propranolol hydrochloride (Amnuaikit et al. 2005), and zidovudine (Narishetty and Panchagnula 2004, 2005). Synergistic effects of terpenes and iontophoresis when used together have also been reported in the literature. Terpenes, such as menthol, cineole, and terpineol, have been shown to increase the flux of buspirone hydrochloride by more than 200-fold compared to a 15-fold increase when

using iontophoresis alone. Of the abovementioned terpenes, menthol yielded a higher flux compared to cineole and terpineol (Al-Khalili et al. 2003).

When compared with other terpenes, viz., terpineol, menthone, pulegone, carvone, etc., menthol has been reported as a more potent penetration enhancer and is comparable to cineole for skin permeation enhancement of imipramine hydrochloride (Jain et al. 2002). Menthol also seems to be a better penetration enhancer than (+)-limonene, (−)-linalool, and carvacrol for the transdermal delivery of propranolol hydrochloride. In one study (Kunta et al. 1997), four terpenes (L-menthol, (+)-limonene, (−)-linalool, and carvacrol) at three different concentrations (1, 5, and 10 % (w/w)) were investigated for their ability to enhance permeation of propranolol hydrochloride in a mouse skin model. The permeation of propranolol was not affected by increasing the concentration of terpene from 5 to 10 % (w/w), with the exception of linalool which shows an exaggerated concentration-dependent effect. Among the above terpenes, menthol (1 %, w/w) was claimed to be the best penetration enhancer, as it showed relatively (flux value 122 μg/cm^{2}h) higher skin permeation with a shorter lag time than the other compounds. Fujii et al. compared the permeation-enhancing effect of L-menthol with p-menthane-3,8-diol (MDO), a metabolite of L-menthol, through Yucatan micropig skin using antipyrine (a hydrophilic drug) and indomethacin (a lipophilic drug) as model drugs. They concluded that both L-menthol and MDO have a similar enhancing effect on the skin permeation of indomethacin, while MDO exhibited a lower effect on percutaneous transport of antipyrine (Fujii et al. 2004).

On the basis of differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FT-IR) studies (Jain et al. 2002; Zhao and Singh 1998, 1999), it was proposed that the major mechanism of action of menthol as a permeation enhancer is the disruption of the hydrogen bond network at the head groups of ceramides in the lipid bilayers of the stratum corneum. DSC and FT-IR techniques are commonly employed for the determination of the mode of action of terpenes on the permeation of actives through the skin (Naik and Guy 1997). Usually, terpenes enhance drug permeation by one of the following three mechanisms: disruption of the highly ordered lipid structure of the stratum corneum (Godwin and Michniak 1999; Vaddi et al. 2002a, b; Zhao and Singh 1998; Barry 1991), increased drug diffusivity, or increased drug partitioning into the stratum corneum (Berry 1987; Cal et al. 2001). The increased electrical conductivity of skin tissues and thereby opening the polar pathways within the stratum corneum has been postulated as another mode of action for terpenoids (Walker and Smith 1996). The mechanism of permeation enhancement induced by terpenes, such as menthone and limonene in combination with ethanol, was studied by FT-IR using propranolol hydrochloride as the permeant and porcine epidermis. The study revealed that the above enhancers showed a decrease in the peak area and height for both symmetric and asymmetric C–H stretching absorbance in comparison with untreated skin, indicating that the aforementioned terpenes act by stratum corneum lipid extraction (Zhao and Singh 1999). This mechanism was later corroborated by using tamoxifen as a model drug (Zhao and Singh 1998).

Nagle et al. investigated the potential benefits of combining menthol with methotrexate MTX in a vesicular gel base. They evaluated the antipsoriatic efficacy of the formulations and observed that menthol not only improved the penetration and dermal availability of MTX, but also rendered it more effective with greater patient acceptability (Nagle et al. 2011). Song et al. investigated the effects of terpenes (cineole, l-menthol, and D-limonene) on the release and permeation of ofloxacin and lidocaine from moisture-activated composite patches. It was found that 0.33 % cineole in the gel base provided the highest permeation flux (77.7 ± 25.3 μg/cm(2)/h) of lidocaine. The permeation of lidocaine was also enhanced by l-menthol, while D-limonene failed to increase the permeation rate of lidocaine (Song et al. 2009).

11.3.5 Carvone

Carvone is found naturally in many essential oils, but is abundant in caraway seed (*Carum carvi*) oil. It is used in aromatherapy and alternative

medicine. A threefold increase in bioavailability of nicardipine hydrochloride across rat skin was reported by Krishnaiah et al. when carvone (8 %, w/w) was incorporated in a hydroxypropyl cellulose (HPC) gel (Krishnaiah et al. 2003). In another study, Gao and Singh compared the effect of four cyclic terpenes (carvone, 1,8-cineole, menthol, and thymol) used in combination with 50 % ethanol, on the transdermal delivery of tamoxifen across porcine epidermis. Carvone enhanced the permeation of tamoxifen more than cineole, thymol, or menthol. Disruption of intercellular lipid structure of the stratum corneum was suggested as the possible mechanism of action of carvone as a penetration enhancer (Gao and Singh 1998).

Varman and Singh have investigated the effects of terpenes (e.g., limonene, p-cymene, geraniol, farnesol, eugenol, menthol, terpineol, carveol, carvone, fenchone, and verbenone) on the conformational stability and biological activity of a model protein lysozyme. The smaller terpenes containing ketones with low lipophilicity (log $K_{o/w}$~2.00) were optimal for preserving conformational stability and biological activity of lysozyme in a transdermal formulation containing terpenes as permeation enhancers (Varman and Singh 2012).

11.3.6 Cineole

Cineole is a cyclic ether and a monoterpene, also known by a variety of synonyms: 1,8-cineole, limonene oxide, cajeputol, 1,8-epoxy-p-menthane, 1,8-oxido-p-menthane, and eucalyptol. It is the primary chemical constituent of *Eucalyptus globulus* and other species of *Eucalyptus*. When compared with menthol in propylene glycol used as a penetration enhancer for propranolol hydrochloride across rat skin, cineole was reported to be the more efficient enhancer (Amnuaikit et al. 2005). In another study (Narishetty and Panchagnula 2004) the mechanism of cineole and other oxygen-containing monoterpenes (menthol, menthone, pulegone, α-terpineol, and carvone) on percutaneous absorption of zidovudine across rat skin was investigated. Cineole was again reported as the most effective enhancer. It was suggested that the possible mechanism of permeation enhancement of zidovudine by terpenes was modification of skin barrier properties by forming hydrogen bonds with lipid head groups of stratum corneum (SC) lipids. Terpenes had no effect on the partition coefficient and thermodynamic activity of the drug.

Narishetty and Panchagnula investigated by DSC and attenuated total reflectance Fourier transform infrared spectroscopic (ATR–FT-IR) studies the effect of 1,8-cineole and menthol on stratum corneum lipids and permeation of zidovudine across human cadaver skin (Narishetty and Panchagnula 2005). Both enhancers, applied at 5 % (w/v) in 66.6 % ethanol as a vehicle, improved the flux of zidovudine across human cadaver skin. DSC and ATR–FT-IR analysis revealed that 1,8-cineole and menthol enhanced permeation of zidovudine by transforming stratum corneum lipids from a highly ordered orthorhombic perpendicular subcellular packing to a less ordered, hexagonal subcellular packing. Both terpenes showed effects on both lipid alkyl tails and polar head groups, as indicated by reduction in transition temperature (Tm) and blue shift in non-hydrogen-bonded amide I stretching frequency, respectively. These findings suggested that the above terpenes mainly act at polar head groups and break interlamellar and intralamellar hydrogen bonding networks (Narishetty and Panchagnula 2005).

Recently, Raman spectroscopy has been used to investigate the interaction of terpenes and human skin (Williams et al. 2006). It is a versatile and nondestructive method to study the skin, and it provides advantages over infrared spectroscopy for examining this type of naturally hydrated tissue. A marked change in the C–H stretching region was observed by the Raman spectrum of the 1,8-cineole-treated excised full-thickness human skin. The application of 1,8-cineole onto excised skin altered the stratum corneum lipid domain, producing interfacial defects between ordered and disordered domains, allowing increased drug delivery through the tissue (Williams et al. 2006).

Seven novel terpenes, namely, iso-eucalyptol, β-citronellene, valencene, rose oxide, safranal, lavandulol acetate, and prenol, were studied as potential penetration enhancers for the delivery of valsartan through rat skin and human cadaver skin (HCS) with reference to the established terpene eucalyptol. Among all terpenes, iso-eucalyptol showed the maximum enhancement through rat skin (enhancement ratio (ER) = 7.4) and HCS (ER = 3.60) over control (Ahad et al. 2011).

Seung et al. have investigated the transdermal penetration of three synthetic peptides in the presence of terpenes together with ethanol (EtOH) (i.e., menthone/EtOH, carveol/EtOH, or cineole/EtOH). Cineole was the most effective enhancer for all three peptides tested. The maximum ER of approximately 2 was achieved by cineole in 50 % ethanol solution (Ham et al. 2007).

11.3.7 Ascaridole

Ascaridole is a peroxide functional group bearing bicyclic monoterpene. It is the primary constituent of the oil of *Chenopodium* or Mexican Tea (*Chenopodium ambrosioides*). Its penetration-enhancing activity has been investigated using 5-fluorouracil as the model drug, and it was found to be comparable to 1,8-cineole and more effective than ylang-ylang oil and anethole (Williams and Barry 1989).

11.3.8 Terpinolene

Terpinolene is most commonly used in the fragrance and textile industries. Monti et al. investigated the permeation of dapiprazole base (DAP-B) using different penetration enhancers including terpenes ((−) α-bisabolol, (±) α-bisabolol, L-carvone, D-limonene, L-limonene, mircene, α-pinene, terpinolene, and eucalyptol) in a series of liquid and semisolid vehicles, through hairless mouse skin (Monti et al. 1995). Terpinolene caused maximum skin permeation of DAP-B from liquid vehicles, whereas D-limonene was the most active enhancer in semisolid vehicles followed by (−) α-bisabolol, L-limonene, terpinolene, and α-pinene.

11.3.9 Nerolidol

Nerolidol is a naturally occurring sesquiterpene found in essential oils of many types of plants and flowers. It is also known as peruviol. Nerolidol is present in neroli, ginger, jasmine, lavender, tea tree, and lemon grass. It is used as a flavoring agent and in perfumery. It is also currently being investigated as a skin penetration enhancer for transdermal delivery of therapeutic drugs. It seems to act by increasing the diffusion coefficient of drugs, such as 5-fluorouracil (Cornwell and Barry 1994).

In another study (El-Kattan et al. 2011), nerolidol was reported as the most efficient penetration enhancer out of four different terpenes (fenchone, thymol, D-limonene, and nerolidol) with respect to four model drugs with differing lipophilicities: nicardipine hydrochloride, hydrocortisone, carbamazepine, and tamoxifen. These terpenes were found more effective at enhancing the penetration of hydrophilic rather than lipophilic drugs.

The effect of terpenes (nerolidol, carvone, and anethole), on permeation of selegiline hydrochloride across rat skin, has also been investigated. Nerolidol provided a 3.2-fold increase in permeation of the above drug across rat skin in comparison to control (Krishnaiah et al. 2006). The amphiphilic structure of nerolidol is attributed to its permeation-enhancing activity, which is suitable for the alignment within the lipid lamellae of the stratum corneum, thus disrupting its highly organized packing (Cornwell and Barry 1994).

11.3.10 Farnesol

Farnesol is a sesquiterpene alcohol, present in many essential oils, such as citronella, neroli, cyclamen, lemon grass, tuberose, rose, musk, balsam, and tolu. It is used in perfumery to accentuate the odors of sweet floral perfumes. It has been reported that farnesol (0.25 %, v/v) was the best skin permeation enhancer for diclofenac sodium, with respect to other terpenes (Nokhodchi et al. 2007). The permeation-enhancing activity was found to be in the following order: farnesol

>carvone >nerolidol >menthone >limonenoxide. However, at 2.5 % (v/v) nerolidol was found to be the best candidate with a 198-fold increase in the permeability coefficient of diclofenac sodium followed by farnesol with a 78-fold increase in permeation.

11.4 Application of Essential Oils as Penetration Enhancers

Essential oils are volatile, odoriferous substances obtained from flowers, fruits, leaves, and roots of certain plants. Essential oils are consisting of mixtures of many diverse and unique chemical compounds (Fox et al. 2011). The components of essential oils (Pinder 1960; Betts 2001; Dudareva et al. 2004) can generally be classified as:

- Terpenes and terpenoids
- Nitrogen- and sulfur-containing compounds (e.g., allylisothiocyanate found in mustard oil)
- Aromatic compounds, which are benzene derivatives (e.g., eugenol which is the main constituent of clove oil)
- Miscellaneous compounds (includes long-chain unbranched substances)

The penetration-enhancing effect of several individual terpenes (as discussed earlier in the chapter) isolated from essential oils has been extensively investigated, while reports of use of essential oils per se as skin sorption promoters are also found in literature and presented in Table 11.3.

11.4.1 Eucalyptus Oil

Eucalyptus oil can be obtained from numerous species of the Myrtaceae family, which includes *Eucalyptus citriodora*, *Eucalyptus dives*, *Eucalyptus globulus*, *Eucalyptus polybractea*, and *Eucalyptus radiata*. It is extracted by steam distillation from the leaves (Essential Science Publishing 2006). The main chemical components of eucalyptus oil are α-pinene, β-pinene, α-phellandrene, 1,8-cineole, limonene, terpinen-4-ol, aromadendrene, epiglobulol, piperitone, and globulol (http://en.wikipedia.org/wiki/Eucalyptus_oil). The penetration-enhancing activity of eucalyptus, *Chenopodium*, ylang-ylang, and anise essential oils has been evaluated in excised human skin using 5-fluorouracil as a model drug. Eucalyptus and *Chenopodium* oils were the most effective drug permeation enhancers, which exhibited almost a 30-fold increase in drug permeability coefficient, followed by ylang-ylang with an approximately 8-fold increase. Anise oil proved to be the least effective permeation enhancer among the above essential oils (Williams and Barry 1989).

Eucalyptus oil enhanced the penetration of chlorhexidine (2 % w/v in 5–50 % v/v of eucalyptus oil) into the dermis and lower layers of the epidermis. The combination of 10 % (v/v) eucalyptus oil with 2 % w/v of chlorhexidine in 70 % (v/v) isopropyl alcohol significantly enhanced the skin penetration of chlorhexidine 2 min after application compared to a solution of chlorhexidine in isopropyl alcohol or neat chlorhexidine (Karpanen et al. 2010).

11.4.2 Niaouli Oil

Niaouli oil is extracted by steam distillation from the leaves and twigs of *Melaleuca quinquenervia*, of the Myrtaceae (myrtle) family (Essential Science Publishing 2006; Stewart 2005). The main chemical constituents of niaouli oil are 55–70 % 1,8-cineole (oxide) and limonene (monoterpene), 7–15 % α-pinene (monoterpene), 2–6 % β-pinene (monoterpene), and 2–6 % viridiflorol (sesquiterpene) (Essential Science Publishing 2006; Stewart 2005). It is useful for treating respiratory/sinus and urinary tract infections, allergies, and hypertension (Essential Science Publishing 2006).

Monti et al. evaluated six essentials oils (cajuput, cardamom, melissa, myrtle, niaouli, and orange oil) using estradiol as a drug model. All essential oils were used at 10 % (w/w) concentration in propylene glycol (PG). Among the oils, niaouli was found to be the best penetration enhancer for estradiol (Monti et al. 2002, 2009). Further, four main terpene components of niaouli

Table 11.3 Application of various essential oils as penetration enhancers

Essential oil	Source	Chemical constituents	Permeant	Description
Eucalyptus oil	*Eucalyptus citriodora*, *Eucalyptus dives*, *Eucalyptus globulus*, *Eucalyptus polybractea*, *Eucalyptus radiata*	a-Pinene, b-pinene, limonene, 1,8-cineole terpinen-4-ol	5-Fluorouracil	Eucalyptus and *Chenopodium* oil have shown a 30-fold increase in drug permeability coefficient followed by ylang-ylang and anise oil (Williams and Barry 1989)
			Chlorhexidine	Eucalyptus oil (10 % v/v) with isopropyl alcohol (70 % v/v) significantly enhanced the skin penetration of chlorhexidine compared to a solution of chlorhexidine/isopropyl alcohol alone (Karpanen et al. 2010)
Niaouli oil	*Melaleuca quinquenervia*	8-Cineole (oxide) Limonene, α-pinene, β-pinene, viridiflorol	Estradiol	Niaouli oil was found to be more effective than cajuput, cardamom, melissa, myrtle and orange essential oils for enhancing the transdermal penetration of estradiol (Monti et al. 2009)
Turpentine oil	*Coniferae* spp.	β-Pinene α-pinene carene, camphene, dipentene, and terpinolene	Flurbiprofen	5 % (v/v) turpentine oil was found to give maximum transdermal penetration rate and significantly more effective than tulsi oil at the same concentration (Charoo et al. 2008)
Alpinia oxyphylla oil	*Alpinia oxyphylla*	Low-polarity fraction estragol, copaene azulene, β-bisabolene α-panasinsen, *p*-cymene high-polarity fraction 1*H*-cycloprop[e] azulene, α-panasinsen, germacrene B, humulene 6,7-epoxide	Indomethacin	High-polarity fraction of *A. oxyphylla* essential oil was more efficient in enhancing the skin permeation of indomethacin at concentrations of 3 and 5 % than the lower-polarity fraction (Fang et al. 2003)
Sweet basil	*Ocimum basilicum*	Estragol linalool eugenol and 1,8-cineole	Indomethacin	It was reported that the skin permeation of indomethacin was more with the low-polarity fraction (Fang et al. 2004)
			Labetalol hydrochloride	Basil oil was the most effective penetration enhancer followed by camphor, geraniol, thymol, and clove oil (Jain et al. 2008)
Tulsi oil	*Ocimum sanctum*	Oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool	Flurbiprofen	The significant enhanced skin penetration was observed at 5 % v/v of tulsi oil when added to a binary mixture of propylene glycol–isopropyl alcohol (30:70 % (v/v)) (Charoo et al. 2008)

(continued)

Table 11.3 (continued)

Essential oil	Source	Chemical constituents	Permeant	Description
Cardamom oil	*Elettaria cardamomum*	α-Terpinyl acetate 1,8-cineole, linalool linalyl acetate limonene	Prednisolone	An acetone extract of cardamon seed was A better skin penetration enhancer of prednisolone than Azone® (Yamahara et al. 1989).
			Indomethacin, diclofenac, and piroxicam	With a 1 % (v/v) concentration of cardamom oil, it was found that the highest penetration index was observed for piroxicam, followed by indomethacin, and then diclofenac (Huang et al. 1995)
Peppermint oil	*Mentha piperita*	Menthol (34–44 %), menthone (12–20 %), menthofurane (4–9 %), 1,8-cineole (2–5 %), pulegone (2–5 %), menthyl acetate (4–10 %)	Benzoic acid	Peppermint oil showed the most significant effect on skin integrity, and the percutaneous penetration of benzoic acid was found protective at the lower concentrations of 0.1 % and 1.0 % (v/v) as compared to eucalyptus oil and tea tree oil (Nielsen 2006)
Black cumin oil	*Cuminum cyminum*	Cuminaldehyde, γ-Terpinene, β-pinene, *p*-mentadienal, *p*-cymene	Carvedilol	Black cumin oil (5 % v/v) was Black cumin found to be a better penetration enhancer with an enhancement factor of 6.40 for carvedilol, when compared to clove oil, eucalyptus oil, tulsi oil, oleic acid and Tween 80 (Amin et al. 2008)
Fennel oil	*Foeniculum vulgare*	*trans*-anethole fenchone (ketone), linalool 3–5 % α-pinene, 2–5 % methylchavicol	Trazodone hydrochloride	The percutaneous penetration flux for TZN with skin pretreatment by 10 % essential oils was found highest for fennel oil followed by eucalyptus oil, citronella oil, and mentha oil (Das et al. 2006)

oil were investigated, i.e., 1,8-cineole, α-pinene, α-terpineol, and D-limonene individually at a 10 % (w/w) concentration in PG. 1,8-cineole was reported as the best skin permeation promoter for estradiol (Monti et al. 2002).

It was demonstrated that the same essential oils (such as niaouli oil) from different plant sources do not necessarily yield similar skin penetration enhancement results. Niaouli oil (10 % (w/w)) from four different sources increased the transdermal flux of estradiol through hairless mouse skin from 41.50- to 84.63-fold compared to the control group, which consisted of vehicle containing PG and estradiol (Monti et al. 2009). These results confirmed that the reason of differences in the biological effects of plant materials is due to their source from which they are obtained, which leads to differences in the chemical composition of plants.

11.4.3 Turpentine Oil

Turpentine oil is obtained after distillation of the resin that is secreted by conifers (*Coniferae* spp.). It is composed of terpenes, mainly the monoterpenes α-pinene and β-pinene with lower amounts of carene, camphene, dipentene, and terpinolene

(http://en.wikipedia.org/wiki/Turpentine). Turpentine oil at 5 % v/v concentration shows an additive effect with the binary solvent vehicle of propylene glycol–isopropyl alcohol [30:70 % (v/v)] on the skin permeation rate of flurbiprofen. At a concentration of 5 % (v/v), turpentine oil provides higher transdermal penetration rate of fluribiprofen than tulsi oil at the same concentration. When the above binary solvent vehicle was used alone, a significantly lower lag time was found for flurbiprofen flux across the skin (Charoo et al. 2008).

11.4.4 *Alpinia oxyphylla* Oil

Alpinia oxyphylla belongs to the ginger (Zingiberaceae) family and is used in oriental herbal medicine (Lee et al. 1998). Essential oils extracted from *A. oxyphylla* are divided into a lower-polarity fraction and a higher-polarity fraction (Fang et al. 2003). The lower-polarity fraction contains eight sesquiterpenes (including estragol, copaene, 1*H*-cycloprop[e]azulene, himachal-2,8-diene, azulene, octahydro-1,8-dimethyl-7-(2-methylethenyl)-naphthalene, β-bisabolene, α-panasinsen), which are mostly hydrocarbon constituents except for the oxygenated constituent estragol and one cyclic monoterpene (*p*-cymene). The high-polarity fraction consists of seven sesquiterpenes (including 1*H*-cycloprop[e]azulene, octahydro-1,8-dimethyl-7-(2-methylethenyl)-naphthalene, α-panasinsen, germacrene B, humulene 6,7-epoxide, *cis*-α-copaene-8-ol, and nootkatone).

It is reported that the high-polarity fraction of *A. oxyphylla* essential oil at concentrations of 3 and 5 % was more efficient in enhancing the ex vivo permeation of indomethacin through dorsal skin of Wistar rats than the lower-polarity fraction. The results were assessed after pretreatment with the two fractions of the essential oil (3 % (v/v)) in carboxymethylcellulose hydrogels (for 1 or 2 h). The permeation of indomethacin was significantly enhanced, and decrease in the lag time was also found. The skin deposition of indomethacin was enhanced nearly fivefold after the pretreatment, indicating that direct action of essential oil on the skin governs the enhanced absorption of indomethacin rather than the release behavior of the vehicle (Fang et al. 2003).

11.4.5 Sweet Basil and Tulsi Oil

Sweet basil oil is obtained from the leaves, stems, and flowers of *Ocimum basilicum* (Lamiaceae or Labiatae family) through steam distillation. The main constituents of this oil include methylchavicol (estragol) (40–80 %, phenol), linalool (5–10 %, alcohol), eugenol (1–10 %, phenol), and 1,8-cineole (1–7 %, oxide). This essential oil has numerous medicinal properties such as anti-inflammatory, antispasmodic, muscle relaxant, antiviral, and antibacterial (Essential Science Publishing 2006; Stewart 2005).

The ex vivo skin permeation effect of basil essential oil extract was investigated by Fang et al. using Franz diffusion cells and dorsal skin of Wistar rats. The low-polarity fraction of the above oil contained predominantly estragol (aromatic ether), followed by squalene (triterpene) and the sesquiterpenes α-bergamotene and θ-muurolene. Phytol (diterpene) was the most occurring compound in the high-polarity fraction along with the other terpenes such as *d*-linalool, estragol, and butylated hydroxytoluene. A hydrocarbon sesquiterpene (+)-*epi-bicyclosesquiphellandrene* was also present. The low-polarity fraction provided a more efficient penetration of indomethacin into the skin (Fang et al. 2004).

Jain et al. investigated the effect of some essential oils and terpenes on the penetration of a hydrophilic drug labetalol hydrochloride across rat abdominal skin. They found that basil oil was the most effective penetration enhancer followed by camphor, geraniol, thymol, and clove oil. A synergistic effect of the vehicle system (ethanol-water, 60:40) and terpenes was observed. Lag time of labetalol hydrochloride was also significantly decreased in the following order: camphor <basil oil <geraniol <thymol <clove oil <vehicle <water (Jain et al. 2008).

Tulsi oil is obtained from *Ocimum sanctum* which is part of the Lamiaceae family. Some of the main chemical constituents of tulsi oil are oleanolic acid, ursolic acid, rosmarinic acid,

eugenol, carvacrol, linalool, β-caryophyllene (about 8 %), β-elemene (11.0 %), and germacrene D (about 2 %) (Padalia and Verma 2011).

Charoo et al. investigated the penetration-enhancing potential of tulsi and turpentine oil applied in a binary solvent mixture of propylene glycol–isopropyl alcohol (30:70 %, v/v) on transdermal delivery of flurbiprofen across the rat abdominal skin. The flux enhancement factor with turpentine oil and tulsi oil was 2.4 and 2.0, respectively, at 5 % (v/v) concentration beyond which there was no significant increase in the flux. The bioavailability of flurbiprofen was reported to increase by 2.97, 3.80, and 5.56 times with transdermal patch formulation without enhancer, tulsi, and turpentine oil containing formulations, respectively, compared to orally administered flurbiprofen in albino rats (Charoo et al. 2008).

11.4.6 Cardamom Oil

Cardamom oil is obtained from *Elettaria cardamomum* (cardamom) which is part of the ginger (Zingiberaceae) family. The main constituents of this oil include α-terpinyl acetate (45–55 %, ester), 1,8-cineole/eucalyptol (16–24 %, oxide), linalool (4–7 %, alcohol), linalyl acetate (3–7 %, ester), and limonene (1–3 %, monoterpene). It is used as an antispasmodic, antiparasitic, and expectorant agent (Essential Science Publishing 2006; Stewart 2005). One study showed that an acetone extract of cardamom seed (*E. cardamomum*) effectively enhances the dermal penetration of prednisolone across abdominal mouse skin. The acetone extract was composed of two fractions which were separated, and the fractions were identified as acetyl terpineol (*d*-α-terpinyl acetate) and terpineol (*d*-α-terpineol). These fractions were found to be better skin penetration enhancers for prednisolone than Azone® (1-dodecylazacycloheptan-2-one) (Yamahara et al. 1989).

In another study, permeation-enhancing ability of cardamom oil (distilled from the seed of *Amomum cardamomum*) was investigated ex vivo through rabbit abdominal skin. The oil induced enhanced penetration of indomethacin, diclofenac, and piroxicam (Huang et al. 1995). It was found that the enhancing effects of cardamom are dependent on its concentration, with a 1 % (v/v) concentration being more effective than 0.5 % (v/v). The penetration index or enhancement ratio of the drugs was determined at pH 5.8 and pH 7.4. Cardamom oil used at 1% provided the highest penetration index for piroxicam, followed by indomethacin and then diclofenac. A shorter lag time was also observed for the permeation of indomethacin and diclofenac across the skin (Huang et al. 1995). Further, in vivo studies showed that a 30 min pretreatment of rabbit abdominal skin with cardamom (5 % v/v) increased 67.09 times the peak area of the plasma concentration time curve of piroxicam (AUC 0–24 h) when compared to nontreatment. In addition, an absolute bioavailability of 83.23 % was obtained. Results after a 60 min pretreatment were not significantly different from that after a 30 min pretreatment (Fox et al. 2011; Huang et al. 1996).

11.4.7 Peppermint Oil

Peppermint oil is extracted from the stems, leaves, and flower buds of the plant *Mentha piperita* (Lamiaceae or Labiatae family) by steam distillation. The important constituents of the oil include menthol (34–44 %, phenolic alcohols), menthone (12–20 %, ketone), menthofurane (4–9 %, furanoids), 1,8-cineole (eucalyptol, 2–5 %, oxide), pulegone (2–5 %, ketone), and menthyl acetate (4–10 %, ester) (Essential Science Publishing 2006; Stewart 2005). It is used to relieve pain, to control appetite, to stimulate digestion/gallbladder function, and as an anti-inflammatory, antitumoral, antiviral, antibacterial, and antiparasitic agent. Nielsen has investigated the effect of three natural oils (eucalyptus oil, tea tree oil, peppermint oil) on the skin integrity and the percutaneous penetration of benzoic acid when applied topically in relevant concentrations. In vitro permeation studies in human breast or abdominal skin were performed by applying the natural oils in 0.1, 1.0, or 5.0 % (v/v) concentrations in aqueous solutions containing 1 % (v/v) polyoxyethylene sorbitan monolaurate (Tween® 20, Sigma, Steinheim, Germany), 0.9 % (w/v) NaCl,

and tritiated water (^3H_2O) (Nielsen 2006). The flux of ^3H_2O is indicative of the integrity of the skin with a high flux value suggesting damage to the skin (Nielsen 2006). This study indicated that peppermint oil showed the highest effect on skin integrity, and when used at the lower concentrations of 0.1 and 1.0 % (v/v) it was found protective (protects penetration) against the percutaneous penetration of benzoic acid (lipophilic). The protective effect of peppermint oil on the penetration of benzoic acid was find to diminish at increasing concentrations of peppermint oil (Nielsen 2006).

11.4.8 Black Cumin Oil

Black cumin essential oil is obtained by steam distillation from the seeds of *Cuminum cyminum* of the Apiaceae or Umbelliferae family (http://www.essentialoils.co.za/essential-oils/black-pepper.htm). It is useful as an immune stimulant, digestive aid, liver protectant, antioxidant, anti-inflammatory, antitumoral, and antiviral. Its main chemical components include cuminaldehyde (16–22 %, aldehyde), γ-terpinene (16–22 %, monoterpene), β-pinene (12–18 %, monoterpene), *p*-mentadienal (25–35 %, aldehydes), and *p*-cymene (3–8 %, monoterpenes) (Essential Science Publishing 2006; Stewart 2005). It was shown in excised rat abdominal skin that black cumin oil (5 % v/v) was a better penetration enhancer with an enhancement factor of 6.40 for carvedilol compared to clove oil, eucalyptus oil, tulsi oil, oleic acid, and polyoxyethylene sorbitan monooleate (Tween® 80, Sigma, Steinheim, Germany) (Amin et al. 2008).

11.4.9 Fennel Oil

Fennel oil is extracted by steam distillation from the crushed seeds of *Foeniculum vulgare*, which is sweet fennel of the Apiaceae or Umbelliferae family (http://www.essentialoils.co.za/essential-oils/fennel.htm). Its key components are 60–80 % *trans*-anethole (phenolic ester), 12–16 % fenchone (ketone), linalool (alcohol), 3–5 % α-pinene (monoterpene), and 2–5 % methylchavicol (phenol) (Essential Science Publishing 2006; Stewart 2005). It is used as a digestive aid, antiseptic, antispasmodic, antiparasitic, and analgesic agent. It also has anti-inflammatory and antitumoral characteristics and can increase metabolism (Essential Science Publishing 2006). Das et al. have investigated the enhancing effect of several essential oils (fennel oil, eucalyptus oil, citronella oil, and mentha oil) on the percutaneous absorption of trazodone hydrochloride (TZN). The oils were applied on the skin membrane in three different ways: included in the transdermal device, as a pretreatment, or both. Results showed that pretreatment of the skin with essential oils increases the flux values of TZN compared with the values obtained when the same essential oils were included in the transdermal devices. The percutaneous penetration flux for TZN with skin pretreatment by 10 % essential oils was found highest for fennel oil followed by eucalyptus oil, citronella oil, and mentha oil. The amount of TZN retained in the skin after pretreatment with essential oils was found to be very similar in all cases and much higher than in the experiments without skin pretreatment (Das et al. 2006).

11.5 Terpenes: Vehicle Interactions

Ethanol and propylene glycol, themselves being the established skin penetration enhancers, have been used with terpenes for synergistic sorption-promoting effect by many workers (Obata et al. 1991; Okabe et al. 1992; Kobayashi et al. 1994; Cornwell and Barry 1995; Vaddi et al. 2002a, b).

Yamane and coworkers investigated the effects of propylene glycol/water co-solvent systems and terpene penetration enhancers (1,8-cineole, menthone, (+)-limonene, and nerolidol) on the absorption rate of 5-fluorouracil using excised human skin. Co-application of each terpene in propylene glycol co-solvent systems at saturation has been found to increase drug flux significantly. Terpenes activity depended on the propylene glycol content in the vehicles. It was reported that the formulations containing the terpenes in 80%

propylene glycol systems showed maximum fluxes (Yamane et al. 1995).

Vaddi et al. investigated the in vitro permeation of haloperidol (HP) through human skin in the presence of various alcoholic terpenes (carvacrol, linalool, and alpha-terpineol) at 5 % w/v in propylene glycol (PG). Linalool showed greatest increase in the permeation of HP followed by carvacrol and terpineol. HP permeation with linalool was predicted to reach a therapeutic plasma concentration (Vaddi et al. 2002a). In another study, Vaddi and coworkers reported the in vitro permeation of haloperidol (HP) in the presence of limonene oxide and pinene oxide used at 5 % w/v concentration in 50 % v/v ethanol and 100 % v/v propylene glycol (PG). The enhancement activity of these terpenes was higher in 50 % v/v ethanol than in 100 % v/v PG. In 50 % v/v ethanol, terpenes presented the required therapeutic plasma concentration and daily permeated amounts of the drug. Limonene oxide showed higher enhancement in both solvents as compared to pinene oxide, which was attributed to its less bulky structure (Vaddi et al. 2002b).

Godwin and Michniak compared the sorption-enhancing effects of various monoterpenes on the skin of hairless mice using three different model drugs (caffeine, hydrocortisone, and triamcinolone acetonide [TA]) with varying lipophilicities. Terpenes were applied at 0.4 M in propylene glycol (PG) to mouse skin. The model drugs were applied as suspensions in PG 1 h following enhancer pretreatment. The combination of terpenes in PG provided significant enhancement of the permeation of caffeine through mouse skin. They concluded that the combination of terpenes with PG can significantly increase the transdermal penetration of the hydrophilic drug caffeine and the polar steroid hydrocortisone (Godwin and Michniak 1999).

11.6 Other Terpenes and Essential Oils

Carvacrol, or cymophenol, is a constituent of the oil of thyme and oil obtained from pepperwort and wild bergamot. Thymol is a monoterpene phenol, found in oil of thyme, with strong antiseptic properties. It is also called "hydroxy cymene." Anethole or trans-anethole is an aromatic compound that accounts for the distinctive "licorice" flavor of anise, fennel, and star anise. Carvacrol, thymol, and t-anethole are reported to be as effective as reference terpene (L-menthol) in enhancing the transport of both zidovudine and pentamidine (Hashida and Yamashita 1995; Kararli et al. 1995). Another group of investigators studied the effects of chemical enhancers (alcohols and fatty acids) and sonophoresis on the transdermal permeation of tizanidine hydrochloride (TIZ) across mouse skin. TIZ formulated as a suspension in 50 % v/v aqueous ethanol containing 5 % v/v citral showed maximum enhancement. Citral, or 3,7-dimethyl-2,6-octadienal or lemonal, is either of a pair or a mixture of terpenoids with the molecular formula $C_{10}H_{16}O$. The two compounds are double bond isomers, known as geranial (citral A) and neral (citral B). The major source are oils obtained from lemon myrtle (90–98%), *Litsea citrata* (90 %), *Litsea cubeba* (70–85 %), lemongrass (65–85 %), lemon tea tree (70–80 %), *Ocimum gratissimum* (66.5 %), *Lindera citriodora* (about 65 %), and *Calyptranthes parriculata* (about 62 %). A synergistic effect was reported when sonophoresis was applied in the presence of chemical enhancers (Mutalik et al. 2009).

The impacts of bicyclo-monoterpene promoters (i.e., borneol and camphor) on the in vitro permeation of ligustrazine (LGT) through the hairless porcine dorsal skin were also reported. The use of borneol led to greater penetration of ligustrazine across porcine skin compared to camphor. It was shown that the permeation enhancement mechanism of bicyclo-monoterpenes on ligustrazine permeation included extracting and disordering lipids of the stratum corneum, which involved the shift changes in C–H stretching and H-bonding action between enhancers and ceramides. As to the penetration-enhancing capability of bicyclo-monoterpenes, the hydroxy groups were more effective than the ketone groups (Zhang et al. 2010).

Conclusion

The enhancement of passive diffusion of the medicinally active substances through the skin barrier has been the greatest challenge for transdermal scientists all over the world. The use of chemicals (solvents) as skin permeation enhancers has been the most traditional approach of all the penetration enhancement strategies. Synthetic solvents may provide better enhancement potential than some of the natural substances, but the former can be irritating and toxic to the skin. Hence natural enhancers have been the most desirable candidates for enhancing percutaneous drug delivery.

Terpenes and essential oils present such a class of natural penetration enhancers, which have shown to be able to significantly enhance the penetration of both hydrophilic and lipophilic substances with little or no adverse cutaneous effects, and they have been granted GRAS status by FDA. Many new terpenes and essential oils have been investigated recently, including β-citronelline, valencene, rose oxide, safranal, and prenol. More important investigations can be expected in this promising category of penetration enhancers in the ensuing times for improved dermal and transdermal drug delivery.

References

Ahad A, Aqil M, Kohli K, Sultana Y, Mujeeb M, Ali A (2011) Role of novel terpenes in transcutaneous permeation of valsartan: effectiveness and mechanism of action. Drug Dev Ind Pharm 37(5):583–596

Al-Khalili M, Meidan VM, Michniak BB (2003) Iontophoretic transdermal delivery of buspirone hydrochloride in hairless mouse skin. AAPS PharmSci 5(2):61–71

Amin S, Kohli K, Khar RK, Mir SR, Pillai KK (2008) Mechanism of in vitro percutaneous absorption enhancement of carvedilol by penetration enhancers. Pharm Dev Technol 13:533–539

Amnuaikit C, Ikeuchi I, Ogawara K, Higaki K, Kimura T (2005) Skin permeation of propranolol from polymeric film containing terpene enhancers for transdermal use. Int J Pharm 289:167–178

Aqil M, Ahad A, Yasmin Y, Ali A (2007) Status of terpenes as skin penetration enhancers. Drug Discov Today 12:1061–1067

Barry BW (1991) Lipid-protein-partitioning theory of skin penetration enhancement. J Control Release 15:237–248

Barry BW (2004) Breaching the skin's barrier to drugs. Nat Biotechnol 22:165–167

Benson HA (2005) Transdermal drug delivery: penetration enhancement techniques. Curr Drug Deliv 2:23–33

Berry BW (1987) Mode of action of penetration enhancers in human skin. J Control Release 6:85–97

Betts TJ (2001) Chemical characterisation of the different types of volatile oil constituents by various solute retention ratios with the use of conventional and novel commercial gas chromatographic stationary phases. J Chromatogr A 936:33–46

Bruneton J (1999) Terpenoids and steroids. Pharmacognosy phytochemistry medicinal plants. Lavoisier Publishing Inc, Secaucus, pp 461–721

Cal K, Janicki S, Sznitowska M (2001) In vitro studies on penetration of terpenes from matrix-type transdermal systems through human skin. Int J Pharm 224:81–88

Charoo NA, Shamsher AA, Kohli K, Pillai K, Rahman Z (2008) Improvement in bioavailability of transdermally applied flurbiprofen using tulsi (*ocinum sanctum*) and turpentine oil. Colloids Surf B Biointerfaces 65:300–307

Clarys P, Alewaeters K, Jadoul A, Barel A, Manadas RO, Préat V (1998) In vitro percutaneous penetration through hairless rat skin: influence of temperature, vehicle and penetration enhancers. Eur J Pharm Biopharm 46:279–283

Cornwell PA, Barry BW (1994) Sesquiterpene components of volatile oils as skin penetration enhancers for the hydrophilic permeant 5-fluorouracil. J Pharm Pharmacol 46:261–269

Cornwell PA, Barry BW (1995) Effects of penetration enhancer treatment on the statistical distribution of human skin permeabilities. Int J Pharm 117:101–112

Cornwell PA, Barry BW, Bouwstra JA, Gooris GS (1996) Modes of action of terpene penetration enhancers in human skin; differential scanning calorimetry, small-angle X-ray diffraction and enhancer uptake studies. Int J Pharm 127:9–26

Das MK, Bhattacharya A, Ghosal SK (2006) Effect of different terpene-containing essential oils on percutaneous absorption of trazodone hydrochloride through mouse epidermis. Drug Deliv 13:425–431

Dudareva N, Pichersky E, Gershenzon J (2004) Biochemistry of plant volatiles. Plant Physiol 135:1893–1902

El-Kattan AF, Asbill CS, Kim N, Michniak BB (2011) The effects of terpene enhancers on the percutaneous permeation of drugs with different lipophilicities. Int J Pharm 215:229–240

Essential Science Publishing (2006) Essential oils: desk reference, 3rd edn. Essential Science Publishing, Orem, Utah, pp 1–75

Fang JY, Leu YL, Hwang TL, Chen HC, Hung CF (2003) Development of sesquiterpenes from alpinia oxyphylla as novel skin permeation enhancers. Eur J Pharm Sci 19:253–262

Fang JY, Leu YL, Hwang TL, Cheng HC (2004) Essential oils from sweet basil (*ocimum basilicum*) as novel enhancers to accelerate transdermal drug delivery. Biol Pharm Bull 27:1819–1825

Femenía-Font A, Balaguer-Fernández C, Merino V, Rodilla V, López-Castellano A (2005) Effect of chemical enhancers on the in vitro percutaneous absorption of sumatriptan succinate. Eur J Pharm Biopharm 61:50–55

Fox LT, Gerber M, Plessis JD, Hamman JH (2011) Transdermal drug delivery enhancement by compounds of natural origin. Molecules 16:10507–10540

Fujii M, Takeda Y, Yoshida M, Matsumoto M, Watanabe Y (2004) Enhancement effect of p-menthane-3,8-diol on in vitro permeation of antipyrine and indomethacin through yucatan micropig skin. Drug Dev Ind Pharm 30:673–677

Gao S, Singh J (1998) In vitro percutaneous absorption enhancement of a lipophilic drug tamoxifen by terpenes. J Control Release 51:193–199

Ghafourian T, Zandasrar P, Hamishekar H, Nokhodchi A (2004) The effect of penetration enhancers on drug delivery through skin: a QSAR study. J Control Release 99:113–125

Godwin DA, Michniak BB (1999) Influence of drug lipophilicity on terpenes as penetration enhancers. Drug Dev Ind Pharm 25:905–915

Ham SW, Kang MJ, Park YM, Oh IY, Kim BG, Im TJ, Kim SH, Choi YW, Lee J (2007) Transdermal penetration of synthetic peptides and their penetration enhancement caused by some terpene compounds. Bull Kor Chem Soc 28(9):1535–1538

Hanif RM, Qineng P, Zhan G (1998) Penetration enhancing effect of tetrahydrogeraniol on the percutaneous absorption of 5-fluorouracil from gels in excised rat skin. J Control Release 55:297–302

Hashida M, Yamashita F (1995) Terpenes as penetration enhancers. In: Smith EW (ed) Percutaneous penetration enhancers. CRC Press, Florida, pp 309–321

Huang YB, Wu PC, Ko HM, Tsai YH (1995) Cardamom oil as a skin permeation enhancer for indomethacin, piroxicam and diclofenac. Int J Pharm 126:111–117

Huang YB, Wu PC, Ko HM, Tsai YH (1996) Effect of pretreatment by cardamom oil on *in vivo* percutaneous absorption of piroxicam gel: pharmacokinetic analysis in rabbits. Int J Pharm 134:183–191

Jain AK, Thomas NS, Panchagnula R (2002) Transdermal drug delivery of imipramine hydrochloride. I. Effect of terpenes. J Control Release 79:93–101

Jain R, Aqil M, Ahad A, Ali A, Khar RK (2008) Basil oil is a promising skin penetration enhancer for transdermal delivery of labetolol hydrochloride. Drug Dev Ind Pharm 34(4):384–389

Kararli TT, Kirchhoff CF, PenzottiJr SC (1995) Enhancement of transdermal transport of azidothymidine (AZT) with novel terpene and terpene-like enhancers: in vivo-in vitro correlations. J Control Release 34:43–51

Karpanen TJ, Conway BR, Worthington T, Hilton AC, Elliott TS, Lambert PA (2010) Enhanced chlorhexidine skin penetration with eucalyptus oil. BMC Infect Dis 10:278–284

Kobayashi D, Matsuzawa T, Sugibayashi KYM, Kimura M (1994) Analysis of the combined effect of *l*-menthol and ethanol as skin permeation enhancers based on a two-layer skin model. Pharm Res 11:96–103

Koyama Y, Bando H, Yamashita F, Takakura Y, Sezaki H, Hashida M (1994) Comparative analysis of percutaneous absorption enhancement by D-limonene and oleic acid based on a skin diffusion model. Pharm Res 11:377–383

Krishnaiah YS, Satyanarayana V, Bhaskar P (2002) Influence of Limonene on the bioavailability of nicardipine hydrochloride from membrane-moderated transdermal therapeutic systems in human volunteers. Int J Pharm 247:91–102

Krishnaiah YS, Satyanarayana V, Bhaskar P (2003) Formulation and in vivo evaluation of membrane-moderated transdermal therapeutic systems of nicardipine hydrochloride using carvone as a penetration enhancer. Drug Deliv 10:101–109

Krishnaiah YS, Al-Saidan SM, Jayaram B (2006) Effect of nerodilol, carvone and anethole on the in vitro transdermal delivery of selegiline hydrochloride. Pharmazie 61:46–53

Krishnaiah YS, Raju V, Shiva Kumar M, Rama B, Raghumurthy V, Ramana Murthy KV (2008) Studies on optimizing in vitro transdermal permeation of ondansetron hydrochloride using nerodilol, carvone, and limonene as penetration enhancers. Pharm Dev Technol 13(3):177–185

Kunta JR, Goskonda VR, Brotherton HO, Khan MA, Reddy IK (1997) Effect of menthol and related terpenes on the percutaneous absorption of propranolol across excised hairless mouse skin. J Pharm Sci 86:1369–1373

Lee E, Park KK, Lee JM, Chun KS, Kang JY, Lee SS, Surh YJ (1998) Suppression of mouse tumor promotion and induction of apoptosis in HL-60 cells by *Alpinia oxyphylla* Miquel (*Zingiberaceae*). Carcinogenesis 19:1377–1381

Lim PF, Liu XY, Kang L, Ho PC, Chan YW, Chan SY (2006) Limonene GP1/PG organogel as a vehicle in transdermal delivery of haloperidol. Int J Pharm 27:157–164

Liu CH, Chang FY, Hung DK (2011) Terpene microemulsions for transdermal curcumin delivery: effects of terpenes and cosurfactants. Colloids Surf B Biointerfaces 82(1):63–70

Moghimi HR, Makhmalzadeh BS, Manafi A (2009) Enhancement effect of terpenes on silver sulphadiazine permeation through third-degree burn eschar. Burns 35:1165–1170

Monti D, Saettone MF, Giannaccini B, Galli-Angeli D (1995) Enhancement of transdermal penetration of dapiprazole through hairless mouse skin. J Control Release 33:71–77

Monti D, Chetoni P, Burgalassi S, Najarro M, Saettone MF, Boldrini E (2002) Effect of different terpene-containing essential oils on permeation of estradiol through hairless mouse skin. Int J Pharm 237: 209–214

Monti D, Tampucci S, Chetoni P, Burgalassi S, Bertoli A, Pistelli L (2009) Niaouli oils from different sources: analysis and influence on cutaneous permeation of estradiol *in vitro*. Drug Deliv 16:237–242

Mutalik S, Parekh HS, Davies NM, Udupa N (2009) A combined approach of chemical enhancers and sonophoresis for the transdermal delivery of tizanidine hydrochloride. Drug Deliv 16(2):82–91

Nagai T, Okabe H, Takayama K, Ogura A (1989) Effect of limonene and related compounds on the percutaneous absorption of indomethacin. In: Proceedings of the 16th international symposium on controlled release bioactive material, Chicago (USA), pp 181–182

Nagle A, Goyal AK, Kesarla R, Murthy RR (2011) Efficacy study of vesicular gel containing methotrexate and menthol combination on parakeratotic rat skin model. J Liposome Res 21(2):134–140

Naik A, Guy RH (1997) Infrared spectroscopic and differential scanning calorimetric investigations of the stratum corneum barrier function. In: Potts RO, Guy RH (eds) Mechanisms of transdermal drug delivery. Marcel Dekker, New York, pp 87–149

Narishetty STK, Panchagnula R (2004) Transdermal delivery of zidovudine: effect of terpenes and their mechanism of action. J Control Release 95:367–379

Narishetty STK, Panchagnula R (2005) Effect of L-menthol and 1,8-cineole on phase behavior and molecular organization of SC lipids and skin permeation of zidovudine. J Control Release 102:59–70

Nielsen JB (2006) Natural oils affect the human skin integrity and percutaneous penetration of benzoic acid dose-dependently. Basic Clin Pharmacol Toxicol 98:575–581

Nokhodchi A, Sharabiani K, Rashidi MR, Ghafourian T (2007) The effect of terpene concentrations on the skin penetration of diclofenac sodium. Int J Pharm 335:97–105

Obata Y, Takayama K, Machida Y, Nagai T (1991) Combined effect of cyclic monoterpenes and ethanol on percutaneous absorption of diclofenac sodium. Drug Des Discov 8:137–144

Okabe H, Takayama K, Ogura A, Nagai T (1989) Effect of limonene and related compounds on the percutaneous absorption of indomethacin. Drug Des Deliv 4: 313–321

Okabe H, Takayama K, Nagai T (1992) Percutaneous absorption of ketoprofen from acyclic gel patches containing D-limonene and ethanol as absorption enhancers. Chem Pharm Bull 40:1906–1910

Okyar A, Nuriyev M, Yildiz A, Pala-Kara Z, Ozturk N, Kaptan E (2010) The effect of terpenes on percutaneous absorption of tiaprofenic acid gel. Arch Pharm Res 33(11):1781–1788

Ota Y, Hamada A, Nakano M, Saito H (2003) Evaluation of percutaneous absorption of midazolam by terpenes. Drug Metab Pharmacokinet 18:261–266

Padalia RC, Verma RS (2011) Comparative volatile oil composition of four Ocimum species from northern India. Nat Prod Res 25(6):569–575

Pinder AR (1960) The chemistry of the terpenes. Wiley, New York, pp 1–223

Sapra B, Jain S, Tiwary AK (2008) Percutaneous permeation enhancement by terpenes: mechanistic view. AAPS J 10(1):120–132

Shah VP (1994) Skin penetration enhancers: scientific perspectives. In: Hsieh DS (ed) Drug permeation enhancement: theory and applications. Marcel Dekker, New York, pp 19–23

Song YH, Gwak HS, Chun IK (2009) The effects of terpenes on the permeation of lidocaine and ofloxacin from moisture-activated patches. Drug Deliv 16(2):75–81

Stewart D (2005) The chemistry of essential oils made simple: God's love manifest in molecules. Care Publications, Marble Hill, pp 55–591

Thakur RA, Wang Y, Michniak BB (2006) Essential oils and terpenes. In: Smith EW, Maibach HI (eds) Percutaneous penetration enhancers, 2nd edn. CRC Press, Boca Raton, pp 159–173

Torssell KBG (1983) Natural product chemistry: a mechanistic and biosynthetic approach to secondary metabolism. Wiley, Chichester, pp 167–225

Vaddi HK, Ho PC, Chan SY (2002a) Terpenes in ethanol: haloperidol permeation and partition through human skin and stratum corneum changes. J Control Release 81:121–133

Vaddi HK, Ho PC, Chan SY (2002b) Terpenes in propylene glycol as skin-penetration enhancers: permeation and partition of haloperidol, Fourier transform infrared spectroscopy and differential scanning calorimetry. J Pharm Sci 91:1639–1651

Varman RM, Singh S (2012) Investigation of effects of terpene skin penetration enhancers on stability and biological activity of lysozyme. AAPS PharmSci Tech 13(4):1084–1090

Vávrová K, Zbytovská J, Hrabálek A (2005) Amphiphilic transdermal permeation enhancers: structure–activity relationships. Curr Med Chem 12:2273–2291

Walker RB, Smith EW (1996) The role of percutaneous penetration enhancers. Adv Drug Deliv Rev 18:295–301

Williams AC, Barry BW (1989) Essential oils as novel human skin penetration enhancers. Int J Pharm 57:R7–R9

Williams AC, Barry BW (1991) Terpenes and the lipid–protein-partitioning theory of skin penetration enhancement. Pharm Res 8:17–24

Williams AC, Barry BW (2004) Penetration enhancers. Adv Drug Deliv Rev 56:603–618

Williams AC, Edwards HGM, Lawson EE, Barry BW (2006) Molecular interaction between the penetration enhancer 1,8-cineole and human skin. J Raman Spectrosc 37:361–366

Yamahara J, Kashiwa H, Kishi K, Fujimura H (1989) Dermal penetration enhancement by crude drugs: *in vitro* skin permeation of prednisolone enhanced by active constituents in cardamom seed. Chem Pharm Bull 37:855–856

Yamane MA, Williams AC, Barry BW (1995) Terpene penetration enhancers in propylene glycol/water

co-solvent systems: effectiveness and mechanism of action. J Pharm Pharmacol 47(12A):978–989

Zhang CF, Zhan W, Yang ZL, Wang YL (2010) Impacts of bicyclo-monoterpene enhancers on transdermal delivery of ligustrazine. Yao Xue Xue Bao 45(11):1452–1458

Zhao K, Singh J (1998) Mechanisms of percutaneous absorption of tamoxifen by terpenes: eugenol, D-limonene and menthone. J Control Release 55:253–260

Zhao K, Singh J (1999) In vitro percutaneous absorption enhancement of propranolol hydrochloride through porcine epidermis by terpenes/ethanol. J Control Release 62:359–366

Zhao K, Singh J (2000) Mechanism(s) of in vitro percutaneous absorption enhancement of tamoxifen by enhancers. J Pharm Sci 89:771–780

Transcutol® (Diethylene Glycol Monoethyl Ether): A Potential Penetration Enhancer

12

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Contents

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12.1 Introduction

The skin is one of the most effective routes among the penetration barriers. Percutaneous absorption includes the passage of the drug molecule from the skin surface into the stratum corneum as a result of a concentration gradient and subsequently its diffusion through the stratum corneum and underlying epidermis and dermis into the blood circulation. The skin in contact with penetrant molecules behaves as a passive barrier. The greatest resistance to the penetration of drugs is provided by the stratum corneum, and it is the rate-limiting step in percutaneous absorption. Penetration enhancers are substances that assist in the absorption of the penetrant through the skin. This is achieved by temporarily diminishing the impermeability of the skin. Ideally, these substances should be pharmacologically inert, nontoxic, nonirritating, nonallergenic, compatible with the drug and excipients, odorless, tasteless, colorless, and inexpensive and have good solvent properties. They should not cause the loss of body fluids, electrolytes, and other endogenous materials. After their removal, the skin should immediately recover its barrier properties (Sinha and Kaur 2000).

There are several methods for modifying the barrier properties of the stratum corneum in order to enhance drug penetration and absorption through skin (Singh and Choudhury 2007).

N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_12

One of the methods includes the use of chemical penetration enhancers, which may act by one or more of three main mechanisms (Barry 1983):

1. By disrupting of the highly ordered structure of stratum corneum lipids
2. By interacting with intercellular proteins
3. By improving partitioning of the drug, co-enhancer, or solvent into the stratum corneum

The type of enhancer employed has a significant impact on the design and development of the product.

Transcutol® or diethylene glycol monoethyl ether (DEGEE) is a liquid that has a long history of use in cosmetic and over-the-counter topically applied products. DEGEE is the official United States Pharmacopeia name for this solvent, while cosmetic products list this substance on their labels as ethoxydiglycol in accordance with the International Nomenclature of Cosmetic Ingredients (INCI) Dictionary. Both official names refer to the pharmaceutical/cosmetic solvent having the trade name TRANSCUTOL. The first FDA-approved prescription formulation containing Transcutol® was 5 % dapsone topical gel. Also some other topical formulations that contain Transcutol® have been approved recently or are currently under development. It is expected that in the foreseeable future, this excipient will be a part of a considerable number of dermatics (Osborne 2011).

In this chapter, some of most important physicochemical properties of Transcutol® will be reviewed, as well as its application, pharmacology, and toxicity.

12.2 Physicochemical Properties of Transcutol

Transcutol® is in liquid state. Its molecular formula is $C_6H_{14}O_3$ ($CH_3CH_2OCH_2CH_2OCH_2$–CH_2OH) and its chemical name is 2-(2-ethoxyethoxy) ethanol [CAS Number 111-90-0]. Figure 12.1 shows the structure formula of Transcutol®.

Transcutol® has been used as an industrial solvent for many years under the trade names

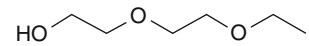

Fig. 12.1 Structure of Transcutol®

Table 12.1 Some of the important physicochemical properties of Transcutol

Property	
Physical state	Liquid
Appearance	Colorless
Odor	Mild odor, fruity odor
Molecular weight	134.18
Class	Glycol ether
Melting point	−76 °C
Boiling point	192–202 °C
Vapor pressure	14 mmHg at 20 °C
Vapor density	4.64
Specific gravity	0.988 at 25 °C
Viscosity	3.85 cP at 25 °C
Flash point	93 °C
Water solubility	100 g/100 g H_2O at 25 °C
Stability	Stable under ordinary condition, hygroscopic

Carbitol™ ® (Dow Chemical Company, USA), Dioxitol® (Shell Oil Company, Houston, USA), Ethoxydiglycol® (Lotioncrafter, Washington, USA), Solvolsol® (JiangSu RuiJia Chemical Co., Ltd, China), and Dowanal DE® (Alibaba Group, Hangzhou, China).

Transcutol® is compatible with some common used solvents such as ethanol, propylene glycol, and oleic acid, but it is not miscible with vegetable oils or mineral oil. The industrial grades of this solvent are contaminated with ethylene glycol and diethylene glycol. Toxicology studies on Transcutol® revealed that prior to the 1990s, the used material that had a purity of 98 %, showing some adverse effects being attributed to the toxicity of ethylene glycol being present in Transcutol® as impurity. Normally, in preparation of pharmaceutical products, USP-NF grades of Transcutol® should contain no more than 50 μ/g 2-methoxyethanol, not more than 160 μ/g 2-ethoxyethanol, not more than 620 μ/g ethylene glycol, and not more than 150 μ/g diethylene glycol. Thus, pharmaceutical Transcutol® has >99.9 % purity (Osborne 2011).

Some of Transcutol® important physicochemical properties are shown in Table 12.1.

12.3 Application

Transcutol® was applied in several commercial formulations and also in many studies as a main ingredient (as an excipient or active excipient with the role to help/increase the drug penetration) of formulations. As mentioned above, the first FDA-approved prescription formulation containing Transcutol® was 5 % dapsone topical gel (Aczone®, Allergan, USA).

Transcutol® was added as a co-surfactant (Mandal and Mandal 2011; Solanki et al. 2012; Goyali et al. 2012; Javadzadeh et al. 2007) and penetration enhancer to many formulations, which were further investigated in different studies. Transcutol® seems to be very attractive as a penetration enhancer due to its non-toxicity, biocompatibility with the skin, miscibility with polar and nonpolar solvents, and optimal solubilizing properties for a large number of drugs (Barakat et al. 2011). Many studies have shown that a permeant's solubility in the skin was enhanced by Transcutol® without significantly influencing its diffusivity in the skin (Osborne 2011). This effect, leading to the formation of an intracutaneous depot of the investigated permeant proves that Transcutol® is able to increase the reservoir capacity of the stratum corneum for some permeants.

On the other hand, Transcutol® is a hygroscopic compound that can absorb water from the skin. Then, by maximizing their thermodynamic activity due to a change in their solubility, improved skin penetration of certain drugs could be expected (Ritschel and Hussain 1988; Biliak et al. 1993).

It is frequently used in dermal formulations, transdermal delivery systems, and ocular and also intranasal delivery systems. It is often used in the formulation of nanoemulsions for transdermal or oral drug delivery systems. Because it spreads easily without streaking, Transcutol® is used in sunless tanning products, which contain high concentrations of Transcutol® (20–40 %) and can be applied frequently to large skin surface areas (Osborne 2011). In a wide range of hair-coloring products that are rinse-off products, it is also contained. Although some of these products are known to be irritating, Transcutol® itself is not considered as irritant substance. However, Transcutol® may promote the delivery of skin irritant excipients that are contained in the product.

In the following section, we are going to review the application of Transcutol® in different fields.

In the study of Mura et al. (2011), the ability of Transcutol® to produce elastic vesicles with soy lecithin (SL) and the influence of the obtained vesicles on in vitro transdermal delivery of minoxidil were investigated. For this mean, penetration enhancer-containing vesicles (PEVs) were prepared using Transcutol® aqueous solutions (5–10–20–30 % v/v) as hydrophilic phase. SL liposomes, without Transcutol®, were used as control. Transdermal delivery of minoxidil was studied by in vitro diffusion experiments through pig skin. Results of in vitro diffusion experiments showed that Transcutol®-containing PEVs are able to deliver minoxidil to deep skin layers without any transdermal permeation.

In the similar study (Mura et al. 2009) for in vitro transdermal delivery of minoxidil, PEVs were prepared as dehydrated–rehydrated vesicles by using soy lecithin and different amounts of three penetration enhancers, Transcutol®, Labrasol®, and cineole. As control, soy lecithin liposomes, without penetration enhancers, were used. In this study, in vitro diffusion experiments were done through newborn pig skin. Results showed that more deformable vesicles than conventional liposomes with a good drug entrapment efficiency and stability were obtained in all the used penetration enhancer-containing formulations. In vitro skin penetration data revealed that PEVs were able to give a statistically significant improvement of minoxidil deposition in the skin without any transdermal delivery in comparison with classic liposomes and penetration enhancer-containing drug ethanolic solutions.

In an attempt to formulate a transdermal delivery system of celecoxib (CXB), Transcutol® was used as a co-surfactant to prepare a nanoemulsion (Baboota et al. 2007). In nanoemulsion formulations, when using a single surfactant, transient negative interfacial tension and fluid interfacial film are rarely achieved, and usually there is a

need for the addition of a co-surfactant. The bending stress of the interface decreases in the presence of co-surfactant and allows the interfacial film sufficient flexibility to take up different curvatures required to form a nanoemulsion over a wide range of compositions. Thus, the co-surfactant selected in this study was Transcutol® with the HLB value of 4.2. Therefore, by combination of Transcutol® with other surfactants such as Tween 80, thermodynamically stable O/W nanoemulsions of CXB were prepared for transdermal drug delivery. Obtained formulation showed improved transdermal delivery of CXB. Compared to CXB gel, permeability parameters like steady-state flux, permeability coefficient, and enhancement ratio were significantly increased in nanoemulsions. This was because of presence of permeation enhancers like Sefsol® 218 (propylene glycol mono caprylic ester), Triacetin® (glycerol triacetate), Tween® 80 (polysorbate 80), and Transcutol®.

Barakat et al. (2011) used Transcutol® for the preparation of indomethacin-loaded nanoemulsion for transdermal delivery. They used Transcutol® as a co-surfactant and also gained from its penetration-enhancing ability. Franz diffusion cell with rabbit skin as permeation membrane was used for ex vivo skin permeation studies. A significant increase in the permeability parameters such as steady-state flux (22.61 ± 3.45 μg/cm^2/h), permeability coefficient ($0.22 \times 10^{-2} \pm 0.0003$ cm/h), and enhancement ratio (8.939) was observed in optimized nanoemulsion formulations compared with the conventional indomethacin gel. The anti-inflammatory effects of nanoemulsion formulations showed a significant increase in percent inhibition value after 4 h when compared with conventional indomethacin gel on carrageenan-induced paw edema in rats. Transcutol®, by increasing the drug solubility, primarily acted as a cosolvent, promoting indomethacin release from the formulation. Therefore, the concentration gradient of the drug was increased, supporting the passage of larger amounts of the drug into the stratum corneum. Transcutol® allowed greater solubilization of the drug in the aqueous phase of the skin as well (Artusi et al. 2004; Baroli et al. 2000; Barry 1987).

In another study, percutaneous permeation of tetrahydropalmatine (THP) was assessed using different transdermal enhancers, i.e., *N-methyl-2*-pyrrolidone (NMP), Transcutol®, and Span 80 at the concentration of 3 % w/w (Li et al. 2011). Vertical Franz diffusion cell was used for transdermal penetration of THP through excised rabbit skin and in vitro release of THP across transparent Cellophane® tests. The results showed that Transcutol® had a slightly lower promoting effect compared with *N*-methyl-2-Pyrrolidone (NMP). The enhancement order for enhancers was NMP > Transcutol® > control ≥ Span 80. The most excellent penetration enhancer was NMP, which had the Q_{36h} up to 118.19 μg/cm^2, providing an almost 2.69-fold increase in permeation amount, followed by Transcutol® with a 1.68-fold increase.

Transdermal delivery of testosterone was also improved by preparing microemulsion formulation using Transcutol® as co-surfactant (Hathout et al. 2010). In this study in vitro testosterone transdermal delivery is evaluated across porcine skin in Franz diffusion cells. Testosterone was delivered successfully across the skin from the microemulsions examined, with the highest flux achieved (4.6 ± 0.6 μg cm^{-2} h^{-1}) from a formulation containing 32 % Transcutol®. The microemulsions considered offer potentially useful vehicles for the transdermal delivery of testosterone.

A reservoir-type transdermal patch for delivery of ketorolac was developed, which contained Transcutol® as a penetration enhancer (Amrish and Kumar 2009). Investigating the in vitro permeation of ketorolac across rat skin, it was observed that Transcutol® possessed higher penetration-enhancing ability than DMSO and d-limonene in the concentration of 5 % w/w. DMSO demonstrated a flux of 27.97 μg/cm^2/h. There was an enhancement of 1.55 times. Transcutol® showed a flux of 29.72 μg/cm^2/h with ER 1.65. These amounts for d-limonene are 18.89 μg/cm^2/h and 1.05 respectively.

Synergic effect of Transcutol® and Azone® in order to increase transdermal penetration of sodium naproxen from Pluronic® F-127 (Sigma-Aldrich, Steinheim, Germany) containing gels was investigated by Escobar-Chaves et al. (2005).

It was found that the combination of these two penetration enhancers, Azone® (1.7 % v/v) and Transcutol® (24.7 % v/v), in Pluronic® F-127 gels enhanced sodium naproxen penetration through human skin in vivo, with enhancement ratios of up to twofold compared with the formulation containing only Transcutol®. These results were confirmed by transepidermal water loss (TEWL) and attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy, suggesting a synergic action for Azone® and Transcutol®.

Harrison et al. (1996) analyzed the mechanism of penetration enhancement of Transcutol® and Azone® as well known potent penetration enhancers. For this reason, changes in the diffusivity and solubility of a model permeant (4-cyanophenol) induced by the aforementioned penetration enhancers were investigated in vitro in human stratum corneum using ATR-FTIR spectroscopy. These results were compared to the effects of the enhancers on permeant's flux obtained using simple Franz-type diffusion cells. It has been shown by both methods that the enhancers increased the flux of cyanophenol across human skin in vitro. In addition, it has been demonstrated by ATR-FTIR that these enhancers are likely to exert their effects by different mechanisms. It was suggested that Azone® reduces the diffusional resistance of the stratum corneum and Transcutol® increases the solubility of the penetrant in this skin layer.

The transdermal permeation of sodium diclofenac was improved using Transcutol® in combination with other enhancers (Escribano et al. 2003). In vitro permeation studies were carried out using human skin as a membrane. The highest permeation values were obtained with the liquid formulation which contained Transcutol® 59.2 %, oleic acid 14.9 %, and d-limonene 5 % (w/w) as permeation enhancers. The anti-inflammatory activity of this formulation was also compared with that of a semisolid formulation on carrageenan-induced paw edema in rats. As expected from in vitro results, the selected formulation showed higher activity in comparison with the semisolid formulation. When tested on rabbits locally in a 72-h trial, no skin irritation was seen. These results suggest that topical delivery of sodium diclofenac with a penetration enhancer such as Transcutol® may be an effective treatment for both dermal and subdermal injuries.

In a study done by Shokri et al. (2012), only burst penetration of piroxicam through the skin was observed from enhancer-contained emulgel formulation in comparison with free enhancer emulgel one. In this study a suitable emulgel formulation of piroxicam was prepared and its percutaneous permeation was investigated using Wistar rat skin and diffusion cells. The effect of three penetration enhancers, 2-hydroxyethyl octadecanoate (Myrj®52), cineole, and Transcutol®, with different concentrations in gel formulation on transdermal permeation of the drug was also evaluated. In all concentrations of applied Transcutol® in the formulations, any transdermal permeation enhancement of drug was shown. Significant enhancement of the flux (9.92-fold) was obtained from the emulgel base (without enhancer) compared to the hydroalcoholic gel. Myrj® 52 at the concentration of 0.25 % showed the highest enhancement ratio. Transcutol® only in concentrations higher than 0.25 % w/w showed burst transportation of the drug through the skin.

In the study performed by Javadzadeh and Hamishekar (2011), a 2 % w/w concentration of Transcutol® in gel formulation showed higher enhancement efficacy in transdermal delivery of methotrexate than common gel formulation. Concerning the potential toxicity related with systemic administration of methotrexate (MTX), a topical formulation might be superior for the treatment of psoriasis and other hyperproliferative skin disorders. Insufficient percutaneous penetration of this drug is one of the presumed reasons for the lack of therapeutic effectiveness of topical MTX formulations in psoriasis. In order to enhance skin penetration of MTX, topical gel formulations were prepared using three types of surfactants (anionic, cationic, and nonionic) in different concentrations (0.5, 1 and 2 % w/w). The results showed that SLS (sodium lauryl sulphate) and alkyl benzyl dimethyl chloride did not show a significant enhancement effect on the penetration of MTX. In contrast, Transcutol®

was able to enhance the penetration of MTX and a higher enhancement ratio was obtained with 2 % (w/w) concentration of Transcutol® than free enhancer base gel formulation. Transcutol® showed a flux of 0.926 μg/cm²/min with ER 3.40. The flux for base gel formulation was 0.272 μg/cm²/min.

Finasteride percutaneous absorption into receiver compartment was also promoted using Transcutol® in the study performed by Javadzadeh et al. (2010). Finasteride is a specific inhibitor of typeII 5α-reductase by irreversibly binding to the enzyme and inhibiting the conversion of testosterone to dihydrotestosterone (DHT). In vitro permeation experiments in rat skin revealed that cationic and anionic surfactants used in various concentrations did not show any enhancement effect on the drug permeation. However, Transcutol® increased significantly the percutaneous penetration/permeation of finasteride. Transcutol® in a concentration of 0.25 % increased skin permeation of the drug nearly 3.6 times in the first 15 min. The highest enhancement ratio was gained in the presence of 1 % Transcutol. In this study, among the different topical finasteride formulations that prepared using three kinds of surfactants with different concentrations, Transcutol® 1 % showed the highest enhancement ratio.

The influence of Transcutol®, alone or in combination with propylene glycol (PG), on clonazepam permeation through an artificial membrane and excised rabbit ear skin from carbomer hydrogels was investigated by Mura et al. (2000). Results showed an increase of drug permeation as a function of Transcutol® concentration (10–50 % w/w) in the formulations without propylene glycol.

Then Transcutol® is an excellent enhancing carrier for clonazepam, giving up an increase of about 3.4 (artificial membranes) or 2.3 (rabbit ear skin) times the flux rate as compared with that of the gel base. Moreover, when Transcutol® was used in combination with PG, a further increase in the flux rate was obtained, up to 6.3 times greater than gel base for the formulation containing 10 % w/w of Transcutol® and 40 % w/w of PG (rabbit ear skin). The proposed mechanisms for the enhancement of drug permeation mainly appear to be related to the solubilizing properties of Transcutol®, combined with its ability to increase drug cutaneous retention and to the penetration and carrier properties of PG across the skin.

The ability of Transcutol® as penetration enhancer to promote dermal delivery of tretinoin in liposomes formulation was evaluated by Manconi et al. (2011a). These vesicles were prepared by adding enhancers to conventional phosphatidylcholine vesicles (control liposomes). The influence of the obtained vesicles on transdermal delivery of tretinoin was studied by ex vivo diffusion experiments through newborn pig skin. Main result of these experiments was an improved cutaneous drug accumulation and a reduced transdermal tretinoin delivery. Amount of tretinoin accumulated into the whole skin and permeated through the skin at the end of the experiment (8 h), local accumulation efficiency values(drug accumulated into the skin/drug permeated through the skin ratio), and transdermal flux (J) for Transcutol®-containing formulations were 13.4 μg/cm², 0.76 μg/cm², and 14 and 95 μg/cm²/h respectively.

Shishu et al. (2009) developed microemulsion-based formulations for topical delivery of acyclovir. Transcutol®, eucalyptus oil, and peppermint oil were used as permeation enhancers. In vitro permeation studies through mice skin were performed using Franz diffusion cells. Results showed that the optimum formulation containing 2.5 % Transcutol® as the penetration enhancer showed 1.7-fold enhancement in flux and permeation coefficient as compared to marketed cream and ointment formulation. In vivo antiviral studies were performed in female Balb/c mice against induced herpes simplex virus I infection. A single application of microemulsion formulation containing 2.5 % Transcutol® given 24 h post-injection resulted in complete suppression of development of herpetic skin lesions.

The pharmacokinetic and bioavailability of fenoterol, a B_2 adrenergic agonist from Transcutol® contained liquid formulation, were studied to determine the feasibility of enhanced transdermal delivery (Elshafeey et al. 2011).

Fenoterol has been widely used to treat asthmatic patients. For comparison, control matrix with fenoterol without any enhancer was also tested. The tested formulations were applied to the shaved back skin of rabbits. Blood samples were collected for 24 h and the plasma concentrations of fenoterol were determined. The results showed a maximum concentration of fenoterol in plasma of 514.8 ng/ml after application of the Transcutol® contained liquid formula while its $AUC_{0-\infty}$ amounted to be 485,972 ng·min/ml with a dose of 3 mg/kg, which is significantly higher than that obtained after application of the control formulation without any enhancer. These values for control formulation were 20.2 ng/ml and 20,715 ng·min/ml respectively. Therefore, the transdermal systems containing Transcutol® as the enhancer will offer an efficient drug delivery system for the treatment of bronchial asthma.

Fini et al. (2008) assessed microemulsion systems containing Transcutol® for topical application of hydrocortisone acetate (HCA). The formulations were tested for the permeation of HCA across an animal membrane. The microemulsion formulation promoted permeation across an ex vivo membrane, examined by means of a Franz cell when compared with gel and ointment formulations without enhancer. Microemulsion formulation showed flux of 133 μg/cm^2/h of the drug, since it contained almost 40 % Transcutol®, a permeation enhancer. Gel and ointment provided lower flux (2 and 0.4 μg/cm^2·h respectively). Transcutol® is present in the microemulsions and it is well known that it significantly increases the percutaneous penetration of various active substances.

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) has several pharmacological effects: antioxidant activity, induction of apoptosis, modulation of cell cycle, antimutagenesis, and anti-inflammatory effect. Oxidative skin damages and the inflammatory processes induced by solar UV radiation were inhibited by quercetin in topical formulations. In the study of Chessa et al. (2011), quercetin (2 mg/mL) was loaded in vesicular penetration enhancer-containing vesicles (PEVs), prepared using a mixture of lipids and one of four selected hydrophilic penetration enhancers: Transcutol®, propylene glycol, polyethylene glycol 400, and caprylocaproyl macrogol 8-glyceride (Labrasol®) at the same concentration (40 % of water phase). The influence of PEVs on ex vivo quercetin transdermal delivery was evaluated using Franz-type diffusion cells, newborn pig skin, and confocal laser scanning microscopy. Results showed that drug delivery is affected by the penetration enhancer used in the PEVs' formulation. All PEVs promoted quercetin deposition in the three main skin strata, showing the same behavior in all samples: the lowest drug accumulation in the stratum corneum, the highest in the epidermis, slightly higher in the dermis than in stratum corneum, and significantly lower in receptor fluid than in epidermis.

In the similar study, a microemulsion of quercetin for the cutaneous release was prepared using Transcutol® (Censi et al. 2011). An aqueous phase, containing 40 % Transcutol® as solubilizing agent and permeation enhancer, was emulsified with oil phase and cosolvent. The in vitro quercetin permeability into and through the abdominal hairless pig skin was determined by vertical Franz's cells.

The highest cumulative amount permeated through the skin was shown for quercetin dissolved in Transcutol®. A good permeation is also observed for quercetin dissolved in the microemulsion (drug dissolution rate are 0.163 and 0.105 μg cm^{-2} min^{-2} respectively), confirming the potential ability of this formulation to promote the skin delivery of quercetin. On the contrary, mixtures not containing Transcutol® demonstrated poor quercetin permeability (0.029 μg cm^{-2} min^{-2} of drug dissolution rate). This study demonstrated that Transcutol® played a major role in acting as absorption promoter for quercetin through the membranes.

Manconi et al. (2011b) used Transcutol® in the form of penetration enhancer-containing vesicles (PEVs) as carriers for transdermal delivery of diclofenac, in the form of either acid (DCF) or sodium salt (DCFNa). Soy phosphatidylcholine and aqueous solutions containing different concentrations of Transcutol® were used for preparation of PEVs. Newborn pig skin was applied for transdermal delivery test using conventional liposomes and a commercial gel as controls.

Obtained results showed that drug accumulation and permeation were closely related to Transcutol® concentration. By increasing Transcutol® concentration, drug deposition into the dermis and diclofenac delivery through the skin improved. The mean amount of the drug permeated after 8 h from conventional liposomes was 16.6 and 7.2 μg/cm^2 for DCF and DCFNa, respectively, while 2.1 μg/cm^2 of DCFNa was delivered by the commercial gel. Highest drug permeation was provided by PEVs through the skin in comparison with the control, and the permeation enhancement was directly proportional to the Transcutol® concentration. DCFNa permeation from 20 to 30 % Transcutol®-PEVs increased dramatically up to 60 μg/cm^2 after 8 h.

The ability of Transcutol® on the in vitro permeation of carvedilol from saturated solutions across porcine skin was investigated by Gannu et al. (2008). Excised porcine ear skin was applied for skin permeation studies using Franz diffusion cells. Phosphate-buffered saline (pH 7.4) solution containing 40 % v/v polyethylene glycol 400 was used as control. The flux of carvedilol from formulations that contain Transcutol® as enhancer (5 % w/v) was 7.6 times higher than that observed using the control. The control sample showed a flux of 0.33 μg/cm^2/h. The present study suggests that Transcutol® at a 5 % w/v may be used as a potential penetration enhancer for transdermal delivery of carvedilol.

Skin accumulation of clobetasol propionate and mometasone furoate was also increased by the use of 20 % Transcutol® as a penetration enhancer (Senyiğit et al. 2009). Pig ear skin was used for in vitro permeation experiments as barrier. The formulations tested were chitosan gels, sodium deoxycholate gels and commercial creams of drugs.

Cázares-Delgadillo et al. (2005) found that sucrose esters (SEs) were effective when combined with Transcutol®. In this study they investigated the effect of sucrose esters (laureate and oleate) in combination with Transcutol®, on the permeation of lidocaine (a weak base) as a function of vehicles' pH value. Porcine ear skin was used as the barrier membrane. According to their results, in the absence of an enhancer, lidocaine flux increased from pH 5 to 9 with a corresponding increase in the level of the unionized base. However, when the skin was pretreated with 2 % laureate in Transcutol® (2 %), drug permeation was higher at pH 5.0 and 7.0 than at 9.0. A different trend was observed in experiments with 2 % oleate in Transcutol® (2 %), where skin flux was maximal at a more basic pH, when the degree of ionization is low. The results propose that sucrose laureate enhances the penetration of the ionized form of the drug (12-fold greater flux), whereas sucrose oleate is more effective in promoting permeation of the unionized species.

According to the previous studies which showed that Transcutol® increased significantly the percutaneous penetration of several drugs, particularly if used in combination with suitable surfactants (Watkinson et al. 1991; Touitou et al. 1994), Cázares-Delgadillo et al. concluded that the presence of a co-solvent, i.e., Transcutol® in this case, promoted the absorption of the surfactant into the skin, favoring its interaction with the stratum corneum lipids. Furthermore, it has also been reported that the addition of a cosolvent may lead to a change in the solubility of a solute, altering its thermodynamic activity and consequently the skin/vehicle partition coefficient (Mura et al. 2000).

As mentioned above, the addition of a cosolvent promotes the penetration of a surfactant into the skin, favoring its interaction with the stratum corneum lipids. Transcutol® has been used as a cosolvent because it can influence the partitioning behavior of a drug or a co-enhancer into the tissue (Mura et al. 2000).

Atenolol, griseofulvin, clebopride, dexamethasone, and ivermectin are pharmaceutical actives listed as being successfully formulated using Transcutol® (Osborne 2011). In these topical formulations, Transcutol® is reported to be a highly effective solubilizer, enabling high drug loading leading to improved skin permeation.

In another study (Liu et al. 2006), the effect of Transcutol® on the corneal permeability of drugs was investigated in vitro, using isolated rabbit corneas. The ocular irritation of Transcutol® was also tested in rabbits in vivo. The results showed that in the presence of Transcutol®, at a concentration of 0.005–0.03 %, the maximum increase

in the apparent permeability coefficient (P_{app}) was 1.5-, 1.5-, 3.0-, and 3.3-fold for ribavirin, gatifloxacin, levofloxacin hydrochloride, and enoxacin, respectively, compared to formulation that was free of enhancer. However, this value was reduced for oxaprozin in the presence of Transcutol®. The results of the ocular irritation studies showed that Transcutol® was non-irritant in the concentrations studied (0.005–0.03 %), but at a concentration of 0.05 %, a slight irritation was seen. It was also found that no visible ocular damage or abnormal clinical signs involving the cornea, iris, or conjunctivae were observed at all used concentrations. It was concluded that Transcutol® as an enhancer may have potential clinical benefits in improving the ocular drug delivery of hydrophilic compounds.

The bioavailability of nitrendipine (NDP), a potent antihypertensive molecule, was enhanced using Transcutol® as solubilizer through formulating a nanoemulsion for its intranasal delivery (Jain and Patravale 2009). In vivo absorption studies showed that NDP absorption from the nanoemulsion had a rapid onset of action and a relative bioavailability of 60.44 %, which was significantly greater than the marketed oral tablets.

12.4 Biomedical Effects and Toxicity

In the non-clinical safety assessment of potential new drugs, the formulation must be done in a manner that used vehicle show any, little, or at least acceptable effects.

Although some of the products containing Transcutol® as an ingredient are known to be irritating, Transcutol® itself is not considered as an irritant substance. However, Transcutol® may promote the delivery of other skin irritant excipients that are contained in the product.

In December 2006, the Scientific Committee on Consumer Products (SCCP) concerned a view on Transcutol®. An excellent toxicological evaluation summary of Transcutol® was provided by this opinion. Evaluation on animals showed that Transcutol® produces little hematotoxicity. According to the two negative in vivo mutagenicity studies and the structure of the substance, SCCP adopted that it will not have relevant mutagenic potential. A series of Gattefosse (Gattefosse Company, French) reports mentioned in the SCCP review indicate that (1) neat Transcutol® dosed at 0.020 mL per about 50 mm^2 of human volunteer skin was well tolerated, and (2) no pathological irritation or sensitization reaction significant to a cutaneous intolerance was noted.

SCCP issued an opinion on the percutaneous absorption of Transcutol® according to the three in vitro studies (Osborne 2011). Based on these results, a significant amount of Transcutol® applied to the skin can enter the systemic circulation. Fortunately, Transcutol®'s low systemic toxicity is in a border of safety score of 102 (MOS score is the nonobserved adverse effect level divided by the systemic exposure dose following topical application). This MOS score means that after applying a 2 % Transcutol®-emulsified formulation, blood levels of Transcutol® are about 100 times lower than the blood level at which it causes first observable adverse effects. Although some products such as hair dyes and sunscreens may have MOS scores above 500, the primary requirement for drugs is that the MOS must be above 1 (Osborne 2011).

However, when Transcutol® is used for the oral or intraperitoneal route, this compound cannot be considered as inert excipient and more pharmacology or toxicology studies are required (Delongeas et al. 2010; Budden et al. 1979). In the study of Budden et al., Transcutol® was screened in vivo for its acute toxicity, influence on the behavior, and sedative and muscle relaxant properties in mice. Based on their obtained results, it was recommended not to exceed a certain concentration (i.e. 0.7 % (w/v)) in the intraperitoneal preparations used for drug screening tests (Budden et al. 1979).

Conclusion

Transcutol® is a safe and well-tolerated solvent that is applied as an inactive ingredient/excipient in cosmetic products and also in prescriptional dermatics for many years as a cosurfactant and a penetration enhancer. This

substance tends to penetrate the skin after topical application. Many studies have shown that the permeant's solubility in the skin is enhanced by Transcutol® without significantly influencing its diffusivity in the skin. The ability of Transcutol® to dissolve poorly soluble active pharmaceutical ingredients in suitable solvents makes it a highly useful pharmaceutical excipient. Transcutol® itself is not considered as an irritant substance. Transcutol® in the skin of human volunteers was well tolerated, and no pathological irritation or sensitization reactions were reported. This solvent is a promising component for many cosmetic products as well as prescriptional dermatics.

References

Amrish C, Kumar SP (2009) Transdermaldelivery of ketorolac. Yakugaku Zasshi 129(3):373–379

Artusi M, Nicoli S, Colombo P, Bettini R, Sacchi A, Santi P (2004) Effect of chemical enhancers and iontophoresis on thiocolchicoside permeation across rabbit and human skin in vitro. J Pharm Sci 93:2431–2438

Baboota S, Shakeel F, Ahuja A, Ali J (2007) Design, development and evaluation of novel nanoemulsion formulations for transdermal potential of celecoxib. Acta Pharm 57:315–332

Barakat N, Fouad E, Elmedany A (2011) Formulation design of indomethacin-loaded nanoemulsion for transdermal delivery. Pharm Anal Acta S2:1–8

Baroli B, Lopez-Quintela MA, Delgado-Charro MB, Fadda AM, Blanco- Mendez J (2000) Microemulsions for topical delivery of 8-methoxsalen. J Control Release 69:209–218

Barry BW (1983) Dermatological formulation: percutaneous absorption. Marcel Dekker, New York

Barry BW (1987) Mode of action of penetration enhancers in human skin. J Control Release 6:85–97

Bialik W, Walters KA, Brain KR, Hadgraft J (1993) Some factors affecting the in vitro penetration of ibuprofen through human skin. Int J Pharm 92:219–223

Budden R, Kühl G, Bahlsen J (1979) Experiments on the toxic, sedative and muscle relaxant potency of various drug solvents in mice. Pharmacol Ther 5:467–474

Cázares-Delgadillo J, Naik A, Kalia YN, Quintanar-Guerrero D, Ganem-Quintanar A (2005) Skin permeation enhancement by sucrose esters: a pH-dependent phenomenon. Int J Pharm 297:204–212

Censi R, Martena V, Hoti E, Malaj L, Di Martino P (2011) Permeation and skin retention of quercetin from microemulsions containing Transcutol® P. Drug Dev Ind Pharm 38(9):1128–1133

Chessa M, Caddeo C, Valenti D, Manconi M, Sinico C, Fadda AM (2011) Effect of penetration enhancer containing vesicles on the percutaneous delivery of quercetin through new born pig skin. Pharmaceutics 3:497–509

Delongeas JL, Conchard GVD, Beamonte A, Bertheux H, Spire C, Maisonneuve C et al (2010) Assessment of Labrasol_/Labrafil_/Transcutol_ (4/4/2, v/v/v) as a non-clinical vehicle for poorly water-soluble compounds after 4-week oral toxicity study in Wistar rats. Regul Toxicol Pharmacol 57:284–290

Elshafeey AH, Hamza YE, Amin SY, Akhlaghi F, Zia H (2011) Enhanced bioavailability of fenoterol transdermal systems in rabbits. J Bioequiv Availab 3(5): 97–100

Escobar-Chávez JJ, Quintanar-Guerrero D, Ganem-Quintanar A (2005) In vivo skin permeation of sodium naproxen formulated in pluronic F-127 gels: effect of Azone and Transcutol. Drug Dev Ind Pharm 31(4–5): 447–454

Escribano E, Calpena AC, Queralt J, Obach R, Doménech J (2003) Assessment of diclofenac permeation with different formulations: anti-inflammatory study of a selected formula. Eur J Pharm Sci 19(4):203–210

Fini A, Bergamante V, Ceschel GC, Ronchi C, De Moraes CAF (2008) Control of transdermal permeation of hydrocortisone acetate from hydrophilic and lipophilic formulations. AAPS Pharm Sci Tech 9(3):762–768

Gannu R, Vishnu YV, Kishan V, Rao YM (2008) In vitro permeation of carvedilol through porcine skin: effect of vehicles and penetration enhancers. PDA J Pharm Sci Technol 62(4):256–263

Goyali U, Arora R, Aggarwal G (2012) Formulation design and evaluation of a self-microemulsifying drug delivery system of lovastatin. Acta Pharm 62:357–370

Harrison JE, Watkinson AC, Green DM, Hadgraft J, Brain K (1996) The relative effect of Azone and Transcutol on permeant diffusivity and solubility in human stratum corneum. Pharm Res 13(4):542–546

Hathout RM, Woodman TJ, Mansour S, Mortada ND, Geneidi AS, Guy RH (2010) Microemulsion formulations for the transdermal delivery of testosterone. Eur J Pharm Sci 40(3):188–196

Jain R, Patravale VB (2009) Development and evaluation of nitrendipine nanoemulsion for intranasal delivery. J Biomed Nanotechnol 5(1):62–68

Javadzadeh Y, Hamishehkar H (2011) Enhancing percutaneous delivery of methotrexate using different types of surfactants. Colloids Surf B Biointerfaces 82: 422–426

Javadzadeh Y, Shokri J, Hallaj-Nezhadi S, Hamishehkar H, Nokhodchi A (2010) Enhancement of percutaneous absorption of Finasteride by cosolvents, cosurfactant and surfactants. Pharm Dev Technol 15(6):619–625

Javadzadeh Y, Siahi MR, Asnaashari S, Nokhodchi A (2007) Liquisolid technique as a tool for enhancement of poorly water-soluble drugs and evaluation of their physicochemical properties. Acta Pharm 57: 99–109

Li C, Liu C, Liu J, Fang L (2011) Correlation between rheological properties, in vitro release, and percutaneous permeation of tetrahydropalmatine. AAPS PharmSciTech 12(3):1002–1009

Liu Z, Li J, Nie S, Guo H, Pan W (2006) Effects of Transcutol P on the corneal permeability of drugs and evaluation of its ocular irritation of rabbit eyes. J Pharm Pharmacol 58(1):45–50

Manconi M, Caddeo C, Sinico C, Valenti D, Mostallino MC, Biggio G, Fadda AM (2011a) Ex vivo skin delivery of diclofenac by transcutol containing liposomes and suggested mechanism of vesicle–skin interaction. Eur J Pharm Biopharm 78:27–35

Manconi M, Sinico C, Caddeo C, Vila AO, Valenti D, Fadda AM (2011b) Penetration enhancer containing vesicles as carriers for dermal delivery of tretinoin. Int J Pharm 412:37–46

Mandal S, Mandal SS (2011) Microemulsion drug delivery system: a platform for improving dissolution rate of poorly water soluble drug. Int J Pharm Sci Nanotech 3(4):1214–1219

Mura P, Faucci MT, Bramanti G, Corti P (2000) Evaluation of transcutol as a clonazepam transdermal permeation enhancer from hydrophilic gel formulations. Eur J Pharm Sci 9(4):365–372

Mura S, Manconi M, Sinico C, Valenti D, Fadda AM (2009) Penetration enhancer-containing vesicles (PEVs) as carriers for cutaneous delivery of minoxidil. Int J Pharm 380:72–79

Mura S, Manconi M, Valenti D, Sinico C, Vila AO, Fadda AM (2011) Transcutol containing vesicles for topical delivery of minoxidil. J Drug Target 19(3): 189–196

Osborne DW (2011) Diethylene glycol monoethyl ether: an emerging solvent in topical dermatology products. J Cosmet Dermatol 10:324–329

Ritschel WA, Hussain AS (1988) Influence of selected solvents on penetration of griseofulvin in rat skin, in vitro. Pharm Ind 50:483–486

Senyiğit T, Padula C, Ozer O, Santi P (2009) Different approaches for improving skin accumulation of topical corticosteroids. Int J Pharm 380(1–2):155–160

Shishu, Rajan S, Kamalpreet (2009) Development of novel microemulsion-based topical formulations of acyclovir for the treatment of cutaneous herpetic infections. AAPS PharmSciTech 10(2):559–562

Shokri J, Azarmi S, Fasihi Z, Hallaj-Nezhadi S, Nokhodchi A, Javadzadeh Y (2012) Effects of various penetration enhancers on percutaneous absorption of piroxicam from emulgels. Res Pharm Sci 7(4):225–234

Sinha VR, Kaur MP (2000) Permeation enhancers for transdermal drug delivery. Drug DevInd Pharm 26(11):1131–1140

Singh PB, Choudhury PK (2007) Penetration enhancers for transdermal drug delivery of systemic agents. J Pharm Res 6:44–50

Solanki SS, Sarkar B, Dhanwani RK (2012) Microemulsion drug delivery system: for bioavailability enhancement of ampelopsin. ISRN Pharm 2012:1–4

Touitou E, Levi-Schaffer F, Dayan N, Alhaique F, Riccieri F (1994) Modulation of caffeine skin delivery by carrier design: liposomes versus permeation enhancers. Int J Pharm 103:131–136

Watkinson AC, Hadgraft J, Bye A (1991) Aspects of the transdermal delivery of prostaglandins. Int J Pharm 74:229–236

13 Surfactants as Penetration Enhancers for Dermal and Transdermal Drug Delivery

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13.1 Introduction

Over the last 30 years, skin has been considered as a vital route for the delivery of various types of drugs incorporated into different topical formulations, including ointments, creams, gels and skin patches, to induce local or systemic effects. On the other hand, unwanted absorption of chemicals (into systemic circulation) to which skin may become exposed to in everyday life may lead to toxicity and health risks (Bataller et al. 2000; Belsey et al. 2011). As a result, the European Commission regulation known as REACH (Registration, Evaluation and Authorisation/Restriction of Chemicals) requires extensive risk assessment of all existing chemicals, including exposure via dermal contact (Commission of the European Communities 2003). Whether it is the barrier or the target, permeation of compounds through skin is an important consideration and focus of extensive research.

N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_13

Skin has become one of the innovative focuses for research in drug delivery with many drugs being evaluated for transdermal or dermal systems (Som et al. 2012). The preference of this type of drug delivery over oral or other routes of delivery is due to many promising advantages associated with drug delivery through the skin. These include:

- A better control of blood levels
- A reduced incidence of systemic toxicity
- Absence of hepatic first pass metabolism
- Suitability for patients suffering from gastrointestinal problems
- Better patient compliance (non-invasive, painless and simple application)
- Easy and quick termination of therapy by removal of the formulation from the skin
- Ease of use in non-responsive patients, unconscious or comatose
- Absence of harmful effect of GI tract on drugs

Despite the many advantages mentioned here, drug delivery via the skin is not a simple task; the outermost layer of the skin, the stratum corneum (SC), is a barrier both to water transport out of the body and to inward chemical permeation. Many strategies have been suggested in order to overcome the low permeation of drugs through the skin. A popular approach is the use of chemical penetration enhancers or accelerants in topical drug delivery systems in order to reduce reversibly the permeability barrier of the SC (Barry 1983).

Topical drug formulations are normally combinations of different chemical substances which produce the final medicinal product. Formulation ingredients can alter the skin penetration of a compound by affecting the barrier properties of the skin or by changing the partitioning of the compound into the SC (Riviere and Brooks 2011). In addition, a step forward has been achieved in understanding, modelling and predicting the effect of complex mixtures (Ghafourian et al. 2010a, b; Samaras et al. 2012). The topical formulations are often a mixture of various components dispersed/dissolved in a hydrophilic or a lipophilic vehicle also called a base. A vehicle can be a single component, e.g. water, or a mixture of components, e.g. water and ethanol, and it can play a very important role in the penetration of a chemical through the skin. According to Roberts et al. (2002), vehicles can affect skin permeability by a range of mechanisms including delipidisation, dehydration, fluidisation, and desmosome disruption in the SC and also by changing the polarity of the formulation mixture which can change the penetrant solubility and partitioning into SC. Generally, modification of organic vehicles has the potential to enhance maximal drug flux. In case there are components in the vehicle that can interact with the intercellular lipids of the SC, then it is possible that permeation may be enhanced or suppressed (Davis et al. 2002). A vehicle can promote the penetration of a chemical into the skin by having low solubility for the penetrant. Hence, in this way a chemical will not be retained in the vehicle (Baker 1972). This is because the partition coefficient of a drug between the skin and the vehicle generally decreases as the drug solubility in the vehicle rises. Polar co-solvent mixtures, such as propylene glycol with water, may produce saturated drug solutions and so maximise the concentration gradient of the drug across the SC (Barry 2007; Wiechers 2005), although they may also affect SC directly.

Apart from the vehicle in which the drug is contained, two major components in pharmaceutical preparations are surfactants and chemical penetration enhancers (Roberts et al. 2002). Chemical penetration enhancers, otherwise known as accelerants, absorption promoters or simply as penetration enhancers, are chemicals that enhance the penetration of topically applied drugs (Williams and Barry 2004). When designing a transdermal drug formulation, it is highly important to first identify chemicals that significantly enhance drug penetration through the epidermis and at the same time do not irritate or damage the skin. The important properties of such chemical permeation enhancers have been referred and described by Barry (1983) and reviewed recently by Williams and Barry (2004). Some well-known enhancers are Azone® (1-dodecylazacycloheptan-2-one or laurocapram), pyrrolidones such as N-methyl-2-pyrrolidone (NMP), isopropyl myristate (Engelbrecht et al. 2012), nicotinic

acid esters (Le and Lippold 1995) and terpenes (Nokhodchi et al. 2007; Kang et al. 2007).

This chapter will focus on the role of surfactants in transdermal and topical delivery of drugs.

13.2 Properties of Surfactants

In terms of the chemical structure, surfactants are composed of a lipophilic alkyl (or aryl chain) group and a hydrophilic head group (Malmsten 2002), as shown in Fig. 13.1. Surfactants owe their unique properties to this distinctive molecular feature.

The term surfactant comes from the phrase 'surface-active agent'. Due to the distinctive polar and lipophilic ends, when dissolved, these molecules will gather at the surface of high surface tension solvents such as water, with the polar head facing water and lipophilic tail looking towards the air. This surface activity will reduce the surface tension of the solvent and, by the same mechanism acting at solid/liquid interfaces, will improve wettability of solid surfaces.

At low concentrations, surfactants may be dissolved in the water medium and gather at the surface in a non-associated form. If the concentration of the surfactant in water is above a certain limit, then it becomes energetically favourable for aggregates to form. In this case, the surfactant molecules will join together with the hydrophobic tails facing each other and the polar heads facing water. This structure is known as micelle, and the concentration in which micelles are formed is known as the critical micelle concentration (CMC). Depending on the polarity of the solvents, micelles can be in different configurations, as shown in Fig. 13.2. It should be noted that micelles are in dynamic equilibrium with the unaggregated surfactant molecules in the solution and theoretically at concentrations above CMC the concentration of unaggregated molecules will remain constant. Most surfactant molecules that undergo self-association in water form an isotropic solution phase (Anderson et al. 1989). The micelles may have various sizes and shapes, but as a rule of thumb for surfactants with a single hydrocarbon chain, the aggregates are approximately spherical and are formed of roughly around 100 monomers (Rosen and Kunjappu 2004). This aggregation number is obviously the mean value of the micelle size distribution. Aggregates that are much smaller than the mean aggregation number (sub-micellar aggregates) are significantly less energetically favourable, and therefore, they exist in much lower concentrations in the system (Nagarajan and Ruckenstein 1991).

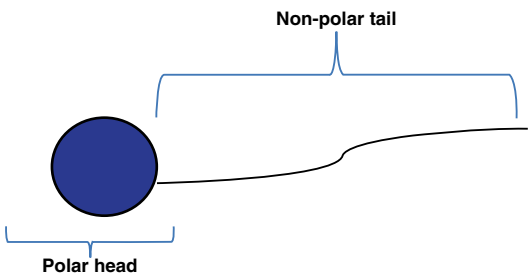


Fig. 13.1 Schematic representation of a surfactant; the non-polar tail consists of a straight or branched hydrocarbon or fluorocarbon chain often containing 8–18 carbon atoms

For any surfactant the balance between hydrophilic and lipophilic regions of the molecules (hydrophilic-lipophilic balance or HLB) can be a useful measure in deciding the appropriate surfactant for a specific application. HLB is the ratio of the size of the hydrophilic (or *polar*) region to the total mass of the molecule as defined by Griffin (1949, 1954). In Griffin's method the HLB for nonionic surfactants is calculated by the equation below in which M_h is the molecular mass of the hydrophilic portion of the molecule and M_t is the total molecular mass (Griffin 1954):

$$\mathrm{HLB} = 20 \times \frac{M_h}{M_t}$$

According to this equation an HLB value of 0 indicates a completely lipophilic surfactant, and a value of 20 corresponds to a completely hydrophilic surfactant. All surfactants combine both hydrophilic and lipophilic groups as shown in Fig. 13.1. As a rule of thumb, a surfactant that has a low HLB number below 9.0 is considered lipophilic, and one that has a high HLB number

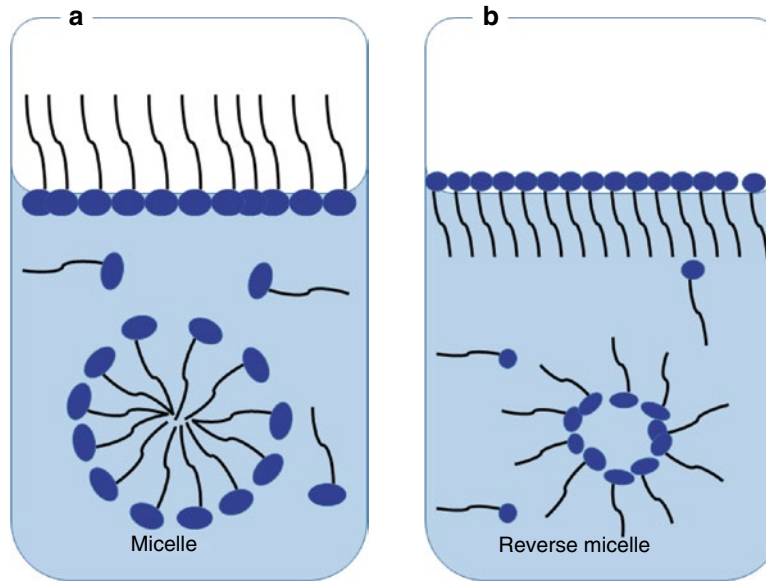


Fig. 13.2 Schematic representation of surfactants in (**a**) polar and (**b**) non-polar solvents

above 11.0 is considered hydrophilic. Those in the range of 9–11 are intermediate. For nonionic surfactants the range of HLB is between 1 and 20, but for ionic surfactants due to the higher strength of the hydrophilic regions, HLB scales will need to consider not only the weight but also the strength of the hydrophilic (and lipophilic) regions (Davis 1957). In this case, the HLB value can go beyond this range if the surfactant ionises. For example, the anionic surfactant sodium lauryl sulphate (SLS) has a HLB of 40.

Because of the unique functional behaviour of surfactants, they have a wide range of applications in pharmaceutical preparations. They can be used as solubilising agents, dissolution enhancers, wetting agents (in suspensions), stabilisers (in emulsions) and controlled flocculators in metered dose inhalers. Surfactants also have applications in other dosage forms such as tablets and capsules (Newton et al. 1971) and suppositories (Hanaee et al. 2004) to enhance the dissolution rate of poorly water-soluble drugs. Furthermore, surfactants are known to affect the permeability characteristics of several biological membranes, including the skin (Florence et al. 1994; Lopez et al. 2000a). Surfactants such as phospholipids have been proposed as useful penetration enhancers of low toxicity in topical formulations (Kato et al. 1987).

13.3 Use of Surfactants in Topical Formulations

Surfactants are used in topical formulations as emulsifying agents in the preparation of creams and lotions and other micro- and nanoemulsion preparations. They are also used in monomeric concentrations or concentrations above CMC as penetration enhancers in drug delivery systems such as skin patches (Mukherjee et al. 2005) and gels (Raut et al. 2010). There are many reports of a wide variety of surfactants enhancing the penetration of compounds across biological membranes (Riegelman and Crowell 1958a, b, c; Aguiar and Weiner 1969). Surfactants are used as penetration enhancers or accelerants as they are capable of promoting penetration of drugs into the skin, or their permeation through the skin, by reversibly reducing the skin barrier resistance. The ideal properties of penetration enhancers including surfactants in order to be considered as penetration enhancers in topical formulations are listed below:

- They should have a good chemical stability and compatibility alongside other ingredients in the formulation.
- They should be pharmacologically and chemically inert.

- They should be non-toxic when applied on the skin.
- They should be effective in low concentrations.
- They should show a rapid onset of action.
- They should have a reproducible and reversible effect.
- Their effect on stratum corneum should be reversible when they are removed from the skin.
- They should be cost effective and affordable.

Below is a description of different types of surfactants and examples of their use in topical formulations.

13.3.1 Natural Surfactants

These are nonionic surfactants often derived from sugars and fatty alcohols. The use of natural surfactants, or 'sugar surfactants', received increased attention in formulation development as co-emulsifying agents for pharmaceutical formulation development (Klang et al. 2010). These surfactants are acetals that are obtained by condensation of glycosides such as saccharose and glucose with fatty alcohols. Natural surfactants which are non-toxic and readily biodegradable include alkyl polyglucosides and sucrose esters (Cázares-Delgadillo et al. 2005; Calderilla-Fajardo et al. 2006; Csóka et al. 2007). Sucrose esters are nonionic surfactants composed of fatty acid esters of sucrose with different fatty acid residues (Csóka et al. 2007). Figure 13.3 gives chemical structures of a few such surfactants.

Natural surfactants have been used for the enhancement of skin permeability for several drugs including cyclosporine A (Lerk and Sucker 1993), oestradiol (Vermeire et al. 1996) and ibuprofen (Csizmazia et al. 2011; Csizmazia et al. 2012) in hydrogel formulations; niflumic acid (Bolzinger et al. 1998) and hydrocortisone (Lehmann et al. 2001) in microemulsion formulations; 4-Hydroxybenzonitrile (Ayala-Bravo et al. 2003), lidocaine, ketoprofen (Okamoto et al. 2005), lidocaine hydrochloride (Cázares-Delgadillo et al. 2005) and propofol (Yamato et al. 2009) in solutions; octyl methoxycinnamate in a nanoemulsion formulation (Calderilla-Fajardo et al. 2006); metoprolol (Csóka et al. 2007) and timolol maleate (El-Laithy 2009) in TTS patch formulations; and vinpocetine in a proniosomal formulation (El-Laithy et al. 2011). In these formulations, there was often a remarkable increase in the skin permeation of the formulated drug upon incorporation of the sugar surfactant. For example, the enhancement ratios for lidocaine skin absorption was in the range 2.67–11.95 (Cázares-Delgadillo et al. 2005) and for propofol was between 2.1 and 2.90 (Yamato et al. 2009) for a variety of the sugar surfactants investigated at 1 % concentration. In another study, sugar surfactants were able to enhance skin absorption of lidocaine and ketoprofen up to 2.75- and 1.62-fold, respectively, for the ionised forms of the drugs (Okamoto et al. 2005).

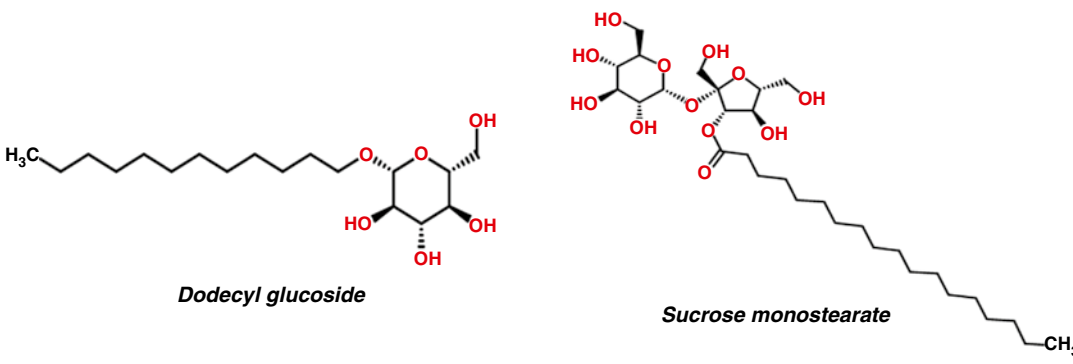


Fig. 13.3 Chemical structures of two sugar surfactants

Cyclodextrins or cyclic oligosaccharides (Fig. 13.4) are other sugar derivatives that have been used in topical formulations. These compounds are not commonly labelled as surfactants but have surfactant properties (Bardi et al. 2000) and have been investigated as natural emulsifying agents for the solubilisation of lipophilic drugs leading to improved drug penetration through the skin (Challa et al. 2005; Loftsson et al. 2007). For example, cyclodextrins have been used to enhance the skin delivery of dexamethasone (Lopez et al. 2000a), 4-biphenylacetic acid (Arima et al. 1990), chloramphenicol (Abdel Rahman et al. 1991), ciprofloxacin and norfloxacin (Udupa and Bhat 1992), ethyl 4-biphenylyl acetate (Arima et al. 1990, 1998), hydrocortisone (Loftsson et al. 1994), indomethacin (Lin et al. 1994), nitroglycerine (Umemura et al. 1990), piroxicam and flurbiprofen (Narasimha Reddy and Udupa 1993), prednisolone (Uekama et al. 1987), prostaglandin E_1 (Uekama et al. 1992) and sulphanilic acid (Okamoto et al. 1986).

Modified cyclodextrins with differing water solubility may enhance the penetration of lipophilic drugs by solubilising various drugs (Felton et al. 2002; Másson et al. 1999). Hydroxypropyl-β-cyclodextrin and 2.6-di-O-methyl-β-cyclodextrin are two examples of modified cyclodextrins that have been evaluated for enhancement of transdermal drug absorption. Hydroxypropyl-β-cyclodextrin can increase transdermal flux of oxybenzone through hairless mouse skin by 36.3-fold at the optimum concentration of 10 % (Felton et al. 2002). Percutaneous absorption of celecoxib through isolated human stratum corneum and epidermis is also increased in a concentration-dependent manner, with 5 % w/v concentration of hydroxypropyl- and dimethyl-β-cyclodextrin showing the highest enhancement of celecoxib flux by 6.32- and 7.13-fold, respectively (Ventura et al. 2006). The effect of several different cyclodextrins on the flux of hydrocortisone from aqueous vehicles containing hydrocortisone/cyclodextrin complexes (Loftsson and Sigurdardottir 1994; Sigurdoardóttir and Loftsson 1995) indicates that maximum flux through the hairless mouse skin is obtained when just enough cyclodextrin is added to the vehicle to keep all hydrocortisone in solution (Loftsson and Masson 2001).

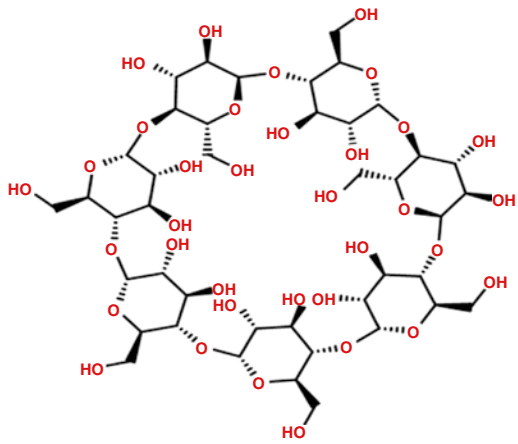


Fig. 13.4 Chemical structure of a cyclodextrin

13.3.2 Anionic Surfactants

Anionic surfactants are surfactants in which the hydrophilic part carries a negative charge, as shown schematically in Fig. 13.5.

This type of surfactants is the most widely used class of surfactants in industrial applications due to their relatively low cost of manufacture; they are used in practically every type of detergent. Anionic surfactants include carboxylates (e.g. soaps), sulphates (e.g. sodium lauryl sulphate (SLS) also known as sodium dodecyl sulphate (SDS)), sulphonates (e.g. dioctyl sodium sulphosuccinate) and phosphate esters. Figure 13.6 shows chemical structures of some of the most commonly used anionic surfactants as chemical permeation enhancers in transdermal drug delivery.

SLS has been shown to increase the skin penetration of many drugs including ketotifen (Kitagawa and Ikarashi 2003), diazepam (Shokri et al. 2001), lorazepam (Nokhodchi et al. 2003), propofol (Yamato et al. 2009), naloxone (Aungst 1986) and foscarnet (Piret et al. 2000). The enhancement ratios as high as 11.30 and 9.3 have been reported for skin permeation coefficient of lorazepam (Nokhodchi et al. 2003) and diazepam

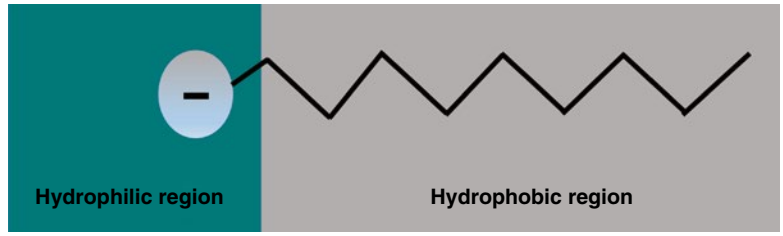
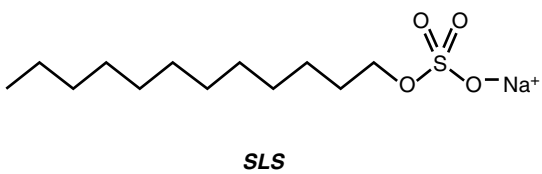
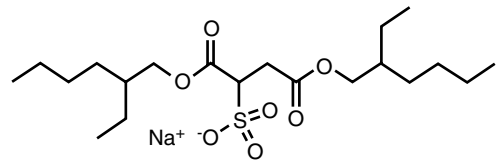


Fig. 13.5 Schematic representation of an anionic surfactant



SLS



Dioctyl sodium sulfosuccinate

Fig. 13.6 Chemical structures of anionic surfactants

(Shokri et al. 2001), respectively, from aqueous solutions containing 5 % SLS.

SLS is a skin irritant (Tupker et al. 1990). This surfactant in combination with low-frequency ultrasound has been shown to increase the transdermal transport of polar chemicals, such as mannitol (Mitragotri et al. 2000). The effect of anionic surfactants on transdermal drug permeation is known to be reversible since the skin tissues return to normal condition upon removal of this surfactant (Legen, et al. 2006; Scheuplein and Ross 1970).

13.3.3 Cationic Surfactants

Figure 13.7 shows a schematic representation of a cationic surfactant and some cationic surfactant examples. Cationic surfactants, such as cetyltrimethyl ammonium bromide (CTAB) and benzalkonium chloride, are often quaternary ammonium compounds with large lipophilic hydrocarbon groups. These two cationic surfactants have been used in transdermal formulations and have shown potential to enhance the permeation of diazepam (Shokri et al. 2001), lorazepam (Nokhodchi et al. 2003), haloperidol (Vaddi et al. 2001), sodium nonivamide acetate (Fang et al. 1997) and methyl nicotinate (Ashton et al. 1992). The skin permeation of methyl nicotinate via human skin treated for 3 days with 1.5 % solution of CATB was increased by 404 % in comparison with the solution without CTAB (Ashton et al. 1992). The enhancement ratios reported for diazepam and lorazepam in the presence of 1 % benzalkonium chloride were 7.98 and 7.66, respectively. When CTAB was used at 5 % w/v, the enhancement ratio of lorazepam increased to 9.82. In case of sodium nonivamide acetate, the reported enhancement ratio was relatively low (1.63). This could be due to the low concentration of CTAB (0.2 % w/v) used.

Both anionic and cationic surfactants have the potential to swell the SC and interact with intercellular keratin (Williams and Barry 2004). It should be kept in mind that both anionic and cationic surfactants have the disadvantage of damaging human skin (Cooper 1984; Gershbein 1979; Farber et al. 1983).

13.3.4 Nonionic Surfactants

Nonionic surfactants are surfactants in which the hydrophilic group is of non-dissociable type, resulting in uncharged polar head group (Fig. 13.8).

These surfactants could be considered as relatively safe. It seems that the skin absorption of some of these surfactants are not high which

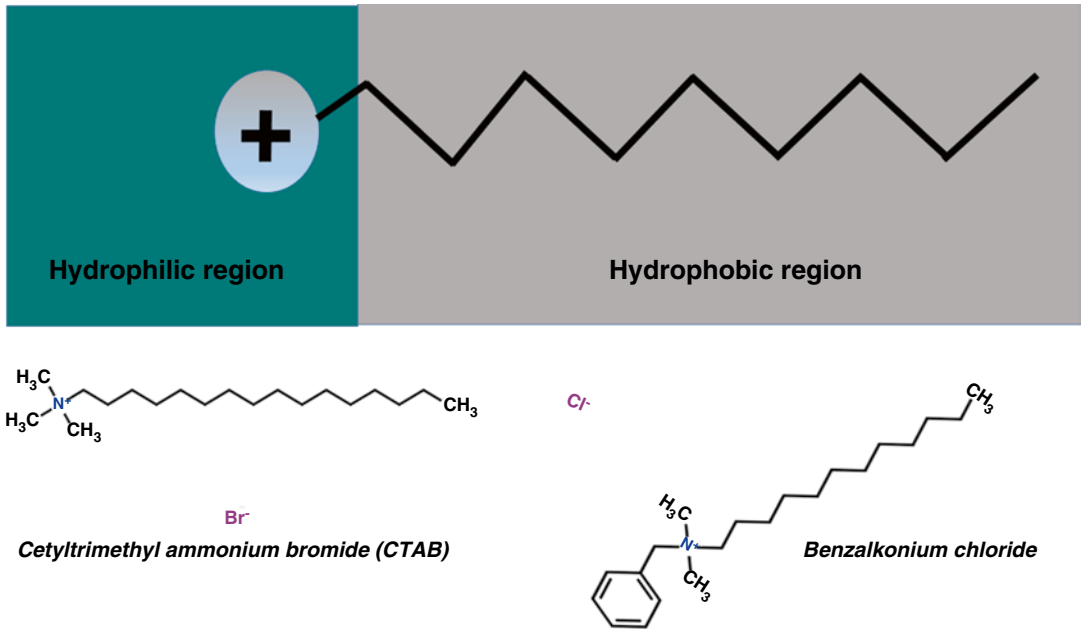


Fig. 13.7 Schematic representation of a cationic surfactant and chemical structures of two commonly used cationic surfactants

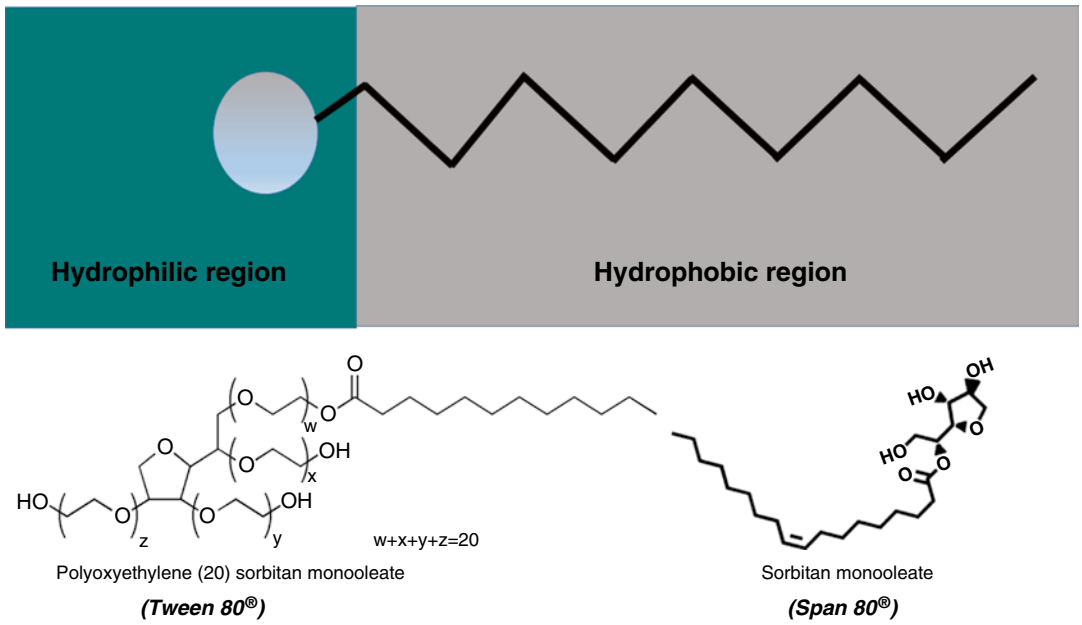


Fig. 13.8 Schematic representation of nonionic surfactants and chemical structures of two representatives

reduces the safety risk. It has been shown that only ~0.5 % of the applied dose of the nonionic surfactant, nonoxynol, crossed human skin in an in vitro skin permeation study (Watkinson et al. 1998). Also, in comparison to anionic and cationic surfactants, nonionic surfactants cause less

irritation and are better tolerated (Effendy and Maibach 2006; Bergh 1999).

Nonionic surfactants could be considered as the most frequently used surfactants in drug delivery (Som et al. 2012). Nonionic surfactants demonstrate improved drug penetration through the skin; however, such effect is dependent on the physical state and concentration of the surfactant (Walters and Hadgraft 1993). Generally, nonionic surfactants show only limited improvement in drug delivery to the skin in comparison with anionic surfactants (Williams and Barry 2004). It has been reported that nonionic surfactants could affect the skin barrier function (Lopez et al. 2000b; Fang et al. 2001) with mechanisms discussed in detail later in this chapter.

Polysorbates (polyoxyethylene (*n*) sorbitan monooleate) (see Fig. 13.8) are safe nonionic surfactants, which provide considerable increase in drug permeation through the skin. Drugs such as salicylic acid and sodium salicylate (Shen et al. 1976), diazepam (Shokri et al. 2001), lorazepam (Nokhodchi et al. 2003), hydrocortisone (Sarpotdar and Zatz 1986a), flufenamic acid (Hwang 1983), tenoxicam (Endo et al. 1996), diclofenac diethylamin (Mukherjee et al. 2005), piroxicam (Shin et al. 2001), lidocaine (Sarpotdar and Zatz 1986b), diltiazem HCl (Limpongsa and Umprayn 2008) and ibuprofen (Park et al. 2000) and alcohols, ethanol and octanol (Cappel and Kreuter 1991) are examples of chemicals with increased transdermal absorption following the incorporation of polysorbates. There are also other nonionic surfactants such as Brij 36T, Brij 35 and Pluronic 127 that have been used to enhance the permeation of drugs such as methyl nicotinate (Ashton et al. 1992) and atenolol (Bhaskaran and Harsha 2000) through the skin.

Nonionic surfactants have been used in the preparation of niosomes and proniosomes aimed for transdermal drug delivery. These formulations are discussed below.

13.3.4.1 Niosomes

Niosomes (nonionic surfactant vesicles) are prepared by encapsulation of drugs into a vesicle derived from nonionic surfactants, such as Tween and Span series (Schreier and Bouwstra 1994). Schematic representation of a typical niosome is shown in Fig. 13.9. Niosomes have been used for topical skin delivery of biopharmaceuticals such as encapsulated DNA-encoding hepatitis B surface antigen (HBsAg) (Vyas et al. 2005), tetanus toxoid (Gupta et al. 2005) and insulin (Pardakhty et al. 2007). For instance, the topical niosomes containing recombinant HBsAg produce a comparable serum antibody titre and endogenous cytokine levels as the intramuscular and topical liposome formulations, and therefore, it can be used as an effective topical immunisation system (Vyas et al. 2005). The transdermal application of niosomes to deliver small molecule drugs such as methotrexate (Lakshmi et al. 2007), clobetasol propionate (Lingan et al. 2011), minoxidil (Mura et al. 2007), terbinafine HCl (Sathali and Rajalakshmi 2010), nimesulide (Shahiwala and Misra 2002), lopinavir (Patel et al. 2012) and chlorpheniramine maleate (Varshosaz et al. 2005) was also reported. Generally, niosomes show in vivo performance similar to liposomes (Azmin et al. 1985; Rogerson et al. 1988; Hofland et al. 1994). However, in comparison to liposomes, niosomes are more attractive due to their better chemical stability and lower cost (Hu and Rhodes 1999). Niosomes are able to carry amphiphilic, hydrophilic and lipophilic drugs.

13.3.4.2 Proniosomes

Proniosomes were proposed and designed in an attempt to overcome the stability issues of niosomes without compromising their properties. Proniosomes (Fig. 13.9) are the dry state of niosomes which can be hydrated immediately before their use to produce aqueous niosome dispersions (Hu and Rhodes 1999). They can overcome problems such as aggregation, fusion and leaking of chemicals from niosomes. Furthermore, proniosomes can provide additional convenience in transportation, distribution, storage, dosing and skin absorption (Shamsheer et al. 2011). The topical application of proniosomes and niosomes has been extensively reviewed and compared (Mahale et al. 2012; Kumar and Rajeshwarrao 2011). Drug-loaded proniosomes have found their application in different therapies including the treatment of hypertension using proniosomal gel containing perindopril (Kute et al. 2012),

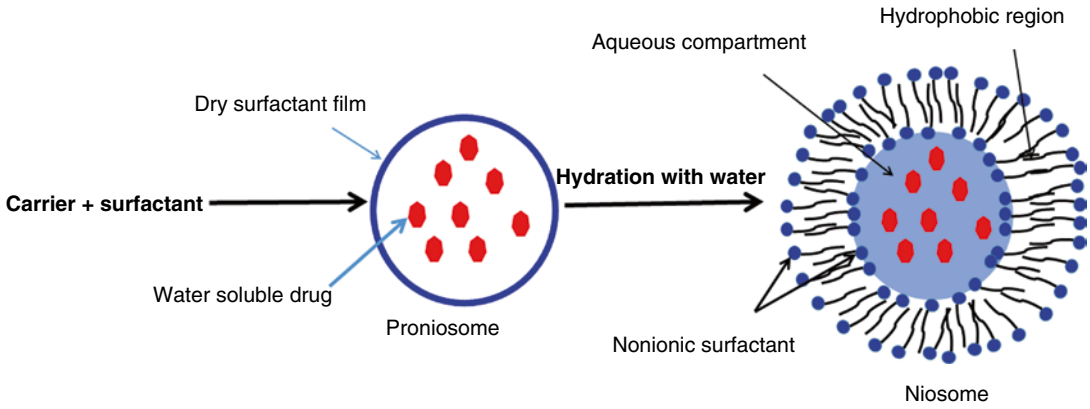


Fig. 13.9 Schematic representation of a proniosome and a niosome

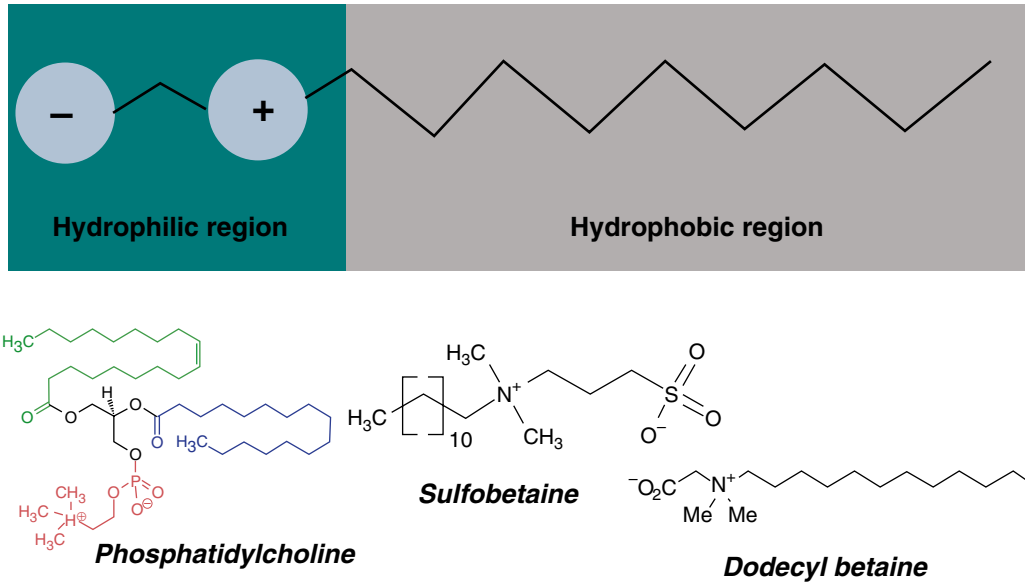


Fig. 13.10 Schematic representation of amphoteric surfactants and chemical structures of two synthetic amphoteric surfactants and of phosphatidylcholine (a type of phospholipid)

inflammation and pain using meloxicam (El-Menshawe and Hussein 2013) and hormonal insufficiencies using estradiol (Fang et al. 2001).

13.3.5 Amphoteric (Zwitterionic) Surfactants

Zwitterionic surfactants are surfactants in which a single molecule shows both anionic and cationic dissociations depending on the pH of the environment they are placed (Fig. 13.10). The positive charge is carried generally by an ammonium group, and the negative charge is often a carboxylate. Common examples are dodecyl betaine and sulphobetaine (Fig. 13.10).

Amphoteric surfactants are less frequent in comparison to anionic, cationic and nonionic surfactants (Som et al. 2012). Amphoteric surfactants include synthetic products such as dodecyl betaines, alkyl sulphobetaines and natural products such as phospholipids. Phospholipids such

as phosphatidylcholine can increase drug penetration by increasing tissue hydration (Kogan and Garti 2006) and interaction with the lipids of the stratum corneum (Kirjavainen et al. 1999). It has been shown that lecithin (which is mainly composed of phospholipids) can enhance the in vitro permeation of flavonoids to a significant extent and the photoprotective activity of flavonoids in human volunteers (Saija et al. 1998).

13.4 Comparison of Surfactants as Penetration Enhancers

Surfactants may solubilise both the lipophilic drug within the formulation and the lipids within the SC (Williams and Barry 2004). Various types of surfactants have been used in topical formulations with a special interest in nonionic surfactants due to the lowest toxicity and skin irritation (Walters 1990). All types of surfactants lead to skin protein denaturation and this may follow the order: anionics > cationics > zwitterionics > amine oxides > polyoxyethylene (POE) nonionics (Miyazawa et al. 1984; Rhein et al. 1986). The skin irritancy of anionics can be diminished by the addition of positively charged materials such as protein hydrolysates (Rosen and Kunjappu 2012). In general, reducing CMC will result in reduced concentration of monomeric anionic surfactant (Hall-Manning et al. 1998) and hence the skin irritation, which is mostly associated with the concentration of monomeric surfactant in the aqueous phase (O'Lenick 2005). In agreement with this hypothesis, it has been observed that addition of polymers that reduce CMC can reduce the skin irritation of anionic surfactants (Mannheimer 1961; Fevola et al. 2010).

It has been suggested that ionic surfactants exert greater effects on the flux of compounds than nonionic surfactants. For example, the in vitro penetration of methyl nicotinate through human skin is increased more by CTAB and SLS (ionic surfactants) than by Brij 36T® (a nonionic surfactant) (Ashton et al. 1992). These results showed that the onset of action is faster with the nonionic surfactants compared to ionic surfactants, which are due to the slow penetration of ionic surfactants into the skin. This supports the hypothesis that in order to change the permeability of SC, surfactants must first penetrate into the skin to elicit any changes in the barrier function (Ashton et al. 1992). Nonionic nonoxynol surfactants (Watkinson et al. 1998) and anionic SLS (Loden 1990) have been shown to permeate skin in small quantities (less than 0.5 % and 3 %, respectively, within 48 h). Higher permeation enhancement by ionic surfactants could be explained by the reports that ionic surfactants can cause extensive damage to the skin and hence cause a large increase in transdermal drug flux (Gershbein 1979). According to Farber et al. (1983) and Cooper (1984), in general cationic surfactants are more damaging and cause a greater increase in the drug flux than anionic surfactants, and anionic surfactants cause greater enhancement and damage than nonionic surfactants. Nonionic surfactants such as Brij 36T® cause relatively little damage to the skin, and their effect on transdermal flux is small (Hwang and Danti 1983). In case of nonionic surfactants, some examples of the most widely used surfactants that are used for improving skin penetration of drugs have been listed in Table 13.1.

Dalvi and Zatz (1981) found that skin permeability was not increased by some nonionic surfactants in purely aqueous media. However, Shahi and Zatz (1978) did report that Tween® 80 was responsible for enhancement of hydrocortisone penetration from isopropyl alcohol:water mixtures. Therefore, it is apparent that the nature of the medium can influence the interaction between nonionic surfactants and the skin barrier (Sarpotdar and Zatz 1986b). Further investigations employing lidocaine solutions in propylene glycol-water vehicles supported this assumption (Sarpotdar and Zatz 1986a). It has been shown that at concentrations of 0.5 and 1 % Tween® 80 increased the skin penetration of chloramphenicol 1.10- and 1.54-fold, respectively, from water (Aguiar and Weiner 1969).

Amongst nonionic surfactants, polyoxyethylene alkyl ethers and esters have been shown to be more effective enhancers of permeation than polysorbates (Walters 1990). The activity of surfactants as penetration enhancers is dependent on

Table 13.1 Examples of the most commonly used nonionic surfactants used as skin enhancer

Group	Chemical name	Trade name	HLB	Example application as transdermal permeation enhancer[a]
Polysorbates	Polysorbates polyoxyethylene (20) sorbitant monolaurate	Tween 20®	16.7	Alfuzosin HCl permeation was increased 2.84- and 3.85-fold at the enhancer concentrations of 1 and 2 %, respectively (Prasanthi and Lakshmi 2012) The highest ER value of 5.08 was reported for ascorbic acid when the concentration of the enhancer was 5 % (Akhtar et al. 2011)
	Polyoxyethylene (20) sorbitant monopalmitate	Tween 40®	15.6	ER value of 6.44 was reported for captopril when 5 % of the surfactant was used (Wu et al. 1996)
	Polyoxyethylene (20) sorbitant monostearate	Tween 60®	14.9	ER value of 5.10 was reported for captopril when 5 % of the surfactant was used (Wu et al. 1996)
	Polyoxyethylene (20) sorbitant monooleate	Tween 80®	15.0	The ER values of 3.75 and 5.68 were reported for lorazepam (Nokhodchi et al. 2003) and diazepam (Shokri et al. 2001) at the surfactant concentration of 1 %. The highest ER of 15.22 was reported for captopril via rabbit skin when 5 % surfactant was used (Wu et al. 1996)
Sorbitan esters	Sorbitan monolaurate	Span 20®	8.6	ER value of 4.39 was observed for diclofenac sodium at the surfactant concentration of 3 % (Arellano et al. 1998) ER value of 6.5 was reported for sumatriptan succinate at 5 % concentration (Femenia-Font et al. 2005)
	Sorbitan monopalmitate	Span 40®	6.7	The presence of this surfactant caused an increase in ER value of estradiol to 1.35 when it was used in proniosomes (the amount of surfactant was 90 mg) (Fang et al. 2001)
	Sorbitan monostearate	Span 60®	4.7	The surfactant increased the penetration of estradiol from proniosome formulations with ER value of 2.12 (the amount of surfactant was 90 mg) (Fang et al. 2001)
	Sorbitan monooleate	Span 80®	4.3	ER value of 4.10 was observed for diclofenac sodium at the surfactant concentration of 3 % (Arellano et al. 1998)
Polyoxyethylene alkyl ethers	Polyoxyethylene (4) lauryl ether	Brij 30®	9.7	ER value of 1.58 was observed for ibuprofen at the surfactant concentration of 15 % (Park et al. 2000) ER value of 4.46 was reported for aceclofenac when the concentration of surfactant was 2 %(Mohamad and Jan 2012)
	Polyoxyethylene (23) lauryl ether	Brij 35®	16.9	ER was 1.27 for mepivacaine permeation (Cho et al. 2011)
	Polyoxyethylene (10) lauryl ether	Brij 36T®		Permeation of methyl nicotinate in presence of 1.5 % Brij 35T increased by 92 % from human skin (Ashton et al. 1992)
	Polyoxyethylene (2) cetyl ether	Brij 52®	5.3	No significant increase in the permeation of ibuprofen was reported (Park et al. 2000)
	Polyoxyethylene (20) cetyl ether	Brij 58®	15.7	ER value of 6.83 was reported for aceclofenac when the concentration of surfactant was 2 % (Mohamad and Jan 2012)
	Polyoxyethylene (2) stearyl ether	Brij 72®	4.9	ER value of 1.61 was observed for ibuprofen at the surfactant concentration of 15 % (Park et al. 2000) ER value for prilocaine was 1.57 (Kang and Shin 2012)
	Polyoxyethylene (20) oleyl ether	Brij 98®	15.3	ER value of 2.78 was observed for ibuprofen at the surfactant concentration of 15 % (Park et al. 2000)

[a]ER is the enhancement ratio

the chemical structure, and both the hydrophobic alkyl chain and the hydrophilic region are significant (Walters et al. 1988). For instance, Walters et al. (1988) have studied the effect of 15 surfactants on skin absorption of methyl nicotinate and showed that both the hydrophobic alkyl chain and the hydrophilic ethylene oxide chain have significant effect on the rate of methyl nicotinate transport across hairless mouse skin. They showed that the permeability of the drug from aqueous solution was increased 100 % when polyoxyethylene (10) lauryl ether (Brij 36T®) was used, but the permeation rate of the drug was unaffected when polyoxyethylene (10) isostearyl ether was incorporated in the aqueous solution. They concluded that nonionic surfactants with greater than eight carbons and an ethylene oxide chain less than 14 are able to increase the skin permeation of the drug, whereas the branched surfactants are ineffective.

Several studies have used quantitative methods to relate the effect of molecular structural properties of the penetration enhancers to the penetration enhancement effects (Ghafourian et al. 2004; Kalhapure et al. 2012). These quantitative structure activity relationships (QSARs) have indicated that the optimum enhancer property is different depending on the properties of the permeating drug (Ghafourian et al. 2004). More precisely, it was shown that for the relatively hydrophilic drugs, 5-fluorouracil and diclofenac sodium, less lipophilic enhancers were the most active, and QSAR indicated the possible involvement of intermolecular electron donor-acceptor interactions either with skin components or between the enhancer and the permeating drug. This was in contrast to the skin permeation promotion of more lipophilic drugs, such as hydrocortisone, estradiol and benazepril by enhancers, where a linear relationship between enhancement activity and n-octanol/water partition coefficients of the enhancers was evident (Ghafourian et al. 2004). Kang et al. (2007) compared the skin penetration-enhancing ability of 49 terpenes and terpenoids on the in vitro permeability coefficient of haloperidol through excised human skin. They concluded that an ideal terpene enhancer for haloperidol should possess at least one or combinations of the following properties: hydrophobicity, liquid state at room temperature, possessing an ester or aldehyde but not carboxylic acid functional group and neither a triterpene nor tetraterpene.

Although nonionic surfactants can improve the penetration of substances through biological or skin membranes, several studies indicate that this enhancement may be dependent on the nature of the permeating drug and the model membrane used in their studies (Chowhan and Pritchard 1978; Goodman and Barry 1989; Rigg and Barry 1990). For example, no significant enhancement of 5-flourouracil absorption was observed following Tween® 20 treatment of human and snake skin, while for mouse skin, the permeation increased significantly when the same formulation of 5-flourouracil was used (Rigg and Barry 1990). In another study, the Wistar rat skin was treated with different concentrations of Tween® 20 (1 and 5 % w/v in ethanolic solution) to enhance the skin penetration of 5-fluorouracil and antipyrine (Lopez et al. 2000b). Tween® 20 was not effective when used at 1 % concentration; however, it increased skin permeation of 5-flurouracil at 5 % concentration. Both concentrations of Tween® 20 were ineffective in enhancing the absorption of antipyrine. Tween® 20 has also been shown to improve the absorption of hydrocortisone and lidocaine through mouse skin (Sarpotdar and Zatz 1986a, b), but it did not enhance the penetration of naloxone (Aungst et al. 1986). It is obvious from the above information that there are conflicting results on the enhancing effect of surfactants on the penetration of drugs through various skin types. This also depends on the nature of the permeating drug and the solvent type.

13.5 Proposed Mechanisms of Penetration Enhancement

Any increase in the transdermal absorption of compounds may be due to the increased diffusivity within the SC or the increased partitioning of compounds between the SC and the formulation medium. Surfactants may interact with skin

components or alter the saturation state of the drug within the formulation. Several mechanisms have been suggested to explain the interaction between surfactants and the skin, and the resulting alteration of the skin permeability characteristics. Some of these suggested mechanisms are summarised below.

In order to change the barrier property of the SC, surfactants need to penetrate this membrane first. Surfactants may interact with protein components and denature the SC proteins, or they may interact with the lipid compartment and increase its fluidity (Barel et al. 2009; Rhein et al. 1986). One hypothesis about the mechanism of penetration enhancement by anionic surfactants is that these surfactants, for example, alkyl sulphates, bind to epidermal proteins (Breuer 1979) and this leads to increased anionic sites in the membrane (Rhein et al. 1986) which may in turn increase the hydration level (Walters and Hadgraft 1993) and therefore permeation rate of the drug.

In a recent thermodynamic study of the hydrated skin samples without surfactants and in the presence of sodium dodecyl sulphate (anionic), cetyl trimethyl ammonium chloride (cationic) and, the nonionic surfactant, lauryl alcohol ethoxylated (12 mol ethylene oxide) (LAE-12OE), differential scanning calorimetric results indicated that the anionic surfactant promoted an elevation of the water content of the tissue, while the cationic surfactant dehydrated the skin and the LAE-12OE provided a non-significant interaction with the skin samples (Baby et al. 2006). In an investigation using sodium salts of n-alkyl sulphates with variable carbon chain length ($n = 8–14$), it was observed that the anionic surfactants promote SC hydration in vitro and in vivo in healthy adult human volunteers (Wilhelm et al. 1993). Although the permeation of surfactants was not measured, it was shown that the hydration was closely correlated with the irritation potential of the investigated compounds. The hydration and irritation initially increased with increasing carbon chain length, and the maximum response was obtained for the C12 analogue (sodium lauryl sulphate) (Wilhelm et al. 1993). It has been suggested that within a specific class of surfactants, the extent of swelling largely corresponds to the known irritancy of these surfactants, but swelling may not be the mechanism of irritancy by all surfactant classes (Rhein et al. 1986). This is because irritation is more complex than just penetration and involves an additional cascade of events after the penetration of surfactant.

Interaction of surfactants with the SC proteins as the main mechanism of penetration enhancement has been challenged recently, by several studies indicating minimal change in the relevant region of the FTIR spectra of stratum corneum samples (Walters et al. 2012). Secondary structure of proteins in the SC is commonly monitored with amide I and amide II band contours between 1480 and 1730 cm^{-1} in FTIR spectra (Mendelsohn et al. 2006). After SLS treatment, only minor changes are observed in this spectral region, demonstrating minimal effect of SLS on keratin structure in the SC (Saad et al. 2012; Mao et al. 2012).

Surfactants are believed to intercalate into lipid bilayers of the SC (Almeida et al. 2010) resulting in interfacial defects and structure disruption providing diffusional paths for applied drug molecules (Hadgraft 2001). Some evidence into this has been provided from membrane fluidity studies following surfactant interactions with erythrocyte lipid bilayer (Martinez et al. 2007). Fluorescent anisotropy measurements using specific probes of known locations within the lipid bilayer indicated alterations in the membrane fluidity on the external (but not internal) region of the membrane by several cationic and anionic amino acid-based surfactants. The surfactants chain lengths were relatively short, between 8 and 14, which may explain the lack of deep incorporation into the membrane bilayer of the SC (Martinez et al. 2007).

Many publications concerning the effect of surfactants on biological membrane permeability describe a concentration-dependent behaviour showing an increase in permeability at low surfactant concentrations, which decreases at higher concentrations. Studies on the effect of surfactant concentrations provide interesting mechanistic insight. In one such investigation, the effect of several surfactant concentrations was investi-

gated on the penetration of diazepam (Shokri et al. 2001) and lorazepam (Nokhodchi et al. 2003) through rat skin in vitro. The surfactants included Tween® 80 (nonionic), SLS (anionic), CTAB (cationic) and benzalkonium chloride (cationic). The plots of enhancement ratios against concentration have been presented in Fig. 13.11.

Figure 13.11 shows that in most surfactant concentrations, the enhancement ratio is above 1 (enhancement effect of all surfactants). It can be seen that enhancement ratio for both drugs increases with surfactant concentration, but for most surfactants, especially the nonionic surfactant (Tween® 80), a reduction in enhancement ratio can be observed at higher concentrations and a concentration optimum is seen. Similar observation has been made with different concentrations of several terpenes as the enhancers of diclofenac sodium (Nokhodchi et al. 2007). This reduction seen in Fig. 13.11 in the enhancement ratio of surfactants (at about 2.5 %) was attributed to the ability of the surfactant to form micelles and the possibility of interaction between micelles and the drugs (Shokri et al. 2001; Nokhodchi et al. 2003). At concentrations below CMC, surfactant molecules exist as monomers, which are able to penetrate the SC and change the barrier property of this membrane. Solubilisation of the drug molecules by surfactant micelles may decrease the thermodynamic activity of the drug and, hence, the driving force for the drug absorption. Therefore, the overall effect of a surfactant on the rate of drug permeation across a membrane will be a combination of the influence of these two opposing effects, the enhancing effect of the surfactant monomers and the solubilising effect of surfactant micelles (Walters and Hadgraft 1993; Nokhodchi et al. 2003).

Interestingly, Fig. 13.11 shows that, in case of ionic surfactants, as the concentration of surfactants increases from 2.5 to 5 % w/w the enhancement ratios increase. According to the widely accepted hypothesis, at concentrations above the CMC of surfactants, the flux of the drug should not increase further as surfactants are believed to be absorbed as monomers and hence exert their effect as monomers (Faucher and Goddard 1978; Loden 1990). However, it has been observed for SLS that, at concentrations that exceed the CMC, the concentration of the surfactant within the epidermis increases with the increase in the total surfactant concentration in the solution contacting the skin (Moore et al. 2003a). This observation may indicate that micellar SLS contributes to drug penetration into the epidermis (Moore, et al. 2003b), or on the other hand, the sub-micellar aggregates may be responsible for the increased drug absorption at higher concentrations than CMC concentrations (James-Smith et al. 2011). Sub-micellar aggregates are aggregates of surfactant in solution containing fewer monomers than the aggregation number of the micelle, e.g.

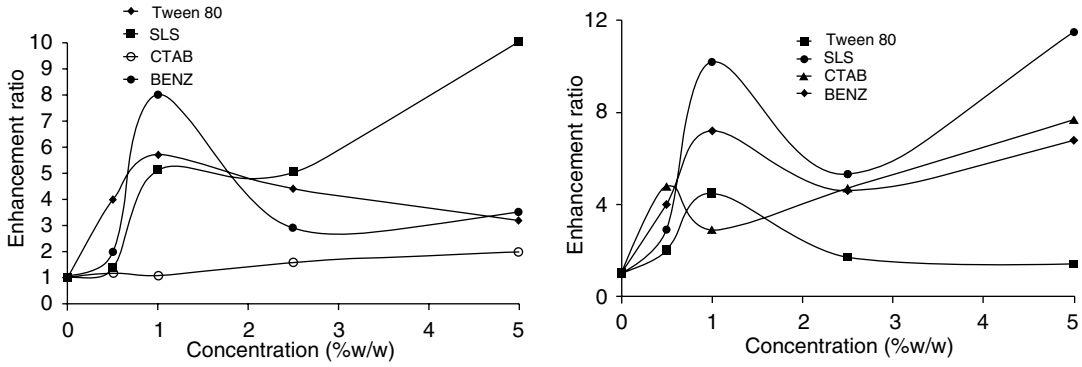


Fig. 13.11 Effect of various surfactants used at different concentrations on skin penetration of lorazepam (*right*) and diazepam (*left*); in the labels Tween® is Tween 80® (The *left* and *right* figures were taken from Shokri et al. (2001) and Nokhodchi et al. (2003) with permission from Elsevier)

dimers, trimers and other multimers (James-Smith et al. 2007). Using ultrafiltration, the concentration of monomers and sub-micellar aggregates has been determined and correlated well with skin SLS concentration (James-Smith et al. 2011). Other investigations have also shown a dose-dependent damage to the skin by surfactants at concentrations above the CMC (Loden 1990). This could be a reason for the increase in drug flux at higher concentrations of ionic surfactants.

13.6 Synergistic Effects of Surfactants and Physical Methods

Apart from the use of surfactants, there are several other techniques to enhance the transdermal delivery of drugs including physical methods such as electroporation, sonophoresis and microneedles; chemical methods such as the use of prodrugs; and different salt forms and ion pairs. Synergistic effects of a combination of chemical enhancers and other enhancing techniques have been investigated in order to increase the transdermal absorption of drugs to the therapeutic levels. The main benefit of using a combination of two skin absorption enhancement techniques is that most surfactants cannot be used in high concentrations due to safety issues. By combining with a second technique, it is possible to reduce the surfactant concentration and yet achieve the desired optimal absorption levels. Combinations of chemical enhancers and iontophoresis (Singh and Singh 1995; Bhatia et al. 1997; Oh et al. 1998; Wearley and Chien 1990; Bhatia et al. 1997), chemicals enhancers and electroporation (Wang et al. 1998; Weaver et al. 1997) and chemical enhancers and ultrasound (Johnson et al. 1996; Mitragotri et al. 2000) have been investigated. It has been shown that the use of chemical enhancers combined with iontophoresis is a promising approach to enhance the permeation of various drugs across the skin, an example being the study conducted by Oh et al. (1998) on transdermal absorption of zidovudine. Mitragotri (2000b) has extensively reviewed the synergistic effects of chemical enhancers in combination with other techniques. Most of the published data shows that the concentration of chemical enhancers can be decreased without compromising their effectiveness on the adequate delivery of drugs across the skin. Pretreatment with surfactants prior to iontophoresis can significantly improve drug bioavailability through human skin, as shown for laureth-3 ethyloxylene ether, laureth-7 ethyloxylene ether and sodium sulphosuccinate (Li et al. 2005a). A drawback of this method is the need for the equipment and also the cutaneous side effects of iontophoresis such as transient mild skin irritation (erythema and oedema) that is increased with surfactant pretreatment (Li et al. 2005b). Recently, Silva et al. (2011) applied three nonionic ether-monohydroxyl surfactants with or without iontophoresis to enhance the skin delivery of ondansetron hydrochloride and diltiazem hydrochloride. They showed that the enhancing effect of various nonionic surfactants depended on the drug used (see Table 13.2). It was observed that the combined use of the surfactants and iontophoresis profoundly increased the flux value. For example, the highest enhancement ratio of 420 for ondansetron hydrochloride was observed for glycol monododecyl ether combined with iontophoresis compared to passive drug permeation without enhancer pretreatment, while in case of diltiazem hydrochloride, octaethylene glycol monododecyl ether produced the highest enhancement ratio of 200. None of the surfactants caused any significant skin damage indicating their suitability for transdermal formulations.

Combined with electroporation, the presence of surfactants often leads to significantly higher transdermal absorption of drugs. For example, it has been shown that SLS improves the efficiency of transdermal delivery of glucose and dextrans of molecular weight 4 and 10 kDa in combination with electroporation (Murthy et al. 2004). In electroporation, short high-voltage pulses are applied on the skin which create aqueous pathways across the SC. Dynamic electrical resistance studies of the skin and the fluorescent imaging of the so-called localised

Table 13.2 Effect of nonionic surfactants alone and in combination with iontophoresis (0.3 mA for 8 h)

Enhancer	Diltiazem HCl		Ondansetron HCl	
	$Flux_{surf}$ (μg cm^{-2} h^{-1})	$Flux_{surf + ionto}$ (μg cm^{-2} h^{-1})	$Flux_{surf}$ (μg cm^{-2} h^{-1})	$Flux_{surf + ionto}$ (μg cm^{-2} h^{-1})
Control (drug-loaded hydrogel only)	0.71 ± 0.26	123.24 ± 12.7	0.043±0.14	16.35 ± 3.46
Propylene glycol (PG)	0.55 ± 0.06	102.09 ± 12.5	0.41 ± 0.27	14.57 ± 2.78
Ethylene glycol monododecyl ether in PG	3.98 ± 1.72	166.25 ± 33.9	1.12 ± 0.21	22.66 ± 3.76
Pentaethylene glycol monododecyl ether in PG	6.68 ± 1.11	181.58 ± 24.7	4.59 ± 0.78	20.79 ± 3.20
Octaethylene glycol monododecyl ether in PG	3.57 ± 0.90	199.00 ± 25.6	2.00 ± 0.69	15.24 ± 3.36

Concentration of the surfactants was 0.16 M

transport regions indicated that surfactants can prolong the lifetime of these aqueous pathways (Jiang et al. 2007). SLS was more effective than Tween® 80 in prolonging the time of recovery of the skin resistance. The effect of both surfactants on transdermal permeation was concentration dependent and increased sharply with increasing the concentration but slacked off beyond their CMC (Jiang et al. 2007). It appears that the presence of SLS during electroporation helps in achieving the desired drug transport with a lower electrical exposure dose (Murthy et al. 2004), due to facilitating the SC barrier disruption during pulse application and also due to prolonging the lifetime of electropores created by the pulse.

Surfactants can also be combined with low-frequency ultrasound which leads to the formation of localised transport regions in the skin being more permeable than the surrounding regions of the skin (Kushner et al. 2004). It has been demonstrated that the incorporation of SLS during sonophoresis leads to enhanced skin perturbation; therefore, desired level of skin perturbation can be achieved by shorter ultrasound exposure time (Kushner et al. 2007). The combination of SLS and sonophoresis has been shown to be an effective transdermal delivery method for hydrophilic permeants, urea, mannitol, raffinose and inulin (Kushner et al. 2008) and hydrophilic macromolecules such as gold nanoparticles and quantum dots (Seto et al. 2010).

13.7 Surfactant Mixtures

Although the penetration enhancement can be achieved by using one surfactant alone, the enhancement is often accompanied by irritation as discussed earlier (Kanikkannan and Singh 2002; Rhein et al. 1990), thus restricting the surfactant application as a penetration enhancer. One approach is to use surfactant mixtures as a potential solution to decouple the enhancement and irritation effects (James-Smith et al. 2011). It has been shown that the mixtures of surfactants can be used to improve the potency of topical formulations without elevating the skin irritation (Karande et al. 2007). Mixing surfactants often leads to reduced denaturation of the SC keratin (Rhein et al. 1990). The use of surfactant mixtures allows the surfactants to control their interactions with the skin through the formation of micellar structures (Fevola et al. 2008; Ghosh and Blankschtein 2008; Moore et al. 2003a; Walters et al. 2008). In a recent study, Moore et al. (2003b) reported that addition of the nonionic surfactant, dodecyl hexa (ethylene oxide) ($C_{12}E_6$), with SLS reduced the amount of SLS penetration into the epidermis. Based on the observation that the skin concentration of SLS increased beyond its CMC, they suggested that, as well as monomers, SLS micelles may be able to penetrate into the skin. Using dynamic light-scattering experiments, the hydrodynamic radii of the SLS/$C_{12}E_6$ micelles were determined to be increasing with increasing the concentration of

$C_{12}E_6$. They suggested that this may account for the reduced penetration of the mixed micelles in comparison with pure SLS micelles with the latter having a smaller size than the typical SC aqueous pore radii reported in the literature. This is in accordance with the hypothesis suggesting the penetration of intact lipid vesicles into the skin (Cevc and Blume 2001, 2003, 2004).

Alternatively, the irritancy reduction of SLS solution in the presence of nonionic surfactant could be due to a reduction in CMC value of SLS, and this in turn can reduce the number of the monomer concentration of SLS in the solution. Recent findings by James-Smith et al. (2011) suggested that the presence of sub-micellar aggregates in the solution plays an important role in the penetration of surfactants into the skin. Generally, when the micelles are more stable, then the concentration of sub-micellar aggregates should be less. This in turn can reduce the penetration enhancement effect of the surfactant. James-Smith et al. (2011) investigated the effect of mixtures of a cationic surfactant, CTAB, and the anionic surfactant, SLS. The measured skin properties including skin conductivity, perturbation of lipid structure and skin concentration of SLS correlated with the concentration of monomers and sub-micellar aggregates and not with the total SLS concentration in the donor compartment. They showed that addition of the cationic surfactant, CTAB, reduced the concentration of monomers and sub-micellar SLS in the donor phase and the perturbation of skin's barrier properties due to SLS.

Overall, these studies indicate that due to the synergistic effects when using combinations of surfactants, it is possible to use lower concentrations of the surfactants compared with when using only one surfactant, without decreasing the effectiveness in terms of the skin permeation enhancement. Further, as lower concentrations of different surfactants are used, the irritancy potential is decreased.

Conclusion

Use of surfactants in transdermal drug delivery is a common practice due to the advantages that this technique offers in terms of the permeation enhancement potential, good safety record of many surfactants and the synergism that is often observed with physical and other chemical methods of permeation enhancement. Nonionic surfactants are the safest and most widely used surfactants for the skin absorption enhancements, but these usually are the least effective enhancers in comparison with the anionic and cationic surfactants. However, in general, the enhancement potential depends not only on the nature of the surfactant's lipophilic and hydrophilic nature but also on the nature of the permeating drug, type of the vehicle in the formulation and also the skin sample source from different animal species. It is recommended to use surfactants in combination with another physical or chemical method in order to achieve the required amount of drug absorption with lower surfactant concentration and/or reduced dose of the second physical or chemical method, e.g. shorter ultrasound exposure time or lower combination surfactant concentration. The anionic surfactant SLS is one of the most studied surfactants as penetration enhancer and also in mechanistic studies. Studies using different concentrations of SLS, use of SLS in different solvents, combination of SLS and other surfactants or physical methods have increased the scientific knowledge with regard to the mechanisms involved in the transdermal permeation enhancement by surfactants.

References

Abdel Rahman A, Khidr S, Ahmed S, Aboutaleb A (1991) Evaluation of chloramphenicol-β-cyclodextrin inclusion complex. Eur J Pharm Biopharm 37:34–37

Aguiar AJ, Weiner MA (1969) Percutaneous absorption studies of chloramphenicol solutions. J Pharm Sci 58:210–215

Akhtar N, Rehman MU, Khan HMS, Rasool F, Saeed T, Murteza G (2011) Penetration enhancing of polysorbate 20 and 80 on the in vitro percutaneous absorption of L-Ascorbic acid. Trop J Pharm Res 10:281–288

Almeida JA, Marques EF, Jurado AS, Pais AA (2010) The effect of cationic gemini surfactants upon lipid membranes. An experimental and molecular dynamics

simulation study. Phys Chem Chem Phys 12:14462–14476
Anderson D, Wennerstrom H, Olsson U (1989) Isotropic bicontinuous solutions in surfactant-solvent systems: the L_3 phase. J Phys Chem 93:4243–4253
Arellano A, Santoyo S, Martn C, Ygartua P (1998) Surfactant effects on the in vitro percutaneous absorption of diclofenac sodium. Eur J Drug Metab Pharmacokinet 23:307–312
Arima H, Adachi H, Irie T, Uekama K (1990) Improved drug delivery through the skin by hydrophilic β-cyclodextrins. Drug Invest 2:155–161
Arima H, Miyaji T, Irie T, Hirayama F, Uekama K (1998) Enhancing effect of hydroxypropyl-β-cyclodextrin on cutaneous penetration and activation of ethyl 4-biphenylyl acetate in hairless mouse skin. Eur J Pharm Sci 6:53–59
Ashton P, Walters KA, Brain KR, Hadgraft J (1992) Surfactant effects in percutaneous absorption I. Effects on the transdermal flux of methyl nicotinate. Int J Pharm 87:261–264
Aungst, B. J., Rogers, N. J., & Shefter, E. (1986). Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactants, sulfoxides and amides. International journal of pharmaceutics, 33(1), 225–234
Ayala-Bravo HA, Quintanar-Guerrero D, Naik A, Kalia YN, Cornejo-Bravo JM, Ganem-Quintanar A (2003) Effects of sucrose oleate and sucrose laureate on in vivo human stratum corneum permeability. Pharm Res 20:1267–1273
Azmin M, Florence A, Handjani-Vila R, Stuart J, Vanlerberghe G, Whittaker J (1985) The effect of nonionic surfactant vesicle (niosome) entrapment on the absorption and distribution of methotrexate in mice. J Pharm Pharmacol 37:237–242
Baby AR, Lacerda AC, Velasco MV, Lopes PS, Kawano Y, Kaneko TM (2006) Evaluation of the interaction of surfactants with stratum corneum model membrane from Bothrops Jararaca by DSC. Int J Pharm 317:7–9
Baker H (1972) The skin as a barrier. In: Rock A (ed) Textbook of dermatology. Blackwell Scientific, Oxford, pp 249–255
Bardi L, Mattei A, Steffan S, Marzona M (2000) Hydrocarbon degradation by a soil microbial population with beta-cyclodextrin as surfactant to enhance bioavailability. Enzyme Microb Technol 27:709–713
Barel AO, Paye M, Maibach HI (2009) Handbook of cosmetic science and technology, 3rd edn. Marcel Dekker, New York
Barry B (1983) Properties that influence percutaneous absorption. In: Dermatological formulations: percutaneous absorption 18:127–233
Barry BW (2007) Transdermal drug delivery. In: Aulton ME (ed) Aulton's Pharmaceutics, The Design and Manufacture of Medicines. Elsevier, Churchill Livingstone, pp 580–585, Chapter 38
Bataller R, Bragulat E, Nogué S, Görbig MN, Bruguera M, Rodés J (2000) Prolonged cholestasis after acute paraquat poisoning through skin absorption. Am J Gastroenterol 95:1340–1343
Belsey NA, Cordery SF, Bunge AL, Guy RH (2011) Assessment of dermal exposure to pesticide residues during re-entry. Environ Sci Technol 45:4609–4615
Bergh M (1999) Allergenic oxidation products. Acta Derm Venereol 79(Supplement 205):5–26
Bhaskaran S, Harsha S (2000) Effect of permeation enhancer and Iontophoresis on permeation of atenolol from transdermal gels. Ind J Pharm Educ 62:424
Bhatia KS, Gao S, Singh J (1997) Effect of penetration enhancers and iontophoresis on the FT-IR spectroscopy and LHRH permeability through porcine skin. J Controlle Release 47:81–89
Bolzinger M, Carduner T, Poelman M (1998) Bicontinuous sucrose ester microemulsion: a new vehicle for topical delivery of niflumic acid. Int J Pharm 176:39–45
Breuer MM (1979) The interaction between surfactants and keratinous tissues. J Soc Cosmet Chem 30:41–64
Calderilla-Fajardo S, Cazares-Delgadillo J, Villalobos-Garcia R, Quintanar-Guerrero D, Ganem-Quintanar A, Robles R (2006) Influence of sucrose esters on the in vivo percutaneous penetration of octyl methoxycinnamate formulated in nanocapsules, nanoemulsion, and emulsion. Drug Dev Ind Pharm 32:107–113
Cappel MJ, Kreuter J (1991) Effect of nonionic surfactants on transdermal drug delivery: I. Polysorbates. Int J Pharm 69:143–153
Cázares-Delgadillo J, Naik A, Kalia Y, Quintanar-Guerrero D, Ganem-Quintanar A (2005) Skin permeation enhancement by sucrose esters: a pH-dependent phenomenon. Int J Pharm 297:204–212
Cevc G, Blume G (2001) New, highly efficient formulation of diclofenac for the topical, transdermal administration in ultradeformable drug carriers, Transfersomes. Biochim Biophys Acta (BBA)-Biomembranes 1514:191–205
Cevc G, Blume G (2003) Biological activity and characteristics of triamcinolone-acetonide formulated with the self-regulating drug carriers, Transfersomes®. Biochim Biophys Acta (BBA)-Biomembranes 1614:156–164
Cevc G, Blume G (2004) Hydrocortisone and dexamethasone in very deformable drug carriers have increased biological potency, prolonged effect, and reduced therapeutic dosage. Biochim Biophys Acta (BBA)-Biomembranes 1663:61–73
Challa R, Ahuja A, Ali J, Khar R (2005) Cyclodextrins in drug delivery: an updated review. AAPS PharmSciTech 6:329–357
Cho C, Choi J, Shin S (2011) Enhanced local anesthetic action of mepivacaine from the bioadhesive gels. Pak J Pharm Sci 24:87–93
Chowhan Z, Pritchard R (1978) Effect of surfactants on percutaneous absorption of naproxen I: comparisons of rabbit, rat, and human excised skin. J Pharm Sci 67:1272–1274
Commission of the European Communities (2003) Proposal for a Regulation of the European Parliament and of the Council Concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European

Chemicals Agency and amending Directive 1999/45/EC and Regulation (EC) {on Persistent Organic Pollutants}

Cooper ER (1984) Increased skin permeability for lipophilic molecules. J Pharm Sci 73:1153–1156

Csizmazia E, Eros G, Berkesi O, Berkó S, Szabó-Révész P, Csányi E (2011) Penetration enhancer effect of sucrose laurate and Transcutol on ibuprofen. J Drug Deliv Sci Tec 21:411–415

Csizmazia E, Eros G, Berkesi O, Berkó S, Szabó-Révész P, Csányi E (2012) Ibuprofen penetration enhance by sucrose ester examined by ATR-FTIR in vivo. Pharm Dev Technol 17:125–128

Csóka G, Marton S, Zelko R, Otomo N, Antal I (2007) Application of sucrose fatty acid esters in transdermal therapeutic systems. Eur J Pharm Biopharm 65:233–237

Dalvi UG, Zatz J (1981) Effects of nonionic surfactants on penetration of dissolved benzocaine through hairless mouse skin. J Soc Cosmet Chem 32:87–94

Davies, J. T. (1957). A quantitative kinetic theory of emulsion type, I. Physical chemistry of the emulsifying agent. In Gas/Liquid and Liquid/Liquid Interface. Proceedings of the International Congress of Surface Activity (pp. 426–438).

Davis AF, Gyurik RJ, Hadgraft J, Pellett MA, Walters KA (2002) Formulation strategies for modulating skin permeation. Drugs Pharm Sci 119:271–318

Effendy I, Maibach HI (2006) Surfactants and experimental irritant contact dermatitis. Contact Dermatitis 33:217–225

El-Laithy HM (2009) Novel transdermal delivery of Timolol maleate using sugar esters: preclinical and clinical studies. Eur J Pharm Biopharm 72:239–245

El-Laithy HM, Shoukry O, Mahran LG (2011) Novel sugar esters proniosomes for transdermal delivery of vinpocetine: preclinical and clinical studies. Eur J Pharm Biopharm 77:43–55

El-Menshawe SF, Hussein AK (2013) Formulation and evaluation of meloxicam niosomes as vesicular carriers for enhanced skin delivery. Pharm Dev Technol 18(4):779–786

Endo M, Yamamoto T, Ljuin T (1996) Effect of nonionic surfactants on the percutaneous absorption Tenoxicam. Chem Pharm Bull 44:865–867

Engelbrecht T, Demé B, Dobner B, Neubert R (2012) Study of the influence of the penetration enhancer isopropyl myristate on the nanostructure of stratum corneum lipid model membranes using neutron diffraction and deuterium labelling. Skin Pharmacol Physiol 25:200–207

Fang J, Fang C, Huang Y, Tsai Y (1997) Transdermal iontophoresis of sodium nonivamide acetate. III. Combined effect of pretreatment by penetration enhancers. Int J Pharm 149:183–193

Fang J, Yu S, Wu P, Huang Y, Tsai Y (2001) In vitro skin permeation of estradiol from various proniosome formulations. Int J Pharm 215:91–99

Farber, E. M., Abel, E. A., & Charuworn, A. (1983). Recent advances in the treatment of psoriasis. Journal of the American Academy of Dermatology, 8(3), 311–321

Faucher JA, Goddard ED (1978) Interaction of keratinous substrates with sodium lauryl sulfate. II. Permeation through stratum corneum. J Soc Cosmet Chem 2:339–352

Felton LA, Wiley CJ, Godwin DA (2002) Influence of hydroxypropyl-beta-cyclodextrin on the transdermal permeation and skin accumulation of oxybenzone. Drug Dev Ind Pharm 28:1117–1124

Femenia-Font A, Balaguer-Fernandez C, Merino V, Rodilla V, Lopez-Castellanoa A (2005) Effect of chemical enhancers on the in vitro percutaneous absorption of sumatriptan succinate. Eur J Pharm Biopharm 61:50–55

Fevola M, LiBrizzi J, Walters R (2008) Reducing irritation potential of surfactant-based cleansers with hydrophobically-modified polymers. Polym Prepr 49:671–672

Fevola MJ, Walters RM, LiBrizzi JJ (2010) A new approach to formulating mild cleansers: hydrophobically-modified polymers for irritation mitigation. In: Morgan SE, Lochhead RY (eds) Polymeric delivery of therapeutics, vol 1053, ACS symposium series., pp 221–242

Florence T, Tuker IG, Walters KA (1994) Interaction of non-ionic alkyl and aryl ethers with membranes and other biological systems. In: Rosen MJ (ed) Structure Performance Relationships in Surfactants. ACS Symp Ser 253:189–207

Gershbein LL (1979) Percutaneous toxicity of thioglycolate mixtures in rabbits. J Pharm Sci 68:1230–1235

Ghafourian T, Zandasrar P, Hamishekar H, Nokhodchi A (2004) The effect of penetration enhancers on drug delivery through skin: a QSAR study. J Control Release 99:113–125

Ghafourian T, Samaras EG, Brooks JD, Riviere JE (2010a) Modelling the effect of mixture components on permeation through skin. Int J Pharm 398:28–32

Ghafourian T, Samaras EG, Brooks JD, Riviere JE (2010b) Validated models for predicting skin penetration from different vehicles. Eur J Pharm Sci 41:612–616

Ghosh S, Blankschtein D (2008) The role of sodium dodecyl sulfate (SDS) micelles in inducing skin barrier perturbation in the presence of glycerol. Int J Cosmet Sci 30:73–73

Goodman M, Barry BW (1989) Lipid-protein-partitioning (LPP) theory of skin enhancer activity: finite dose technique. Int J Pharm 57:29–40

Griffin WC (1949) Classification of surface-active agents by 'HLB'. J Soc Cosmet Chem 1:311

Griffin WC (1954) Calculation of HLB values of Non-ionic surfactants. J Soc Cosmet Chem 5:249

Gupta PN, Mishra V, Singh P, Rawat A, Dubey P, Mahor S et al (2005) Tetanus toxoid-loaded transfersomes for topical immunization. J Pharm Pharmacol 57:295–301

Hadgraft J (2001) Skin, the final frontier. Int J Pharm 14(224):1–18

Hall-Manning TJ, Holland GH, Rennie G, Revell P, Hines J, Barratt MD, Basketter DA (1998) Skin irritation

potential of mixed surfactant systems. Food Chem Toxicol 36:233–238

Hanaee J, Javadzadeh Y, Taftachi S, Farid D, Nokhodchi A (2004) The role of various surfactants on the release of salbutamol from suppositories. Il Farmaco 59: 903–906

Hofland HE, van der Geest R, Bodde HE, Junginger HE, Bouwstra JA (1994) Estradiol permeation from nonionic surfactant vesicles through human stratum corneum in vitro. Pharm Res 11:659–664

Hu C, Rhodes DG (1999) Proniosomes: a novel drug carrier preparation. Int J Pharm 185:23–35

Hwang CC, Danti AG (1983) Percutaneous absorption of flufenamic acid in rabbits: Effect of dimethyl sulfoxide and various nonionic surface-active agents. J Pharm Sci 72(8):857–860

James-Smith M, Shekhawat D, Shah D (2007) APPLICATION-Importance of micellar lifetime and Sub-micellar aggregates in detergency processes. Tenside Surf Deterg 44:142–153

James-Smith MA, Hellner B, Annunziato N, Mitragotri S (2011) Effect of surfactant mixtures on skin structure and barrier properties. Ann Biomed Eng 39:1215–1223

Jiang G, Zhu D, Zan J, Ding F (2007) Transdermal drug delivery by electroporation: the effects of surfactants on pathway lifetime and drug transport. Chin J Chem Eng 15:397–402

Johnson ME, Mitragotri S, Patel A, Blankschtein D, Langer R (1996) Synergistic effect of ultrasound and chemical enhancers on transdermal drug delivery. J Pharm Sci 85:670–679

Kalhapure RS, Salunke CL, Akamanchi KG (2012) QSAR model for chemical penetration enhancers containing long hydrocarbon chain. Chemom Intell Lab 118:267–270

Kang C, Shin S (2012) Development of prilocaine gels for enhanced local anesthetic action. Arch Pharm Res 35:1197–1204

Kang L, Yap CW, Lim PF, Chen YZ, Ho PC, Chan YW, Wong GP, Chan SY (2007) Formulation development of transdermal dosage forms: quantitative structure-activity relationship model for predicting activities of terpenes that enhance drug penetration through human skin. J Control Release 120:211–219

Kanikkannan N, Singh M (2002) Skin permeation enhancement effect and skin irritation of saturated fatty alcohols. Int J Pharm 248:219–228

Karande P, Jain A, Arora A, Ho MJ, Mitragotri S (2007) Synergistic effects of chemical enhancers on skin permeability: a case study of sodium lauroylsarcosinate and sorbitan monolaurate. Eur J Pharm Sci 31:1–7

Kato A, Ishibashi Y, Miyake Y (1987) Effect of egg yolk lecithin on transdermal delivery of bunazosin hydrochloride. J Pharm Pharmacol 39:399–400

Kirjavainen M, Mönkkönen J, Saukkosaari M, Valjakka-Koskela R, Kiesvaara J, Urtti A (1999) Phospholipids affect stratum corneum lipid bilayer fluidity and drug partitioning into the bilayers. J Control Release 58:207–214

Kitagawa S, Ikarashi A (2003) Enhanced skin permeation of cationic drug ketotifen through excised guinea pig dorsal skin by surfactants with different electric charges. Chem Pharm Bull 51:1183–1185

Klang V, Matsko N, Zimmermann A, Vojnikovic E, Valenta C (2010) Enhancement of stability and skin permeation by sucrose stearate and cyclodextrins in progesterone nanoemulsions. Int J Pharm 393: 153–161

Kogan A, Garti N (2006) Microemulsions as transdermal drug delivery vehicles. Adv Colloid Interface Sci 123:369–385

Kumar GP, Rajeshwarrao P (2011) Nonionic surfactant vesicular systems for effective drug delivery—an overview. Acta Pharmaceutica Sinica B 1:208–219

Kushner J, Blankschtein D, Langer R (2004) Experimental demonstration of highly permeable localized transport regions in low-frequency sonophoresis. J Pharm Sci 93:2733–2745

Kushner J, Kim D, So PT, Blankschtein D, Langer RS (2007) Dual-channel two-photon microscopy study of transdermal transport in skin treated with low-frequency ultrasound and a chemical enhancer. J Invest Dermatol 127:2832–2846

Kushner J, Blankschtein D, Langer R (2008) Evaluation of hydrophilic permeant transport parameters in the localized and non-localized transport regions of skin treated simultaneously with low-frequency ultrasound and sodium lauryl sulfate. J Pharm Sci 97:906–918

Kute A, Goudanavar P, Hiremath D, Reddy SR (2012) Development and characterization of perindopril erbumine loaded proniosomal gel. Asian J Pharm Tech 2:54–58

Lakshmi P, Devi GS, Bhaskaran S, Sacchidanand S (2007) Niosomal methotrexate gel in the treatment of localized psoriasis: phase I and phase II studies. Indian J Dermatol Venereol Leprol 73:157

Le VH, Lippold BC (1995) Influence of physicochemical properties of homologous esters of nicotinic acid on skin permeability and maximum flux. Int J Pharm 124:285–292

Legen I, Kračun M, Salobir M, Kerč J (2006) The evaluation of some pharmaceutically acceptable excipients as permeation enhancers for amoxicillin. Int J Pharm 308:84–89

Lehmann L, Keipert S, Gloor M (2001) Effects of microemulsions on the stratum corneum and hydrocortisone penetration. Eur J Pharm Biopharm 52:129–136

Lerk PC, Sucker H (1993) Application of sucrose laurate in topical preparations of cyclosporine A. Int J Pharm 92:197–202

Li GL, de Vries JJ, van Steeg TJ, van den Busche H, Maas HJ, Reeuwijk HJ, Danhof M, Bouwstra JA, van Laar T (2005a) Transdermal iontophoretic delivery of apomorphine in patients improved by surfactant formulation pretreatment. J Control Release 101:199–208

Li GL, Van Steeg TJ, Putter H, Van Der Spek J, Pavel S, Danhof M, Bouwstra JA (2005b) Cutaneous side-effects of transdermal iontophoresis with and without surfactant pretreatment: a single-blinded, randomized controlled trial. Br J Dermatol 153:404–412

Limpongsa E, Umprayn K (2008) Preparation and evaluation of diltiazem hydrochloride diffusion-controlled transdermal delivery system. AAPS PharmSciTech 9:464–470

Lin S, Wouessidjewe D, Poelman M, Duchene D (1994) In vivo evaluation of indomethacin/cyclodextrin complexes gastrointestinal tolerance and dermal anti-inflammatory activity. Int J Pharm 106:63–67

Lingan MA, Sathali AAH, Kumar MV, Gokila A (2011) Formulation and evaluation of topical drug delivery system containing clobetasol propionate niosomes. Sci Rev Chem Commun 1:7–17

Loden M (1990) The simultaneous penetration of water and sodium lauryl sulphate through isolated human skin. J Soc Cosmet Chem 41:227–233

Loftsson T, Masson M (2001) Cyclodextrins in topical drug formulations: theory and practice. Int J Pharm 225:15–30

Loftsson T, Sigurdardottir A (1994) The effect of polyvinylpyrrolidone and hydroxypropyl methylcellulose of HPβCD complexation of hydrocortisone and its permeability through hairless mouse skin. Eur J Pharm Sci 2:297–301

Loftsson T, Frioriksdottir H, Ingvarsdottir G, Jonsdottir B, Siguroardottir A (1994) The influence of 2-hydroxypropyl-β-cyclodextrin on diffusion rates and transdermal delivery of hydrocortisone. Drug Dev Ind Pharm 20:1699–1708

Loftsson T, Vogensen SB, Brewster ME, Konráðsdóttir F (2007) Effects of cyclodextrins on drug delivery through biological membranes. J Pharm Sci 96:2532–2546

Lopez A, Llinares F, Cortell C, Herraez M (2000a) Comparative enhancer effects of Span® 20 with Tween® 20 and Azone® on the in vitro percutaneous penetration of compounds with different lipophilicities. Int J Pharm 202:133–140

Lopez RF, Collett JH, Bentley M (2000b) Influence of cyclodextrin complexation on the in vitro permeation and skin metabolism of dexamethasone. Int J Pharm 200:127–132

Mahale N, Thakkar P, Mali R, Walunj D, Chaudhari S (2012) Niosomes: novel sustained release nonionic stable vesicular systems-an overview. Adv Colloid Interface Sci 183–184:46–54

Malmsten M (2002) Surfactants and polymers in drug delivery. Marcel Dekker, New York

Mannheimer HS (1961) Baby shampoo. American Perfumer 76:36–37

Mao G, Flach CR, Mendelsohn R, Walters RM (2012) Imaging the distribution of sodium dodecyl sulfate in skin by confocal Raman and infrared microspectroscopy. Pharm Res 29:2189–2201

Martinez V, Sanchez L, Busquets MA, Infante MR, Vinardell MP, Mitjans M (2007) Disturbance of erythrocyte lipid bilayer by amino acid-based surfactants. Amino Acids 33:459–462

Másson M, Loftsson T, Másson G, Stefánsson E (1999) Cyclodextrins as permeation enhancers: some theoretical evaluations and in vitro testing. J Control Release 59:107–118

Mendelsohn R, Flach CR, Moore DJ (2006) Determination of molecular conformation and permeation in skin via IR spectroscopy, microscopy, and imaging. Biochim Biophys Acta (BBA)-Biomembranes 1758:923–933

Mitragotri S (2000) Synergistic effect of enhancers for transdermal drug delivery. Pharm Res 17(11): 1354–1359

Mitragotri S, Ray D, Farrell J, Tang H, Yu B, Kost J et al (2000) Synergistic effect of low-frequency ultrasound and sodium lauryl sulfate on transdermal transport. J Pharm Sci 89:892–900

Miyazawa K, Ogawa M, Mitsui T (1984) The physicochemical properties and protein denaturation potential of surfactant mixtures. Int J Cosmet Sci 6:33–46

Mohamad M, Jan R (2012) Challenges in transdermal formulation: in vitro evaluation. Int J Pharm 2:362–365

Moore PN, Puvvada S, Blankschtein D (2003a) Challenging the surfactant monomer skin penetration model: penetration of sodium dodecyl sulfate micelles into the epidermis. J Cosmet Sci 54:29–46

Moore PN, Shiloach A, Puvvada S, Blankschtein D (2003b) Penetration of mixed micelles into the epidermis: effect of mixing sodium dodecyl sulfate with dodecyl hexa (ethylene oxide). J Cosmet Sci 54:143–160

Mukherjee B, Kanupriya MS, Das S, Patra B (2005) Sorbitan monolaurate 20 as a potential skin permeation enhancer in transdermal patches. J Appl Res 1:96–108

Mura S, Pirot F, Manconi M, Falson F, Fadda AM (2007) Liposomes and niosomes as potential carriers for dermal delivery of minoxidil. J Drug Target 15:101–108

Murthy SN, Sen A, Hui SW (2004) Surfactant-enhanced transdermal delivery by electroporation. J Control Release 98:307–315

Nagarajan R, Ruckenstein E (1991) Theory of surfactant self-assembly: a predictive molecular thermodynamic approach. Langmuir 7:2934–2969

Narasimha Reddy D, Udupa N (1993) Formulation and evaluation of oral and transdermal preparations of flurbiprofen and piroxicam incorporated with different carriers. Drug Dev Ind Pharm 19:843–852

Newton J, Rowley G, Törnblom J (1971) Further studies on the effect of additives on the release of drug from hard gelatin capsules. J Pharm Pharmacol 23(S1):156S–160S

Nokhodchi A, Shokri J, Dashbolaghi A, Hassan-Zadeh D, Ghafourian T, Barzegar-Jalali M (2003) The enhancement effect of surfactants on the penetration of lorazepam through rat skin. Int J Pharm 250:359–369

Nokhodchi A, Sharabiani K, Rashidi M, Ghafourian T (2007) The effect of terpene concentrations on the skin penetration of diclofenac sodium. Int J Pharm 335:97–105

O'Lenick AJ (2005) Surfactants: Strategic Personal Care Ingredients. Allured Pub Corp, Carol Stream

Oh S, Jeong S, Park T, Lee J (1998) Enhanced transdermal delivery of AZT (Zidovudine) using iontophoresis and penetration enhancer. J Control Release 51:161–168

Okamoto H, Komatsu H, Hashida M, Sezaki H (1986) Effects of β-cyclodextrin and di-O-methyl-β-

cyclodextrin on the percutaneous absorption of butylparaben, indomethacin and sulfanilic acid. Int J Pharm 30:35–45

Okamoto H, Sakai T, Danjo K (2005) Effect of sucrose fatty acid esters on transdermal permeation of lidocaine and ketoprofen. Bio Pharm Bull 28:1689–1694

Pardakhty A, Varshosaz J, Rouholamini A (2007) In vitro study of polyoxyethylene alkyl ether niosomes for delivery of insulin. Int J Pharm 328:130–141

Park ES, Chang SY, Hahn M, Chi SC (2000) Enhancing effect of polyoxyethylene alkyl ethers on the skin permeation of ibuprofen. Int J Pharm 209:109–119

Patel KK, Kumar P, Thakkar HP (2012) Formulation of niosomal gel for enhanced transdermal Lopinavir delivery and its comparative evaluation with ethosomal gel. AAPS PharmSciTech 13:1502–1510

Piret J, Désormeaux A, Cormier H, Lamontagne J, Gourde P, Juhász J et al (2000) Sodium lauryl sulfate increases the efficacy of a topical formulation of foscarnet against herpes simplex virus type 1 cutaneous lesions in mice. Antimicrob Agents Chemother 44:2263–2270

Prasanthi D, Lakshmi P (2012) Effect of chemical enhancers in transdermal permeation of alfuzosin hydrochloride. ISRN Pharm 2012: article ID 965280, 1–8

Raut S, Suruse PB, Shivhare UD, Bhusari KP (2010) Development and evaluation of non-ionic surfactant based organogels for transdermal delivery of zidovudine. Pharmacie Globale Int J Comprehensive Pharm 3:1–7

Rhein L, Robbins C, Fernee K (1986) Surfactant structure effects on swelling of isolated human. J Soc Cosmet Chem 37:125–139

Rhein LD, Simion F, Hill R, Cagan R, Mattai J, Maibach H (1990) Human cutaneous response to a mixed surfactant system: role of solution phenomena in controlling surfactant irritation. Dermatology 180:18–23

Riegelman S, Crowell W (1958a) The kinetics of rectal absorption II. The absorption of anions. J Am Pharm Assoc 47:123–127

Riegelman S, Crowell WJ (1958b) The kinetics of rectal absorption I. Preliminary investigations into the absorption rate process. J Am Pharm Assoc 47:115–122

Riegelman S, Crowell WJ (1958c) The kinetics of rectal absorption III. The absorption of undissociated molecules. J Am Pharm Assoc 47:127–133

Rigg PC, Barry BW (1990) Shed snake skin and hairless mouse skin as model membranes for human skin during permeation studies. J Invest Dermatol 94:235–240

Riviere JE, Brooks JD (2011) Predicting skin permeability from complex chemical mixtures: dependency of quantitative structure permeability relationships (QSPR) on biology of skin model used. Toxicol Sci 119:224–232

Roberts MS, Cross SE, Pellett MA (2002) Skin transport. Drugs Pharm Sci 119:89–196

Rogerson A, Cummings J, Willmott N, Florence A (1988) The distribution of doxorubicin in mice following administration in niosomes. J Pharm Pharmacol 40: 337–342

Rosen MJ, Kunjappu JT (2004) Surfactants and interfacial phenomena. Wiley, Hoboken

Rosen MJ, Kunjappu JT (2012) Tenside surfactants detergents. In: Surfactants and interfacial phenomena 49: 361

Saad P, Flach CR, Walters RM, Mendelsohn R (2012) Infrared spectroscopic studies of sodium dodecyl sulphate permeation and interaction with stratum corneum lipids in skin. Int J Cosmet Sci 34:36–43

Saija A, Tomaino A, Trombetta D, Giacchi M, De Pasquale A, Bonina F (1998) Influence of different penetration enhancers on in vitro skin permeation and in vivo photoprotective effect of flavonoids. Int J Pharm 175:85–94

Samaras EG, Riviere JE, Ghafourian T (2012) The effect of formulations and experimental conditions on in vitro human skin permeation-Data from updated EDETOX database. Int J Pharm 15:434280–434291

Sarpotdar P, Zatz JL (1986a) Percutaneous absorption enhancement by nonionic surfactants. Drug Dev Ind Pharm 12(11–13):1625–1647

Sarpotdar PP, Zatz JL (1986b) Evaluation of penetration enhancement of lidocaine by nonionic surfactants through hairless mouse skin in vitro. J Pharm Sci 75:176–181

Sathali AAH, Rajalakshmi G (2010) Evaluation of transdermal targeted niosomal drug delivery of terbinafine hydrochloride. Int J PharmTech Res 2:2081–2089

Scheuplein R, Ross L (1970) Effects of surfactants and solvents on the permeability of epidermis. J Soc Cosmet Chem 21:853–873

Schreier H, Bouwstra J (1994) Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery. J Control Release 30:1–15

Seto JE, Polat BE, Lopez RFV, Blankschtein D, Langer R (2010) Effects of ultrasound and sodium lauryl sulfate on the transdermal delivery of hydrophilic permeants: comparative in vitro studies with full-thickness and split-thickness pig and human skin. J Control Release 145:26–32

Shahi V, Zatz JL (1978) Effect of formulation factors on penetration of hydrocortisone through mouse skin. J Pharm Sci 67:789–792

Shahiwala A, Misra A (2002) Studies in topical application of niosomally entrapped nimesulide. J Pharm Pharm Sci 5:220

Shamsheer A, Sabareesh M, Patan R, Sai K, Sudheer B (2011) Formulation and evaluation of lisinopril dehydrate transdermal proniosomal gels. J App Pharm Sci 1:181–185

Shen WW, Danti AG, Bruscato FN (1976) Effect of nonionic surfactants on percutaneous absorption of salicylic acid and sodium salicylate in the presence of dimethyl sulfoxide. J Pharm Sci 65:1780–1783

Shin S, Cho C, Oh I (2001) Effects of non-ionic surfactants as permeation enhancers towards piroxicam from the poloxamer gel through rat skins. Int J Pharm 222:199–203

Shokri J, Nokhodchi A, Dashbolaghi A, Hassan-Zadeh D, Ghafourian T, Barzegar JM (2001) The effect of surfactants on the skin penetration of diazepam. Int J Pharm 228:99–107

Sigurdoardóttir AM, Loftsson T (1995) The effect of polyvinylpyrrolidone on cyclodextrin complexation of hydrocortisone and its diffusion through hairless mouse skin. Int J Pharm 126(1):73–78

Silva S, Hu L, Sousa JJ, Pais AA, Michniak-Kohn BB (2012) A combination of nonionic surfactants and iontophoresis to enhance the transdermal drug delivery of ondansetron HCl and diltiazem HCl. Eur J Pharm Biopharm 80(3):663–673

Singh J, Singh S (1995) Transdermal iontophoresis: effect of penetration enhancer and iontophoresis on drug transport and surface characteristics of human epidermis. Curr Probl Dermatol 22:179

Som I, Bhatia K, Yasir M (2012) Status of surfactants as penetration enhancers in transdermal drug delivery. J Pharm Bioallied Sci 4(1):2

Tupker R, Pinnagoda J, Nater J (1990) The transient and cumulative effect of sodium lauryl sulphate on the epidermal barrier assessed by transepidermal water loss: inter-individual variation. Acta Derm Venereol 70:1–5

Udupa N, Bhat L (1992) Evaluation of FEW ciprofloxacin (CIP) and norfloxacin (NOR) formulations. Drug Dev Ind Pharm 18:2197–2205

Uekama K, Arimori K, Sakai A, Masaki K, Irie T, Otagiri M (1987) Improvement in percutaneous absorption of prednisolone by β-and γ-cyclodextrin complexations. Chem Pharm Bull 35:2910–2913

Uekama K, Adachi H, Irie T, Yano T, Saita M, Noda K (1992) Improved transdermal delivery of prostaglandin E1 through hairless mouse skin: combined use of carboxymethyl-ethyl-β-cyclodextrin and penetration enhancers. J Pharm Pharmacol 44:119–121

Umemura M, Ueda H, Tomono K, Nagai T (1990) Effect of diethyl-beta-cyclodextrin on the release of nitroglycerin from formulations. Drug Des Deliv 6:297–310

Vaddi H, Wang L, Ho P, Chan S (2001) Effect of some enhancers on the permeation of haloperidol through rat skin in vitro. Int J Pharm 212:247–255

Varshosaz J, Pardakhty A, Mohsen S, Baharanchi H (2005) Sorbitan monopalmitate-based proniosomes for transdermal delivery of chlorpheniramine maleate. Drug Deliv 12:75–82

Ventura C, Tommasini S, Falcone A, Giannone I, Paolino D, Sdrafkakis V et al (2006) Influence of modified cyclodextrins on solubility and percutaneous absorption of celecoxib through human skin. Int J Pharm 314:37–45

Vermeire A, Muynck C, Vandenbossche G, Eechaute W, Geerts M, Remon JP (1996) Sucrose laurate gels as a percutaneous delivery system for oestradiol in rabbits. J Pharm Pharmacol 48:463–467

Vyas S, Singh R, Jain S, Mishra V, Mahor S, Singh P et al (2005) Non-ionic surfactant based vesicles (niosomes) for non-invasive topical genetic immunization against hepatitis B. Int J Pharm 296:80–86

Walters KA (1990) Surfactants and percutaneous absorption. In: Predictions of percutaneous penetration. IBC Technical Services, London

Walters KA, Hadgraft J (1993) Pharmaceutical skin penetration enhancement, vol 59. Marcel Dekker, New York

Walters KA, Walker M, Olejnik O (1988) Non-ionic surfactant effects on hairless mouse skin permeability characteristics. J Pharm Pharmacol 40:525–529

Walters RM, Fevola MJ, Librizzi JJ, Martin K (2008) Designing cleansers for the unique needs of baby skin. Cosmet Toiletries 123:53–60

Walters RM, Mao GM, Gunn ET, Hornby S (2012) Cleansing formulations that respect skin barrier integrity. Dermatol Res Pract 2012:article 495917, 1–9

Wang S, Kara M, Krishnan T (1998) Transdermal delivery of cyclosporin-A using electroporation. J Control Release 50:61–70

Watkinson A, Green D, Brain K, James V, Walters K, Azri-Meehan S et al (1998) Skin penetration of a series of nonoxynol homologues. In: Brain KR, James VJ, Waters KS (eds) Perspectives in percutaneous penetration, vol. 5b. STS Publishing, Cardiff, pp 54–59

Wearley L, Chien YW (1990) Enhancement of the in vitro skin permeability of azidothymidine (AZT) via iontophoresis and chemical enhancer. Pharm Res 7:34–40

Weaver JC, Vanbever R, Vaughan TE, Prausnitz MR (1997) Heparin alters transdermal transport associated with electroporation. Biochem Biophys Res Commun 234:637–640

Wiechers J (2005) Optimizing skin delivery of active ingredients from emulsions: from theory to practice. In: Delivery system handbook for personal care and cosmetic products-technology, applications, and formulations. William Andrew Pub, Norwich

Wilhelm KP, Cua AB, Wolff HH, Maibach HI (1993) Surfactant-induced stratum corneum hydration in vivo: prediction of the irritation potential of anionic surfactants. J Invest Dermatol 101:310–315

Williams AC, Barry BW (2004) Penetration enhancers. Adv Drug Deliv Rev 56:603–618

Wu P, Huang Y, Lin H, Tsai Y (1996) In vitro percutaneous absorption of captopril through excised rabbit skin. Int J Pharm 143:119–123

Yamato K, Takahashi Y, Akiyama H, Tsuji K, Onishi H, Machida Y (2009) Effect of penetration enhancers on transdermal delivery of propofol. Bio Pharm Bull 32:677–683

14 Natural Emulsifiers of the Alkyl Polyglucoside Type and Their Influence on the Permeation of Drugs

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14.1 Introduction

Surfactants, emulsifiers or surface-active agents have found vast application in many fields. However, when considering topical pharmaceutical formulations, they mostly serve as diverse stabilising agents (Lopez et al. 2000; Shokri et al. 2001). Additionally, surfactants have long been recognised to modify the permeability of various biological membranes, thus acting as penetration enhancers as well (Casiraghi et al. 2012). Although nowadays much is known on skin structure, a precise mechanism of such an action still remains to be discerned. It has been suggested that the enhancement occurs upon penetration of surfactant molecules into the stratum corneum intercellular matrix, which is followed by binding to keratin filaments, and finally resulting in disruption of the existing order within the corneocytes and lipid bilayers of the skin. Consequently, this leads to an increase in diffusion coefficient and hence permeability (Subedi et al. 2010).

However, satisfactory physical stability of a pharmaceutical preparation often requires the presence of emulsifier(s) in certain concentrations which may compromise skin safety. Anionic and cationic surfactants are known to impair human skin. Among them, sodium lauryl sulphate (SLS) is an efficient emulsifier shown to act as a potent skin irritant, now often used for

N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_14

deliberate transepidermal water loss (TEWL) elevation in human volunteers during in vivo skin tolerance studies (Williams and Barry 2004). When compared to the aforementioned classes, nonionic surfactants are mostly regarded as safe. Moreover, while reviewing current regulatory status in the field of emulsifiers, it could be noticed that a trend in replacing potentially harmful nonionic surfactants with their 'green' alternatives is encouraged whenever applicable (Jurado et al. 2010). Nevertheless, the fact that skin irritation may often be exerted not by an individual substance but by combined use of substances in a final preparation is often disregarded (Hall-Manning et al. 1998).

When considering the development of emulsifiers, influences coming from the cosmetic industry are evident. Recent trends and advances in formulation of cosmetic products have resulted in the fact that patients today have similar high expectations of pharmaceutical topical preparations as well. Apart from the sensorial requirements, both the industry and the final user are increasingly aware that the use of conventional, synthetic surfactants has taken its toll on our environment (Piispanen et al. 2004). Having that in mind, there is an increasing demand in development and application of natural-origin, biodegradable and skin-friendly emulsifiers. Among these, a group of sugar-based surfactants, including sugar esters, sugar amides, glycosides and similar derivatives are being evaluated (Pantelic and Cuckovic 2014). The present chapter aims to provide a deeper insight into the group of alkyl polyglucoside emulsifiers and their impact on drug percutaneous permeation.

14.2 Alkyl Polyglucosides

Alkyl polyglucosides (APGs) represent a group of nonionic emulsifiers based on sugar and fatty alcohol components. Although the first production process of APGs was described more than 100 years ago, these emulsifiers were hastily disregarded until the 1980s, when large-scale cost-efficient production was achieved (Iglauer et al. 2009). The industrial production is based on Fischer synthesis and converts natural fats (e.g. plant oils) or non-natural fatty alcohols, and starches or glucose to APGs (Stubenrauch 2001; Zgola-Grzeskowiak et al. 2008). Since the initially obtained technical products comprised a mixture of different components and stereoisomers, they were named alkyl polyglucosides. Nowadays, stereospecific procedures involving protective groups result in the production of specific components. The APGs that have found application so far differ in the length of the alkyl chain and the average number of monosaccharide units linked to it (Kühn and Neubert 2004).

Additional feature which distinguishes APGs from other conventionally synthesised emulsifiers is that their production takes place without an ethoxylation step (Konya et al. 2004), and for that reason, they were found to be more mild towards the skin and possess considerably lower irritation potential when compared to other emulsifier classes (Tasic-Kostov et al. 2014). As already mentioned, APGs owe their growing popularity to the fact that they are biodegradable and produced from renewable raw materials; hence, they are often labelled as environment-friendly (Garcia et al. 1997).

Apart from the aforementioned biocompatibility and biodegradability, APGs were shown to possess interesting interfacial properties, allowing them to act as efficient pharmaceutical excipients as well (Jurado et al. 2010; Savic et al. 2014). For that reason, this group of natural-origin emulsifiers is envisioned to gradually replace other nonionic emulsifiers derived from petrochemicals (Castro et al. 2006).

Having the APGs mild action in mind, one could ask what could be their expected mechanisms of penetration enhancement. Generally, surfactants are thought to affect skin permeability by the following mechanisms (Casiraghi et al. 2012):

1. The lipid-based mechanism claims surfactants to penetrate into the intercellular regions of the stratum corneum and subsequently increase the membrane fluidity and solubilise or extract its lipid constituents, thus reducing diffusional resistance.

2. The protein-based mechanism relies on surfactant interaction and consecutive binding to keratin filaments which results in corneocytes disordering.

Since the proposed mechanisms are based on the same principles found to be behind the surfactant-induced skin irritation as well (Hall-Manning et al. 1998), one could question the penetration-enhancing potential of these mild natural-origin emulsifiers. However, apart from the suggested mechanisms which come to effect upon direct contact with the skin, nonionic surfactants could also influence percutaneous absorption through modification of a drug's thermodynamic activity within the vehicle. Therefore, the interested researchers commenced a comprehensive physicochemical investigation of alkyl polyglucosides per se along with the containing delivery systems, ranging from structure-activity relationship studies to case-by-case dermal availability evaluations.

14.2.1 Physicochemical Characterisation

Having the proposed mechanisms of penetration enhancement in mind, it is evident that the size as well as the shape of both the fatty unit (alkyl tails) and sugar unit (polar heads) (Fig. 14.1) may influence the penetration-modifying ability of APGs (Casiraghi et al. 2012).

Besides many conducted structure-activity relationship studies in the field, some aspects remain poorly understood. It is believed for some time that satisfactory enhancing properties of a surfactant lay in the optimum alkyl chain length between C8 and C14, while saturated dodecyl tail was frequently distinguished to possess the strongest enhancing properties (Lopez et al. 2000). Although some of the APGs in use possess the recommended alkyl chain length (C8/10, C10, C12, C12/14, etc.) (Patel and Joshi 2008), it can be noticed that long hydrophobic tail tends to dominate the structure of newly synthesised APGs (e.g. C20/22) without significantly altering their enhancing potential (Lukic et al. 2013). Therefore, the enhancing properties demonstrated by the APGs cannot be interpreted by the mere length of the carbohydrate moiety.

Even the early investigations of the simple binary (APG emulsifier/water) and ternary (APG emulsifier/water/oil) systems revealed interesting interfacial properties of these natural-origin emulsifiers leading to the formation of specific colloidal structures (Lukic et al. 2013; Savic et al. 2005). Apart from providing an insight into the samples' microstructure, polarised light microscopy is able to record specific birefringence as a result of the formation of anisotropic structures within both binary and ternary systems. According to Eccleston (Eccleston 1990), the observed distorted Maltese crosses imply the presence of the liquid lamellar phase (Fig. 14.2a). Naturally, the number of the recorded Maltese crosses tends to increase with gradual increase in the emulsifier concentration until the crystals of pure emulsifier are apparent. With the introduction

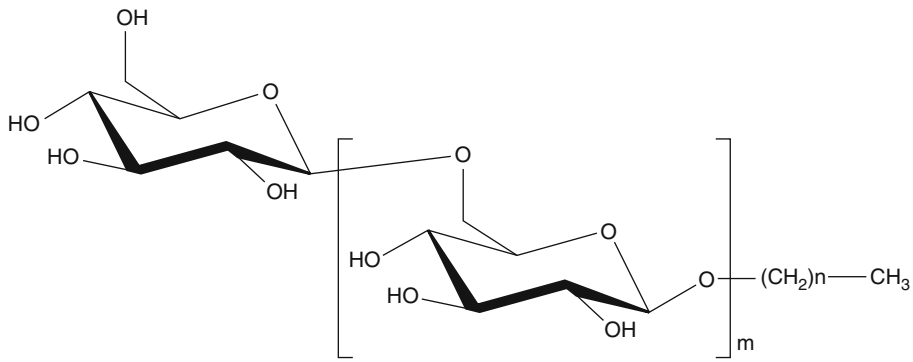

Fig. 14.1 Schematic representation of an alkyl polyglucoside

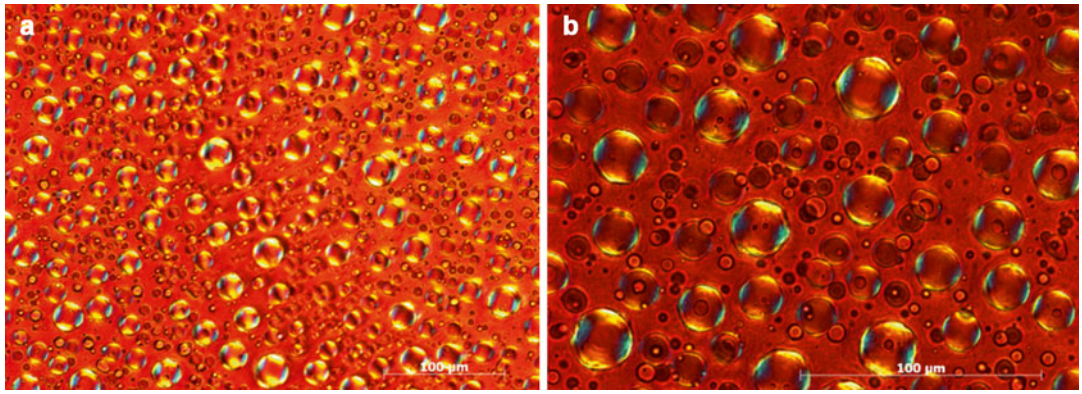

Fig. 14.2 Polarisation micrographs of a C16/18 mixed APG emulsifier (cetearyl glucoside and cetearyl alcohol) stabilised colloidal structure depicting: (**a**) numerous deformed Maltese crosses and (**b**) lamellar gel network layers around larger droplets, as fundamental stabilisation mechanisms

of the oily phase in the system, the captured micrographs transform to more conventional oil droplets packaging. Nevertheless, natural emulsifier-based texture still shows characteristic anisotropic droplets, commonly referred to as 'onion rings', uniformly dispersed in the continuous phase. Additionally, remnants of the lamellar gel network could also be observed as complex layers surrounding the larger droplets or floccules of smaller ones (Fig. 14.2b) (Jaksic et al. 2012). The observed *lamellar liquid crystal and lamellar gel phases* which represent the basic mechanism of physical stabilisation by the APGs have prompted the researchers to investigate whether the potential penetration enhancement lies on the same foundation.

However, a deeper insight into the type of the structures formed can be substantiated by a more sophisticated technique such as freeze-fracture transmission electron microscopy (TEM), while true confirmation may be achieved through small and wide-angle X-ray diffraction (SAXD and WAXD). Typical features of the expected lamellar structures produced by the APGs provided by TEM are widespread lamellar sheets of planar arrangement as a sign of highly ordered lamellar gel phase. Although inherently prone to confounding influence of artefacts, careful consideration of the TEM replicas implied complex water incorporation within the APG-stabilised systems (Savic et al. 2007). The precise structural information on the APG-based systems was further obtained through SAXD and WAXD providing insight into specific diffraction patterns, interlayer spacings and intermolecular distances. Single sharp reflections in the range of 0.415 and 0.42 nm detected in samples based on APG emulsifiers varying in alkyl chain length confirm the predominant presence of α-crystalline gel phase (L_β) within APG-based systems (Lukic et al. 2013; Savic et al. 2006). Interpretation of SAXD patterns seems to be more influenced by the investigated system's composition, namely, the ratio of the incorporated oily phase. In binary systems, the repeated distance tends to increase with the emulsifier concentration from 5.7 to 6.2 nm, while the addition of the oily phase expands the long spacing in APG-based samples (Savic et al. 2005). Soon it became apparent that similar diffraction patterns could be observed in the inherent structure of the stratum corneum, a principal barrier for penetration of xenobiotics.

In spite of the numerous publications available on the subject of skin structure, many things remain to be discovered. When focusing on the problem of percutaneous penetration enhancement, nearly every paper starts its elaboration with the obvious statement that stratum corneum stands in the way of the desired drug penetration. Although commonly represented as a two-compartmental model, it is clear that stratum corneum is not a homogeneous membrane. Investigations of its intercellular spaces via electron microscopy have recorded an unusual

lamellar ordering, namely, a repeating pattern of multiple lamellar structures, further assessed through SAXD and WAXD. SAXD revealed a lamellar phase of long periodicity (approx. 13 nm) and small periodicity (approx. 6 nm) (Bolzinger et al. 2012), while WAXD studies confirmed the possibility of gel phase ordering of physiological lipids (Suhonen et al. 1999). This apparent similarity between APG-stabilised systems and stratum corneum lipid ordering gave rise to hypothesis that possible mechanism of APG penetration enhancement lies in their matching balance between order and fluidity structures (Lagerwall and Scalia 2012).

Despite the fact that emulsifiers of the APG type have been on the market for some time, vehicles for dermatological preparations are mostly stabilised by conventional anionic and nonionic surfactants. However, for some time, in the eyes of both pharmacists and their patients, many of the traditionally used vehicles are considered aesthetically unsuitable and hence are applied unwillingly, directly affecting compliance (Piacquadio and Kligman 1998). On the other hand, constant promotion of natural-origin products by the cosmetic industry has led to corresponding demands of a modern patient, and the pharmaceutical topical dosage forms have to live up to these expectations (Lourith and Kanlayavattanakul 2009). Consequently, there is a need for development of pharmaceutical vehicles with improved sensorial characteristics and their introduction to dermatological practice (Lukic et al. 2014).

Rheological properties of pharmaceutical semisolids not only reflect physical stability but undoubtedly affect drug release behaviour (Dragicevic-Curic et al. 2009). Dermal absorption of a topically applied drug depends on its release and permeability to the treated site, making the satisfactory release rate of a drug from the carrier a prerequisite for the therapeutic efficacy (Li et al. 2011). Furthermore, flow properties may be directly correlated to their applicative properties, which are needless to say considered of great importance. Flow parameters obtained by continual and/or oscillatory rheological measurements for samples stabilised with two mixed APG emulsifiers of different alkyl chain length independently: cetearyl glucoside and cetearyl alcohol (C16/18) and arachidyl glucoside and arachidyl alcohol and behenyl alcohol (C20/22) have shown that all samples exhibited shear-thinning, time-dependent, thixotropic behaviour, as confirmed by the appearance of the samples' flow curves and hysteresis loop areas in the plot of shear stress versus shear rate (Lukic et al. 2013; Jaksic et al. 2012). This reversible and time-dependent rheological manifestation of flow-induced structural changes is considered universally desirable for topically applied preparations (Tadros 2004). Additionally, with proper study design, rheological measurements may simulate actual application of a topical product, i.e. the rubbing-in phase, and provide information on the type of changes the preparation may undergo, whether in composition or structure (Schaefer and Redelmeier 1996). This is of particular importance if a sample comprises volatile components such as ethanol, isopropyl alcohol and even glycerol or propylene glycol to some extent, as commonly used co-solvents in topical dosage forms (Pantelic et al. 2014a). Finally, the fact that APGs are available as odourless and tasteless materials contributes to the overall cosmetic acceptability and appropriate skin feel (Williams and Barry 2004; Allen and Tao 1999).

Thermal analysis, mostly differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), proved to be highly useful in the evaluation of emulsion systems, especially semisolid structures (Konya et al. 2003). The shape of the obtained curves was shown to provide accurate information on the mode of water incorporation. The state of water within such systems is of particular importance when assessing drug penetration since water is long considered to be the safest penetration enhancer (Hadgraft 1999). Due to its inherent nature, water is believed to interact with the polar head groups of the stratum corneum lipid bilayer, thus disturbing its ordering and resulting in more fluid domains facilitating the diffusion of (predominantly) polar penetrants (Suhonen et al. 1999).

The water content of human stratum corneum is approximately 15–20 % of tissue dry weight,

allowing for variations depending on environment conditions (Williams and Barry 2004). One of the commonly applied methods to increase the amount of water present in the skin barrier is by means of occlusion, whether using an occlusive vehicle (typically ointments) or a suitable occlusive cover. Anyway, the intention is to prevent transepidermal water loss and enable the water content of the stratum corneum to reach water levels of the underlying tissues (Williams and Barry 2004). Either way this approach is not well accepted by the patients.

Here lies another possible mechanism of APG-mediated penetration enhancement. The complex lamellar structures which dominate the texture of APG-stabilised systems revealed by the polarisation microscopy, and further confirmed through TEM, SAXD and WAXD measurements, inevitably induce specific distribution of the water phase. For such an evaluation, TGA is considered the most useful technique providing precise insight into the mode of water distribution within the system and, hence, a deeper comprehension of the nature of the colloidal structure. Results of TG analysis should be discussed both in direct assessment of TGA profiles, which enables insight into partial weight loss over different temperature ranges, and indirectly – through comparison of first derivative TGA curves (DTG profiles) for the evaluation of evaporation rates. Alongside TGA, DSC is regarded as a complementary technique, enabling comparison of phase transitions of the investigated bases, commonly reflected through the shape of the curves, peak temperatures and total enthalpies of the melting process.

As postulated by Junginger (1997) and further elaborated by Eccleston (1997), creams of o/w type stabilised with mixed emulsifier should be regarded as systems consisting of at least four phases. When considering the presence of water within such systems, two phases should be distinguished:

- Crystalline/hydrophilic gel phase, which is composed of bilayers of surfactant and fatty amphiphile with inserted water molecules, thus forming interlamellar water and
- Bulk water, which, along with the interlamellarly fixed water, forms the continuous (external) phase of the system.

However, it is assumed that molecules of the interlamellarly fixed water exhibit different physicochemical and biopharmaceutical properties than those of the bulk water phase.

Considering the structure of APGs, it seems safe to say that a certain amount of water will be entrapped by hydrogen bonding. Additionally, the complex lyotropic lamellar crystalline and gel structures, dominating the APG-based systems, inevitably lead to formation of diverse water fractions within the colloidal structures. By systematic evaluation of binary, ternary and cream samples, Savic et al. (2010) have distinguished the following fractions:

1. Free (bulk) water
2. Secondary water, entrapped within the lipophilic gel phase
3. Interlamellarly fixed water between crystal lipid bilayers (hydrophilic gel phase)
4. Interlamellarly fixed water between lipid bilayers, existing in the form of liquid crystals

When dividing the obtained TG weight loss plots into three temperature segments – 25/30–50, 50–70 and 70–100/110 °C – a specific pattern of water distribution within APG-stabilised systems is revealed (Table 14.1).

Results indicate that, irrespective of the alkyl chain length, within samples stabilised by both APG-mixed emulsifiers, water is predominantly incorporated as bulk water and hence mainly evaporated at the third temperature range (70–110 °C). Having in mind the aforementioned complexity of these colloidal structures, this probably occurs upon the disruption of the formed lamellar phases. However, these observations were valid only for samples of a simple composition, comprising emulsifier, oily phase and preserved water. The addition of other co-solvents such as isopropyl alcohol (IPA; 10 % w/w), glycerol (Gly; 20 % w/w) or propylene glycol (PG; 20 % w/w), which is considered

Table 14.1 Percentage of partial weight loss over specified temperature ranges expressed as mean ± SD

Emulsifier type and content in the sample (% w/w)	30–50 °C (% ± SD)	50–70 °C (% ± SD)	70–110 °C (% ± SD)
$APG_{C16/18}$ (8 %)	9.70 ± 1.07	19.47 ± 1.80	41.48 ± 3.12
$APG_{C20/22}$ (10 %)	6.04 ± 0.23	26.30 ± 1.23	63.49 ± 0.72
$APG_{C16/18}$ + IPA	11.77 ± 0.88	59.58 ± 0.67	3.79 ± 1.11
$APG_{C16/18}$ + PG	9.32 ± 0.81	45.12 ± 0.30	20.20 ± 1.60
$APG_{C16/18}$ + Gly	9.56 ± 0.49	22.45 ± 1.00	26.31 ± 0.95

[a]$APG_{C16/18}$ stands for cetearyl glucoside and cetearyl alcohol mixture, and $APG_{C20/22}$ for arachidyl glucoside and arachidyl alcohol and behenyl alcohol-mixed emulsifier

customary in pharmaceutical practice, has altered water distribution. For IPA- and PG-loaded samples, the highest mass loss was observed in the second temperature range (50–70 °C). This could be partially attributed to the volatility of the added solvents (namely, IPA) or more likely their infiltration in the lipid lamellae. On the other hand, the addition of glycerol to the APG-stabilised vehicles has induced certain equilibrium between interlamellarly fixed water and bulk water fractions.

Since an enhancer may become a retardant for a given drug depending on the chosen vehicle (Kaushik and Michniak-Kohn 2010), the vehicle in which the penetrant is dissolved or dispersed is of outmost importance, and it is evident that the drug's percutaneous penetration is controlled by the vehicle's physicochemical properties (Ghafourian et al. 2010). Therefore, comprehensive physicochemical characterisation is essential before proceeding to dermal availability studies.

14.2.2 Quantification of APG-Mediated Enhancing Effects: In Vitro and In Vivo Skin Absorption Studies

So far, permeation studies involving APG-stabilised vehicles have encompassed several model drugs: salicylic acid (Baudonnet et al. 2004), hydrocortisone (Savic et al. 2006, 2007; Pantelic et al. 2014b), diclofenac diethylamine (Vucinic-Milankovic et al. 2007; Pantelic et al. 2014c), ascorbic acid (Pakpayat et al. 2009), lycopene (Pepe et al. 2012), diclofenac sodium and caffeine (Savic et al. 2009), linoleic acid (Goebel et al. 2010), 5-fluorouracil (ElMeshad and Tadros 2011), tacrolimus (Goebel et al. 2011), ketoprofen (Jaksic et al. 2012), tretinoin (Manconi et al. 2006), miconazole nitrate (Peira et al. 2008), etc. APG-based delivery systems investigated in previously cited publications are summarised in Table 14.2, along with the applied characterisation method(s).

Although all of the outlined studies have found APG-stabilised systems to provide either enhanced or in general highly satisfactory drug delivery when compared to that achieved by appropriate reference samples, certain differences could be noticed depending on the selected model drug, indicating a need for further case-by-case studies. It was apparent that the APG colloidal structure strongly influences a drug's thermodynamic activity, providing an additional mechanism for percutaneous penetration enhancement. Also, the aforementioned similarity of the APG-mediated lamellar liquid crystalline structures with the inherent stratum corneum lipid matrix, suggests their considerable capacity for incorporation of both lipophilic and hydrophilic actives, as well as the ability to provide sustained skin hydration (Tasic-Kostov et al. 2011a).

Long has it been known that the presence or the extent of a penetration-enhancing effect cannot be claimed without knowledge of the drug's thermodynamic activity within the carrier (so-called push effect as opposed to the pull effect induced by chemical enhancers) (Bach and Lippold 1998). Here, once again the importance of physicochemical characterisation is brought to light. Even a straightforward technique such as (polarised) light microscopy may provide insight

Table 14.2 Review of the published percutaneous absorption studies involving APG-based drug delivery systems indicating the selected model drug and the applied evaluation method

Investigated APG	Model drug	Dosage form	Characterisation technique	Reference
Cetearyl glucoside and cetearyl alcohol	Salicylic acid	o/w emulsions	Diffusion cells/hydrophilic cellophane membrane	Baudonet et al. (2004)
Octyl-decyl polyglucoside or decyl polyglucoside	Tretinoin	Multilamellar (MLV) and Unilamellar (UV) vesicular formulations	Franz cells/newborn pig skin; followed by tape stripping	Manconi et al. (2006)
Cetearyl glucoside and cetearyl alcohol	Hydrocortisone	Creams	Franz cells/artificial skin constructs	Savic et al. (2006, 2007)
Sorbitan stearate and sucrose cocoate Cetearyl glucoside and cetearyl alcohol	Diclofenac diethylamine	o/w creams	VanKel enhancer cells/ regenerated cellulose membrane	Vucinic-Milankovic et al. (2007)
Decyl polyglucoside	Miconazole nitrate	Microemulsions	Franz cells/pig ear skin; followed by skin accumulation assessment	Peira et al. (2008)
Decyl glucoside and sorbitan monolaurate	Ascorbic acid	Microemulsions	Franz cells/pig ear skin	Pakpayat et al. (2009)
Cetearyl glucoside and cetearyl alcohol	Diclofenac sodium and caffeine	Creams	Franz cells/artificial skin constructs	Savic et al. (2009)
Lauryl glucoside and cetearyl glucoside	Linoleic acid	Microemulsions	Franz cells/excised human skin	Goebel et al. (2010)
Decyl glucoside	Tacrolimus	Microemulsions	Franz cells/excised human skin	Goebel et al. (2011)
Coco glucoside or decyl glucoside	5-fluorouracil	w/o emulsions	Franz cells/skin of newly born mice	ElMeshad and Tadros (2011)
Cetearyl glucoside and cetearyl alcohol	Ketoprofen	Multiphase creams	VanKel enhancer cells/ regenerated cellulose membrane Franz cells/artificial skin constructs Franz cells/excised human stratum corneum In vivo skin-stripping technique	Jaksic et al. (2012)
Decyl glucoside	Lycopene and ascorbic acid	Microemulsions	Franz cells/porcine ear skin, followed by tape stripping	Pepe et al. (2012)
Cetearyl glucoside and cetearyl alcohol	Diclofenac diethylamine	Multiphase creams	VanKel enhancer cells/ regenerated cellulose membrane Franz cells/artificial skin constructs Franz cells/excised human stratum corneum In vivo skin-stripping technique	Pantelic et al. (2014a)
Cetearyl glucoside and cetearyl alcohol	Hydrocortisone	Multiphase creams	In vivo skin-blanching assay; in vivo skin-stripping technique	Pantelic et al. (2014b)

into a drug's thermodynamic activity through determination of its saturation concentration, C_s.

Although penetration enhancement is often linearly correlated to cutaneous adverse reactions (Asbill and Michniak 2000), the mildness of these natural-origin emulsifiers provides an opportunity to investigate their prospective combinations with co-solvents/potential penetration enhancers that may result in a synergistic effect. These combinations offer the possibility of tailoring drug's thermodynamic activity within a vehicle by mere addition of a co-solvent. Cetearyl glucoside and cetearyl alcohol mixture, being the most extensively evaluated APG-mixed emulsifier, so far has been allied to glycerol (Pantelic et al. 2014b; Savic et al. 2009), propylene glycol (Savic et al. 2009) and isopropyl alcohol (Jaksic et al. 2012; Pantelic et al. 2014c) as commonly used co-solvents in dermopharmaceutics.

Apart from being frequently incorporated into pharmaceutical preparations as a co-solvent, due to its ability to bind and hold water, glycerol has proved to be an efficient humectant and moisturiser as well (Karadzovska et al. 2013). Savic et al. (2009) have compared diclofenac sodium and caffeine skin permeation from APG-based vehicles varying in glycerol and propylene glycol in concentrations up to 20 % (w/w). These model drugs were selected for their different physicochemical properties, i.e. a salt of a weak acid and a weak base, respectively. While propylene glycol proved to be an efficient enhancer in the case of diclofenac sodium, the same performance was not observed in the case of the nonelectrolyte model drug. However, in spite of the instrumentally recorded skin moisturisation, it is interesting to note that in both cases glycerol acted as a permeation retardant, rather than enhancer, stressing the importance of the applied vehicle composition.

Vehicles based on the same APG mixture were evaluated by Baudonet et al. (2004) as prospective carriers for salicylic acid. It was shown that depending on the moment of drug incorporation, various thermodynamic activities could be achieved leading to the release of more than 40 % of salicylic acid when the drug remained suspended throughout the vehicle. Although in pharmaceutical settings, the state (and content/concentration) of the drug within a vehicle is principally determined by its physicochemical properties and allowed therapeutic concentration, the addition of volatile components to the vehicle may easily alter its thermodynamic activity upon topical application, possibly even leading to supersaturation.

If taken that isopropyl alcohol penetration enhancement conveys the same basic principles attributed to ethanol, due to being a small polar molecule, this alcohol leads to penetration enhancement similarly to propylene glycol and dimethyl sulfoxide (DMSO), i.e. by disrupting the skin surface and thus reducing its barrier function. Furthermore, alcohols are known to extract lipids from cell membranes, while propylene glycol diffuses into the skin and ameliorates the solubility of these lipids to the penetrant (Karadzovska et al. 2013). Admittedly, these effects were observed while investigating absorption from vehicles containing as much as 50 % of these solvents or more, which is not likely to be the case with emulsion systems aiming for satisfactory long-term stability.

Isopropyl alcohol was a co-solvent of interest for Jaksic et al. (2012) and Pantelic et al. (2014b, c). The authors intended to formulate physicochemically stabile and yet aesthetically appealing emulsion systems (creams) stabilised with cetearyl glucoside and cetearyl alcohol that could successfully sustain diverse co-solvents, among them isopropyl alcohol in the amount of 10 % (w/w). The co-solvent's influence on thermodynamic activity of several drugs was subsequently evaluated (Fig. 14.3). In case of diclofenac diethylamine as the model drug, the addition of isopropyl alcohol induced a sevenfold increase in the calculated C_s values when isopropyl alcohol-loaded alkyl polyglucoside sample was compared to the reference one, stabilised with a conventional nonionic emulsifier. Since the alkyl polyglucoside-based vehicle itself (without the added co-solvents) managed to provide a 3.5-fold increase in C_s relative to the designated reference, the synergistic effect of the investigated alkyl polyglucoside-mixed emulsifier and isopropyl alcohol as the co-solvent on diclofenac

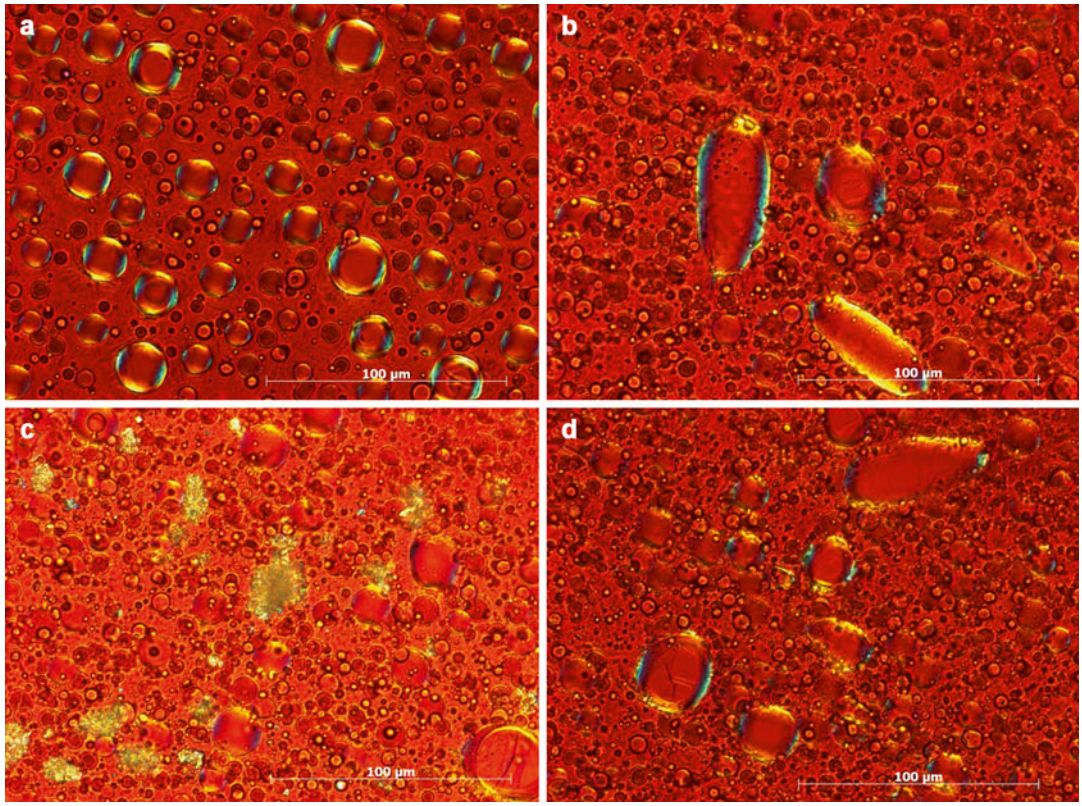

Fig. 14.3 Polarisation micrographs of cetearyl glucoside and cetearyl alcohol-stabilised samples as an illustration of the influence of isopropyl alcohol as the added co-solvent and potential penetration enhancer on thermodynamic activity of two model drugs (ketoprofen and diclofenac diethylamine): (**a**) basic APG sample, (**b**) APG sample loaded with 10 % (w/w) isopropyl alcohol, (**c**) APG sample with isopropyl alcohol and ketoprofen (2.5 % w/w) and (**d**) APG sample with isopropyl alcohol and diclofenac diethylamine (1.16 % w/w)

diethylamine thermodynamic activity is even more apparent. As for hydrocortisone, the second model drug used by this group of researchers, due to its low inherent solubility, all the samples remained to be of suspension type, i.e. the addition of the varied co-solvents/ potential penetration enhancers was not sufficient to significantly influence their thermodynamic activity, as a main driving force for drug diffusion from its delivery system (Mitriaikina and Müller-Goymann 2009; Moser et al. 2001). Nevertheless, certain differences in respective C_s values could still be observed, thus implying subtle tailoring of hydrocortisone thermodynamic activity.

Quantification of dermal absorption may be assessed by various in vitro and in vivo methods (Goi et al. 2010; Narkar 2010). Although in vitro methods are accepted to provide good approximation of the penetration/permeation occurring in vivo, preferable ranking order set by most regulatory agencies is: studies of pharmacodynamic effect > bioequivalence studies with clinical end points > in vitro studies (Tsai et al. 2004; Guidance 1995). However, only a few pharmacological groups of drugs possess a measurable pharmacodynamic response that could be used in such a manner. For other drugs, several in vivo methods are under evaluation, such as skin stripping, dermal microdialysis, NIR spectroscopy and skin biopsy (Boix-Montanes 2011). Apart from skin biopsy which is applied reluctantly, the remaining techniques are considered non-invasive and safe. Although microdialysis and NIR spectroscopy may offer more detailed information, distinguishing the fraction of the drug that has crystallised in the skin and the one

that is in solution (Mugglestone et al. 2012), these techniques require application of sophisticated and expensive equipment operated by trained researchers and, hence, appear hardly applicable routinely.

Therefore, tape-stripping technique stands out as a simple and robust in vivo method for skin absorption assessment which is considered non-invasive and yet reflects real in-use conditions of topical drug application (Pantelic et al. 2014d). Interest in the method was shown by regulatory agencies as well, providing a draft Guidance in 1998 (US FDA Guidance for Industry 1998). The suggested guideline was withdrawn mid-2002, due to discrepancies observed during validation testing by two independent laboratories. Still, the final protocol remains to be defined, as the results obtained by this method may be influenced by diverse intrinsic and external factors, stressing the need for further standardisation of the technique (Lademann et al. 2009; Dragicevic-Curic et al. 2010). However, tape stripping is continually being evaluated as a tool for bioavailability and bioequivalence assessment of topical products (Narkar 2010).

On the other hand, Franz diffusion cells are commonly considered an appropriate method for biopharmaceutical assessment of topical dosage forms. However, since it is postulated that drug penetration through the stratum corneum is the rate-limiting step, skin stripping is considered to be a useful supplementary technique, easily performed in vivo on human volunteers.

With this rational, Jaksic et al. (2012) have conducted a comprehensive skin absorption study of ketoprofen from cetearyl glucoside and cetearyl alcohol-stabilised creams, encompassing both in vitro and in vivo settings. Permeation and penetration profiles of four samples were compared: basic APG delivery system, APG system with 10 % (w/w) of isopropyl alcohol, reference cream stabilised with conventional nonionic emulsifiers (polysorbate 60 and cetostearyl alcohol), also with and without the same content of isopropyl alcohol. Statistically significant enhancement in ketoprofen penetration was observed for both APG creams relative to reference ones. However, this was only apparent during the in vivo assessment, implying the importance of the interaction of APG-based colloidal structure with the skin. As for the addition of isopropyl alcohol, although the synergistic effect with the colloidal structure was not as prominent as expected, a trend of enhanced ketoprofen permeation and penetration could be observed, especially several hours after the application. This was probably due to the relatively low concentration of the added alcohol.

This group of researchers (Pantelic et al. 2014b, c) continued their investigation of cetearyl glucoside and cetearyl alcohol mixture using diclofenac diethylamine and hydrocortisone as subsequent model drugs, thus covering three anti-inflammatory drugs of diverse physicochemical characteristics. In case of diclofenac diethylamine, isopropyl alcohol was chosen as the varied co-solvent and potential penetration enhancer, while hydrocortisone was additionally investigated with glycerol (20 % (w/w)) as well.

Diclofenac diethylamine samples (both alkyl polyglucoside-based and reference ones) were evaluated through several methods, taking into consideration that drug transport from the vehicle to the target site is literally a multistep process. Therefore, the evaluation commenced with in vitro methods. Enhancer cells equipped with synthetic membranes were deemed useful for the initial screening of diclofenac diethylamine liberation from the alkyl polyglucoside vehicles. The authors reported a statistically significant increase in diclofenac diethylamine release from the cetearyl glucoside- and cetearyl alcohol-stabilised sample loaded with isopropyl alcohol, relative to both reference samples (with or without isopropyl alcohol as the potential penetration enhancer).

This finding is in line with the aforementioned values of saturation concentration, C_s, underlining the combined effect of the specific APG-stabilised colloidal structure and certain co-solvents/penetration enhancers, such as isopropyl alcohol. In this case, it may be assumed that isopropyl alcohol as a volatile penetration enhancer rapidly evaporates from the application site, thus accelerating the initial drug liberation from the vehicle and its subsequent transfer to the skin.

Further, diclofenac diethylamine transport was evaluated via permeation studies using Franz diffusion cells and both artificial skin constructs (dermis equivalent was produced using human dermal fibroblasts and covered with an epidermis equivalent employing keratinocytes from a stable HaCaT-line (Savic et al. 2006)) and isolated stratum corneum (stratum corneum excised from the abdomen skin of a donor after plastic surgery). Naturally, the use of artificial skin constructs as the membrane separating donor and receptor compartments in permeation studies resulted in higher levels of diclofenac diethylamine relative to the isolated stratum corneum, which is generally considered to be a more relevant membrane. Therefore, as expected, only the permeation study across the isolated stratum corneum managed to discern differences between the assessed APG-stabilised formulations (with and without the addition of isopropyl alcohol), which appeared around the 13th hour of the experiment, indicating that an enhanced permeation of diclofenac diethylamine may be expected in the long run, but exclusively from the APG-stabilised bases with isopropyl alcohol.

Taking into consideration the basic arrangement of in vitro permeation studies, where the selected membrane tends to be exposed to the receptor medium for several hours (Dias et al. 2007), the contribution of some penetration enhancers may be easily overlooked. Therefore, whenever applicable, it is recommended to include an in vivo method to the overall study design. For that reason, Pantelic et al. (Pantelic et al. 2014c) concluded diclofenac diethylamine skin absorption assessment with a skin-stripping study, which can, unlike the previously discussed in vitro methods, provide information on skin penetration.

When diclofenac diethylamine concentration was plotted versus the relative stratum corneum depth profiles, statistical analysis revealed that the addition of isopropyl alcohol to the cetearyl glucoside and cetearyl alcohol-stabilised vehicle resulted in an increase in drug cutaneous penetration when compared to the sample without this penetration enhancer. The alkyl polyglucoside vehicle with isopropyl alcohol provided significantly higher drug levels at all depths into the stratum corneum, indicating that isopropyl alcohol as the added enhancer along with the specific alkyl polyglucoside colloidal structure have increased the extent of diclofenac diethylamine delivery. Still, the same could not be said for the rate of diclofenac diethylamine transport, because the formulation without isopropyl alcohol has managed to induce deeper penetration of the model drug.

Since chaotropic molecules such as diclofenac diethylamine possess a natural tendency to infiltrate interfacial areas and interact with polar moieties of the lipids, in case of alkyl polyglucoside-based liquid crystalline mesophases, this action may sustain drug delivery (Yariv et al. 2010). However, in the study reported by Pantelic et al. (2014c), the addition of isopropyl alcohol as a penetration enhancer to the cetearyl glucoside and cetearyl alcohol-stabilised samples has managed to induce an enhanced penetration in vivo. The authors awarded this to the ability of isopropyl alcohol to reduce the interface packing of the surfactant molecules.

On the other hand, skin absorption of hydrocortisone as the second model drug selected by Pantelic et al. (2014b) was assessed only in vivo. However, due to the corticosteroid nature of the drug, two in vivo methods were available for that purpose: skin-blanching assay and skin-stripping technique. Apart from isopropyl alcohol (10 % (w/w)), in this study, glycerol (20 % (w/w)) was also assessed as a potential penetration enhancer.

The skin-blanching response produced by the shortest drug exposure (1.5 h) of all the tested formulations showed that significant vasoconstriction was induced only by the cetearyl glucoside- and cetearyl alcohol-based formulation containing isopropyl alcohol. The same trend remained after the skin has been exposed for 3 h, with an enhanced response observed from the basic alkyl polyglucoside sample as well (without added penetration enhancers). After 6 h exposure, the isopropyl alcohol-loaded sample still remained the most efficacious, especially when compared to the sample containing glycerol as a co-solvent known to act as skin-hydrating agent as well.

It should be stressed that the reference sample loaded with 10 % (w/w) of isopropyl alcohol has

induced an increase in skin erythema observed after 3 h of application. Obviously, the conventional emulsifiers used for stabilisation of the reference sample were not able to prevent skin sensitisation induced by the addition of this penetration enhancer.

Permeation parameters obtained during this multipoint study (i.e. 1.5, 3 and 6 h dose durations) implied a highly satisfactory performance of the alkyl polyglucoside-stabilised vehicles when compared to reference ones.

After such a pharmacodynamic response-oriented study, tape-stripping technique was conducted as a dermatopharmacokinetic approach. In this case, significant difference in drug penetration was observed only for the alkyl polyglucoside sample containing isopropyl alcohol, when compared to the corresponding reference sample, which implies that the addition of isopropyl alcohol itself does not universally elicit penetration enhancement from any vehicle, suggesting favourable features of cetearyl glucoside and cetearyl alcohol-stabilised samples.

On the other hand, the addition of glycerol (20 % (w/w)) significantly influenced corneocytes cohesion which resulted in deeper hydrocortisone penetration, probably via skin moisturisation (Karadzovska et al. 2013). Although isopropyl alcohol probably induces a more aggressive impact on superficial corneocytes, glycerol provides a more profound effect. This is possibly founded on their evaporation rates (Kasting and Miller 2006).

ElMeshad and Tadros (2011) investigated whether transdermal delivery of 5-fluorouracil could be enhanced by APG-based w/o emulsions. Apart from coco glucoside or decyl glucoside, the assessed samples comprised lecithin, water, oleoyl macrogolglycerides and ethanol (declared ethanol content was 12.15–17.01 % w/w). The evaluated APGs showed high stability towards electrolyte addition, since their phase behaviour remained unchanged despite varying two different APGs. Although decyl glucoside-based systems showed significantly higher permeation parameters (namely, cumulative drug amount permeated after 24 h, steady-state flux and permeability coefficient), it was suggested that the increase in oil content might also influence thermodynamic activity of the drug, acting as a driving force for its release. The importance of the selected oily/lipid phase was also observed by Savic et al. (2007) while investigating the influence of oil polarity on hydrocortisone permeation. Skin-blanching assay implied that permeation of this topical corticosteroid was retarded in the presence of medium chain triglycerides, whereas less polar isopropyl myristate or non-polar light liquid paraffin enhanced it.

APG-stabilised microemulsions are also some of the extensively evaluated vehicles (Ryan and Kaler 2001). In spite of the lack of pharmaceutical microemulsions for dermal application on the market (Karadzovska et al. 2013), the interest in these thermodynamically stable carriers remains, due to their high solubilisation potential, among other qualities. However, a known drawback of these colloidal systems is their susceptibility to induce acute skin irritation due to high amounts of surfactants/co-surfactants. Therefore, their stabilisation with skin-friendly natural-origin surfactants such as APGs is considered a prospective concept for overcoming these safety issues.

Dermal delivery of ascorbic acid via microemulsions stabilised by decyl glucoside (as the surfactant) and sorbitan monolaurate (co-surfactant) combination was assessed by Pakpayat et al. (2009). The conducted permeation studies revealed the possibility of targeting specific skin layers: the sample labelled as ME 1 (surfactant/co-surfactant ratio 0.30/0.70) enabled ascorbic acid accumulation in stratum corneum and epidermis in satisfactory quantities (21.65 % of the applied ascorbic acid), while a simple reformulation has led to predominant localisation of ascorbic acid in the dermis and the receptor medium.

Favourable reports of APG-stabilised microemulsions as innovative drug carriers were also published by Goebel et al. (2010, 2011). With the aim of developing well-tolerated colloidal carrier systems for linoleic acid and tacrolimus, lauryl glucoside and cetearyl glucoside and decyl glucoside were selected as the investigated APGs, respectively. Penetration studies were performed ex vivo through excised human breast skin from reduction mammoplasty (full-thickness skin).

The obtained results were compared to those obtained for the reference samples: a linoleic acid-loaded w/o cream and commercially available tacrolimus ointment (Protopic®, Astellas Pharma GmbH, Munich, Germany). Both model drugs showed significantly enhanced permeation profiles from APG-based microemulsions. Tacrolimus-loaded microemulsions resulted in higher concentrations in deeper skin layers in comparison to the applied standard vehicle, while linoleic acid penetrated the stratum corneum (3.64 ± 0.10 %) and the viable epidermis (7.37 ± 1.50 %) in significantly higher amounts ($p < 0.01$) when compared to the reference cream.

Naturally, when considering the ongoing trend in nanosystems, efforts have been made in developing APG-stabilised nanocarriers. However, in spite of initially promising results, these sophisticated systems still leave a lot to be desired, especially concerning stability and drug entrapment efficiency. Nevertheless, these sugar-based emulsifiers are even being assessed for stabilisation of peptide and protein therapeutics (Graf et al. 2008; Patel et al. 2008).

Recently, Keck et al. (2014) investigated various alkyl polyglucoside surfactants as prospective stabilisers for solid lipid nanoparticles (SLN) intended for dermal application. After caprylyl/capryl glucoside and coco glucoside proved to be suitable for stabilisation of both SLN and nanostructured lipid carrier (NLC) dispersions (Kovacevic et al. 2011, 2014), C8-10 fatty alcohol glucoside, C8-14 fatty alcohol glucoside, C10-16 fatty alcohol glucoside and C12-16 fatty alcohol glucoside were subsequently investigated for such purpose. Obtained results imply that alkyl polyglucosides with short to medium alkyl chain lengths possess optimal characteristics for stabilisation of SLN dispersions. However, their efficacy in drug delivery is yet to be seen.

14.2.3 Skin Tolerance Assessment: In Vitro and In Vivo Safety Evaluation

In spite of being provided with such an outstanding barrier for percutaneous penetration such as stratum corneum, skin is nowadays exceedingly prone to manifesting adverse reactions (Menon et al. 2012). While the researchers are aiming to discern effective carriers for dermal/transdermal administration, in most cases, this is accomplished along with a linear correlation between penetration enhancement and skin irritation (Asbill and Michniak 2000). Having in mind that the thickness of the stratum corneum barrier varies depending on the anatomic location of the body (Aungst 2012), development of a universal, well-tolerated formulation with satisfactory drug delivery becomes a challenge. Additionally, the highest concentration of an enhancer that can be applied to the skin is limited by safety issues (Mitragotri 2000). When considering the safety aspect of penetration enhancement, it is considered acceptable to temporarily weaken the skin barrier resistance (Novotný et al. 2009). Still, an ideal penetration enhancer should be effective and yet non-irritating, since stratum corneum takes as much as 25–30 days to regenerate (Kang et al. 2007).

Naturally, in order to be approved for pharmaceutical applications, safety of each emulsifier as prospective pharmaceutical excipient must be well demonstrated. This is commonly done by the manufacturer and/or interested researchers and further validated by the appropriate regulatory agencies. Nevertheless, cutaneous reactions are sometimes observed only after topical application of the final preparation, being a manifestation of a complex interplay between its constituents.

For surfactants in general, the irritation potential could be related to competitive hydrogen bonding that may alter the native hydrogen bonding in proteins and lead to their unfolding (Subedi et al. 2010). Having in mind the displayed structure of APGs, such a mechanism for inducing irritation could be possible. However, these concerns were soon cast aside, proving this class of surfactants to have highly satisfactory dermatological properties (Pezron et al. 1996).

When considering mixed surfactants, which are commonly employed in pharmaceutical emulsion systems in search for satisfactory physical stability, skin irritation cannot be regarded as a simple additive effect. While investigating these

natural-origin emulsifiers, it was shown that mixtures of sodium lauryl sulphate (SLS) and an APG resulted in significantly lower irritation potential than observed by SLS alone (Hall-Manning et al. 1998).

Highly informative method for gathering initial safety data is an in vitro skin irritation test commonly referred to as the cytotoxicity assay. Here, artificial skin constructs such as HaCaT cell lines are being exposed to a range of test samples' concentrations, after which a modified Mosmann's MTT reduction method is applied (Faller et al. 2002; Mosmann 1983). Although for topical preparations a cut-off limit defined for relative cell viability is 50 %, contemporary pharmaceutical preparations should aim for more favourable cytotoxicity endpoints. While evaluating safety potential of relatively simple cetearyl glucoside and cetearyl alcohol (8 % w/w) creams varying in the addition of isopropyl alcohol (10 % w/w), propylene glycol (20 % w/w) and glycerol (20 % w/w) as common pharmaceutical co-solvents and potential penetration enhancers, it was observed that APG-stabilised colloidal structure managed to preserve satisfactory cell viabilities, most of which were over 80 % (Fig. 14.4). It is interesting to note that both the basic formulation (here labelled APG) and the isopropyl alcohol-loaded one (APG + IPA) showed excellent results irrespective of the applied sample concentration, which could only be attributed to the prevailing influence of the vehicle itself (Jaksic et al. 2012). Naturally, when evaluating active samples, the presence of the drug inevitably influences cytotoxicity results as well (Savic et al. 2009).

In spite of the fact that the cytotoxicity evaluation conducted on such three-dimensional human skin models is considered to mimic well the conditions cells experience in vivo (Wiegand and Hipler 2009), whenever possible results should be discussed in correlation with those obtained by subsequent in vivo skin performance evaluation. Although even in the recent publications readers could come across visual assessment of skin irritation (ElMeshad and Tadros 2011), researchers have various instrumental non-invasive in vivo bioengineering techniques at their disposal such as measurement of transepidermal water loss (TEWL), stratum corneum hydration level (SCH), electrical capacitance (EC), skin pH and erythema index (EI).

In vivo performance of various APG-stabilised creams was extensively evaluated by Savic et al. (2007, 2009, 2011). Since most of the publications relate acute skin irritation potential, rather than cumulative irritancy which is more appropriate for real in-use conditions (Hall-Manning

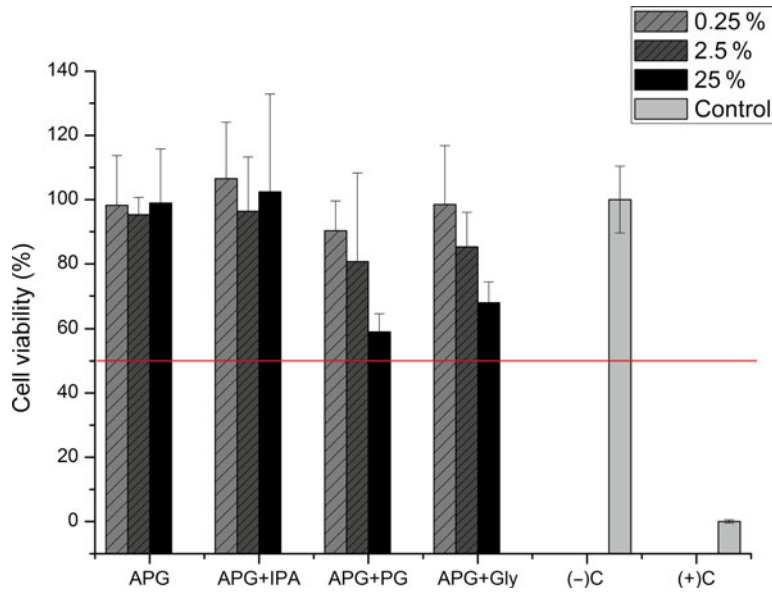

Fig. 14.4 In vitro cytotoxicity assay performed on cetearyl glucoside and cetearyl alcohol-stabilised bases varying in co-solvent addition: concentration-viability histograms of basic sample (*APG*), isopropyl alcohol- (*APG* + *IPA*), propylene glycol- (*APG* + *PG*) and glycerol-loaded samples (*APG* + *Gly*), along with pure Krebs-Ringer buffer (KRB) and 1 % SLS in KRB as negative (−*C*) and positive controls (+*C*), respectively. *Horizontal line* indicates the accepted 50 % cut-off limit for cell viability

et al. 1998), these studies were performed under occlusion in order to additionally enhance the possible irritation effects. However, with such study design, one should keep in mind that the occlusion itself generally modifies the assessed in vivo parameters. Hence, for valid conclusions, suitable non-treated control sites should be tested as well. The obtained results confirmed the mild nature of the APG-based samples indicated by the aforementioned in vitro assay, since all samples induced a decrease in EI values compared to a non-treated control without occlusion. However, it should be noted that the recorded decrease could not be considered statistically significant. On the other hand, APG samples varying in propylene glycol and glycerol have induced a significant increase in SCH and TEWL, unrelated to the occlusion effects. This is a clear confirmation of the penetration-modifying abilities of the two polyols within the APG-based colloidal structure. Yet, due to the known different mechanisms of action, permeation studies further elaborated that propylene glycol acted as penetration enhancer, while glycerol proved to be a retardant.

Recently, prospective use of APGs as stabilisers for highly acidic actives was investigated by Tasic-Kostov et al. (2010, 2011a, b, 2012). In such cases, formulating stable emulsion systems is challenging and mainly relies on conventional acid-stable nonionic polyethylene glycol (PEG) emulsifiers, like ethoxylated fatty alcohols and fatty amphiphiles. This issue was further assessed using two acidic dermopharmaceutic/dermocosmetic actives: glycolic acid as the representative of alphahydroxyacids (AHAs) and lactobionic acid as a polyhydroxy acid (PHA) (Green et al. 2009; Yu and Van Scott 2004). These acidic actives were incorporated in various APG bases and suitable reference carriers (commonly used oil-in-water creams and water-based xanthan gum gels) in the concentration of 6 % (w/w) of glycolic, and 6 or 10 % (w/w) of lactobionic acid. Expectedly, the inclusion of highly acidic actives lowered the pH values of the prepared samples, indicating the necessity for their neutralisation should the preparations be in pH range acceptable for topical application. The following APG emulsifiers were evaluated: C12/14 (cetearyl alcohol and coco glucoside) co-stabilised with myristyl alcohol and myristyl glucoside (Tasic-Kostov et al. 2010, 2011a) and C16/18 (cetearyl alcohol and cetearyl glucoside) (Tasic-Kostov et al. 2012). In their work, the authors mainly focused on stability concerns of such emulsion systems, as well as their skin performance. Although the evaluated acids influenced the characteristic anisotropy of the APG-stabilised systems, it is interesting to note that certain lamellar liquid crystalline structures were even more promoted by the presence of lactobionic acid and its salts that formed after pH adjustment. Along with the favourable results of other physicochemical characterisation, this suggested satisfactory APG capacity to stabilise emulsion systems at very low pH values. Moreover, satisfactory safety profile and moisturising capacity was observed even in case of high concentrations of incorporated lactobionic acid (10 % w/w). In vitro findings showed acceptable cell viability, while in vivo studies implied ameliorated skin performance when compared to the assessed reference carriers.

Finally, several studies showed favourable safety profile of APG-based microemulsions, as vehicles likely to exert irritation potential (Goebel et al. 2010, 2011). For these systems, skin tolerability is usually investigated through hen's egg test (HET) using chorioallantoic membrane (CAM).

In 2007, Mehling et al. (2007) published a comprehensive study on the irritation potential of 18 surfactants, among which were three surfactants of alkyl polyglucoside type: coco glucoside, lauryl glucoside and decyl glucoside. The ocular irritation potential of surfactants was studied using the red blood cell test (RBC), HET-CAM and the Skinethic ocular tissue model, while the skin irritation potential was assessed after conducting a 24 h epicutaneous patch test (ECT) and a soap chamber test (SCT) on human volunteers. In all experiments, alkyl polyglucosides were among the mildest surfactants, their influence classified either as not irritating or slightly irritating. This is in line with the recently published study by Gijbels et al. (2014) who compiled data of nearly 12,000 patients with suspected contact dermatitis from the early 1990s. In

only 30 patients, alkyl polyglucosides were acknowledged as culprits for skin sensitisation.

14.3 Concluding Remarks and Future Prospects

Albeit many diverse substances have been investigated for penetration-enhancing properties, to date none has been discerned as ideal. Driven by demand for naturals, progressive replacement of synthetic materials with 'green' alternatives has distinguished a group of alkyl polyglucoside surfactants. Research conducted so far has suggested several possible mechanisms of APG-mediated percutaneous penetration enhancement which are carefully elaborated throughout the chapter:

- The observed similarity between complex structures which dominate APG-stabilised systems (mainly lamellar liquid crystal and lamellar gel phases) and inherent stratum corneum lipid ordering could enhance penetration on skin contact due to optimal balance between their order and fluidity.
- The complex APG-stabilised colloidal structure may lead to diverse modes of water distribution within these systems, providing sustained skin hydration and hence improved skin permeability.
- The impact on a drug's thermodynamic activity, easily tailored by the addition of various co-solvents/potential penetration enhancers.

Despite the fact that a vast array of membranes used in the reported permeation studies (synthetic, animal, human, etc.) sometimes makes the extrapolation of the obtained results difficult, almost each protocol reported enhanced delivery of APG-based drug delivery systems as opposed to an appropriate reference. Whether one or all of the proposed mechanisms stand behind the described highly satisfactory drug delivery by the APG-based carriers, another advantage of these emulsifiers lays in their excellent dermatological properties. The reported mildness of APGs opens an exciting possibility to combine them with various chemical enhancers.

Nevertheless, in spite of the comprehensive characterisation of some of the APG representatives, certain structure-activity relationships are still poorly understood. Only with a comprehensive knowledge of these emulsifiers and the containing systems it is possible to design effective and safe drugs. In effort to commit itself to principles of sustainable development, pharmaceutical industry will need to rapidly consider inclusion of these natural-origin emulsifiers in dermatological practice. Future studies will inevitably focus on the prospect of developing more advanced delivery systems such as APG-stabilised nanocarriers, as well as the comprehensive characterisation of newly synthesised APGs. Additionally, effort should be made to incorporate these novel carriers such as microemulsions, nanoemulsions and nanoparticles in some patient-friendly dosage forms.

Acknowledgments The authors would like to acknowledge the financial support provided by the Ministry of Education and Science, Republic of Serbia, through project TR 34031.

References

Allen DK, Tao BY (1999) Carbohydrate-alkyl ester derivatives as biosurfactants. J Surfact Deterg 2(3):383–390

Asbill CS, Michniak BB (2000) Percutaneous penetration enhancers: local versus transdermal activity. Pharm Sci Technolo Today 3(1):36–41

Aungst BJ (2012) Absorption enhancers: applications and advances. AAPS J 14(1):10–18

Bach M, Lippold BC (1998) Percutaneous penetration enhancement and its quantification. Eur J Pharm Biopharm 46:1–13

Baudonnet L, Grossiord J-L, Rodriguez F (2004) Physicochemical characterization and in vitro release of salicylic acid from O/W emulsions prepared with montanov 68: effect of formulation parameters. Drug Dev Ind Pharm 30(9):975–984

Boix-Montanes A (2011) Relevance of equivalence assessment of topical products based on the dermatopharmacokinetics approach. Eur J Pharm Sci 42:173–179

Bolzinger M-A, Briançon S, Pelletier J, Chevalier Y (2012) Penetration of drugs through skin, a complex rate-controlling membrane. Curr Opin Colloid Interface Sci 17:156–165

Casiraghi A, Di Grigoli M, Cilurzo F, Gennari CGM, Rossoni G, Minghetti P (2012) The influence of the

polar head and the hydrophobic chain on the skin penetration enhancement effect of poly(ethylene glycol) derivatives. AAPS PharmSciTech 13(1):247–253
Castro M, Fernández Cirelli A, Kovensky J (2006) Synthesis and interfacial properties of sugar-based surfactants composed of homo- and heterodimers. J Surfact Deterg 9:279–286
Dias M, Hadgraft J, Lane M (2007) Influence of membrane–solvent–solute interactions on solute permeation in model membranes. Int J Pharm 336:108–114
Dragicevic-Curic N, Winter S, Stupar M, Milic J, Krajisnik D, Gitter B, Fahr A (2009) Temoporfin-loaded liposomal gels: viscoelastic properties and in vitro skin penetration. Int J Pharm 373:77–84
Dragicevic-Curic N, Gräfe S, Gitter B, Winter S, Fahr A (2010) Surface charged temoporfin-loaded flexible vesicles: in vitro skin penetration studies and stability. Int J Pharm 384:100–108
Eccleston GM (1990) Multiple-phase oil-in-water emulsions. J Soc Cosmet Chem 41:1–22
Eccleston GM (1997) Functions of mixed emulsifiers and emulsifying waxes in dermatological lotions and creams. Colloids Surf A Physicochem Eng Asp 123–124:169–182
ElMeshad AN, Tadros MI (2011) Transdermal delivery of an anti-cancer drug via w/o emulsions based on alkyl polyglucosides and lecithin: design, characterization, and in vitro evaluation of the possible irritation potential in rats. AAPS PharmSciTech 12(1):1–9
Faller C, Bracher M, Dami N, Roguet R (2002) Predictive ability of reconstructed human epidermis equivalents for the assessment of skin irritation of cosmetics. Toxicol In Vitro 16:557–572
Garcia MT, Ribosa I, Campos E, Sanchez LJ (1997) Ecological properties of alkylglucosides. Chemosphere 35(3):545–556
Ghafourian T, Samaras EG, Brooks JD, Riviere JE (2010) Validated models for predicting skin penetration from different vehicles. Eur J Pharm Sci 41:612–616
Gijbels D, Timmermans A, Serrano P, Verreycken E, Goossens A (2014) Allergic contact dermatitis caused by alkyl glucosides. Contact Dermatitis 70:175–182
Goebel A, Knie U, Abels C, Wohlrab J, Neubert R (2010) Dermal targeting using colloidal carrier systems with linoleic acid. Eur J Pharm Biopharm 75:162–172
Goebel A, Neubert R, Wohlrab J (2011) Dermal targeting of tacrolimus using colloidal carrier systems. Int J Pharm 404:159–168
Goi N, Morishita K, Taniguchi A, Ishii T, Saitoh K (2010) Evaluation of percutaneous permeation of flurbiprofen and ketoprofen after application of transdermal patches using a lateral sectioning approach in hairless rats. Pharm Dev Technol 15(6):658–665
Graf A, Ablinger E, Peters S, Zimmer A, Hook S, Rades T (2008) Microemulsions containing lecithin and sugar-based surfactants: nanoparticle templates for delivery of proteins and peptides. Int J Pharm 350:351–360
Green B, Yu RJ, Van Scott EJ (2009) Clinical and cosmeceutical uses of hydroxyacids. Clin Dermatol 27:495–501
Hadgraft J (1999) Passive enhancement strategies in topical and transdermal drug delivery. Int J Pharm 184:1–6
Hall-Manning TJ, Holland GH, Rennie G, Revell P, Hines J, Barratt MD, Basketter DA (1998) Skin irritation potential of mixed surfactant systems. Food Chem Toxicol 36:233–238
Iglauer S, Wu Y, Shuler P, Tang Y, Goddard WA III (2009) Alkyl polyglycoside surfactant-alcohol cosolvent formulations for improved oil recovery. Colloids Surf A Physicochem Eng Asp 339:48–59
Jaksic I, Lukic M, Malenovic A, Reichl S, Hoffmann C, Müller-Goymann C, Daniels R, Savic S (2012) Compounding of a topical drug with prospective natural surfactant-stabilized pharmaceutical bases: physicochemical and in vitro/in vivo characterization – a ketoprofen case study. Eur J Pharm Biopharm 80:164–175
Junginger HE (1997) Multiphase emulsions. In: Rieger MM, Rhein LD (eds) Surfactants in cosmetics. Marcel Dekker, New York, pp 155–182
Jurado E, Vicaria JM, Fernandez-Arteaga A, Chachalis P, Garcia-Martin JF (2010) Wetting power in aqueous mixtures of alkylpolyglucosides and ethoxylated fatty alcohols. J Surfact Deterg 13:497–501
Kang L, Poh AL, Fan SK, Ho PC, Chan YW, Chan SY (2007) Reversible effects of permeation enhancers on human skin. Eur J Pharm Biopharm 67:149–155
Karadzovska D, Brooks JD, Monteiro-Riviere NA, Riviere JE (2013) Predicting skin permeability from complex vehicles. Adv Drug Deliv Rev 65(2):265–277
Kasting GB, Miller MA (2006) Kinetics of finite dose absorption through skin 2: volatile compounds. J Pharm Sci 95:268–280
Kaushik D, Michniak-Kohn B (2010) Percutaneous penetration modifiers and formulation effects: thermal and spectral analyses. AAPS PharmSciTech 11(3):1068–1083
Keck CM, Kovacevic A, Müller RH, Savic S, Vuleta G, Milic J (2014) Formulation of solid lipid nanoparticles (SLN): the value of different alkyl polyglucoside surfactants. Int J Pharm 474(1–2):33–41
Konya M, Sorrenti M, Ferrari F, Rossi S, Csoka I, Caramella C, Bettinetti G, Eros I (2003) Study of the microstructure of oil/water creams with thermal and rheological methods. J Therm Anal Calorim 73:623–632
Konya M, Bohus P, Paglino L, Csoka I, Csanyi E, Eros I (2004) Coherent emulsions containing alkylpolyglucoside esters as emulsifiers. Prog Colloid Polym Sci 125:161–166
Kovacevic A, Savic S, Vuleta G, Müller RH, Keck CM (2011) Polyhydroxy surfactants for the formulation of lipid nanoparticles (SLN and NLC): effects on size, physical stability and particle matrix structure. Int J Pharm 406(1–2):163–172
Kovacevic A, Müller RH, Savic S, Vuleta G, Keck CM (2014) Solid lipid nanoparticles (SLN) stabilized with polyhydroxy surfactants: preparation, characterization and physical stability investigation. Colloids Surf A Physicochem Eng Asp 444:15–25

Kühn A, Neubert R (2004) Characterization of mixtures of alkyl polyglycosides (plantacare) by liquid chromatography-electrospray ionization quadrupole time-of-flight mass spectrometry. Pharm Res 21(12):2347–2353

Lademann J, Jacobi U, Surber C, Weigmann H-J, Fluhr JW (2009) The tape stripping procedure- evaluation of some critical parameters. Eur J Pharm Biopharm 72:317–323

Lagerwall J, Scalia G (2012) A new era for liquid crystal research: applications of liquid crystals in soft matter nano-, bio- and microtechnology. Curr Appl Phys 12:1387–1412

Li C, Liu C, Liu J, Fang L (2011) Correlation between rheological properties, in vitro release, and percutaneous permeation of tetrahydropalmatine. AAPS PharmSciTech 12(3):1002–1010

Lopez A, Llinares F, Cortell C, Herraez M (2000) Comparative enhancer effects of Span®20 with Tween®20 and Azone® on the in vitro percutaneous penetration of compounds with different lipophilicities. Int J Pharm 202:133–140

Lourith N, Kanlayavattanakul M (2009) Natural surfactants used in cosmetics: glycolipids. Int J Cosmet Sci 31:255–261

Lukic M, Pantelic I, Daniels R, Müller-Goymann C, Savic M, Savic S (2013) Moisturizing emulsion systems based on the novel long-chain alkyl polyglucoside emulsifier the contribution of thermoanalytical methods to the formulation development. J Therm Anal Calorim 111:2045–2057

Lukic M, Pantelic I, Savic S (2014) Emulsion systems: from stability concerns to sensory properties. In: Pantelic I (ed) Alkyl polyglucosides: from natural-origin surfactants to prospective delivery systems. Woodhead Publishing, Cambridge, pp 73–105

Manconi M, Sinico C, Valenti D, Lai F, Fadda AM (2006) Niosomes as carriers for tretinoin III. A study into the in vitro cutaneous delivery of vesicle-incorporated tretinoin. Int J Pharm 311:11–19

Mehling A, Kleber M, Hensen H (2007) Comparative studies on the ocular and dermal irritation potential of surfactants. Food Chem Toxicol 45:747–758

Menon GK, Cleary GW, Lane ME (2012) The structure and function of the stratum corneum. Int J Pharm 435(1):3–9

Mitragotri S (2000) Synergistic effect of enhancers for transdermal drug delivery. Pharm Res 17(11):1354–1359

Mitriaikina S, Müller-Goymann CC (2009) Comparative permeation studies of non-diluted and diluted betamethasone-17-valerate semisolid formulations through isolated human stratum corneum and artificial skin constructs. Skin Pharmacol Physiol 22:142–150

Moser K, Kriwet K, Naik A, Kalia Y, Guy R (2001) Passive skin penetration enhancement and its quantification in vitro. Eur J Pharm Biopharm 52:103–112

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63

Mugglestone C, Mariz S, Lane M (2012) The development and registration of topical pharmaceuticals. Int J Pharm 435:22–26

Narkar Y (2010) Bioequivalence for topical products – an update. Pharm Res 27:2590–2601

Novotný J, Kovaříková P, Novotný M, Janůšová B, Hrabálek A, Vávrová K (2009) Dimethylamino acid esters as biodegradable and reversible transdermal permeation enhancers: effects of linking chain length, chirality and polyfluorination. Pharm Res 26(4):811–821

Pakpayat N, Nielloud F, Fortuné R, Tourne-Peteilh C, Villarreal A, Grillo I, Bataille B (2009) Formulation of ascorbic acid microemulsions with alkyl polyglycosides. Eur J Pharm Biopharm 72:444–452

Pantelic I, Cuckovic B (2014) Alkyl polyglucosides: an emerging class of sugar surfactants. In: Pantelic I (ed) Alkyl polyglucosides: from natural-origin surfactants to prospective delivery systems. Woodhead Publishing, Cambridge, pp 1–19

Pantelic I, Lukic M, Vuleta G, Savic S (2014a) Towards alkyl polyglucoside-stabilized formulations: influence of some common excipients. In: Pantelic I (ed) Alkyl polyglucosides: from natural-origin surfactants to prospective delivery systems. Woodhead Publishing, Cambridge, pp 53–72

Pantelic I, Lukic M, Markovic B, Daniels R, Vesic S, Vuleta G, Savic S (2014b) Effect of small changes in natural origin-based emulsion systems on hydrocortisone skin absorption and performance: a comparison of two in vivo methods. Pharm Dev Technol 19(1):55–64

Pantelic I, Lukic M, Markovic B, Lusiana, Hoffmann C, Müller-Goymann C, Milic J, Daniels R, Savic S (2014c) Development of a prospective isopropyl alcohol-loaded pharmaceutical base using simultaneous in vitro/in vivo characterization methods of skin performance. Drug Dev Ind Pharm 40(7):960–971

Pantelic I, Lukic M, Daniels R, Savic S (2014d) Alkyl polyglucoside-based delivery systems: in vitro/in vivo skin absorption assessment. In: Pantelic I (ed) Alkyl polyglucosides: from natural-origin surfactants to prospective delivery systems. Woodhead Publishing, Cambridge, pp 107–134

Patel AR, Joshi VY (2008) Evaluation of SLS: APG mixed surfactant systems as carrier for solid dispersion. AAPS PharmSciTech 9(2):583–590

Patel AR, Kulkami S, Nandeker TD, Vavia PR (2008) Evaluation of alkyl polyglucoside as an alternative surfactant in the preparation of peptide-loaded nanoparticles. J Microencapsul 25(8):531–540

Peira E, Carlotti ME, Trotta C, Cavalli R, Trotta M (2008) Positively charged microemulsions for topical application. Int J Pharm 346:119–123

Pepe D, Phelps J, Lewis K, Dujack J, Scarlett K, Jahan S, Bonnier E, Milic-Pasetto T, Hass MA, Lopes LB (2012) Decylglucoside-based microemulsions for cutaneous localization of lycopene and ascorbic acid. Int J Pharm 434(1-2):420–428

Pezron I, Galet L, Clausse D (1996) Surface interaction between a protein monolayer and surfactants and its correlation with skin irritation by surfactants. J Colloid Interface Sci 180:285–289

Piacquadio D, Kligman A (1998) The critical role of the vehicle to therapeutic efficacy and patient compliance. J Am Acad Dermatol 39:67–73

Piispanen P, Persson M, Claesson P, Norin T (2004) Surface properties of surfactants derived from natural products. Part 1: syntheses and structure/property relationships—solubility and emulsification. J Surfact Deterg 7(2):147–159

Ryan LD, Kaler EW (2001) Alkyl polyglucoside microemulsion phase behaviour. Colloids Surf A Physicochem Eng Asp 176:69–83

Savic S, Vuleta G, Daniels R, Müller-Goymann C (2005) Colloidal microstructure of binary systems and model creams stabilized an alkylpolyglucoside non-ionic emulsifier. Colloid Polym Sci 283:439–451

Savic S, Savic M, Vesic S, Vuleta G, Müller-Goymann CC (2006) Vehicles based on a sugar surfactant: colloidal structure and its impact on in vitro/in vivo hydrocortisone permeation. Int J Pharm 320:86–95

Savic S, Savic M, Tamburic S, Vuleta G, Vesic S, Müller-Goymann CC (2007) An alkylpolyglucoside surfactant as a prospective pharmaceutical excipient for topical formulations: the influence of oil polarity on the colloidal structure and hydrocortisone in vitro/in vivo permeation. Eur J Pharm Sci 30:441–450

Savic S, Weber C, Tamburic S, Savic M, Müller-Goymann C (2009) Topical vehicles based on natural surfactant/fatty alcohols mixed emulsifier: the influence of two polyols on the colloidal structure and in vitro/in vivo skin performance. J Pharm Sci 98:2073–2090

Savic S, Tamburic S, Savic M (2010) From conventional towards new – natural surfactants in drug delivery systems design: current status and perspectives. Expert Opin Drug Deliv 7:353–369

Savic S, Lukic M, Jaksic I, Reichl S, Tamburic S, Müller-Goymann C (2011) An alkyl polyglucoside-mixed emulsifier as stabilizer of emulsion systems: the influence of colloidal structure on emulsions skin hydration potential. J Colloid Interface Sci 358:182–191

Savic S, Pantelic I, Lukic M, Markovic B, Milic J (2014) Behind the alkyl polyglucoside-based structures: lamellar liquid crystalline and lamellar gel phases in different emulsion systems. In: Pantelic I (ed) Alkyl polyglucosides: from natural-origin surfactants to prospective delivery systems. Woodhead Publishing, Cambridge, pp 21–52

Schaefer H, Redelmeier TE (1996) Skin barrier: principles of percutaneous absorption. Karger, Basel

Shokri J, Nokhodchi A, Dashbolaghi A, Hassan-Zadeh D, Ghafourian T, Barzegar Jalali M (2001) The effect of surfactants on the skin penetration of diazepam. Int J Pharm 228:99–107

Stubenrauch C (2001) Sugar surfactants – aggregation, interfacial, and adsorption phenomena. Curr Opin Colloid Interface Sci 6:160–170

Subedi RK, Oh SY, Chun M-K, Choi H-K (2010) Recent advances in transdermal drug delivery. Arch Pharm Res 33(3):339–351

Suhonen TM, Bouwstrab JA, Urtti A (1999) Chemical enhancement of percutaneous absorption in relation to stratum corneum structural alterations. J Control Release 59:149–161

Tadros T (2004) Application of rheology for assessment and prediction of the long-term physical stability of emulsions. Adv Colloid Interface Sci 108–109:227–258

Tasic-Kostov M, Savic S, Lukic M, Tamburic S, Pavlovic M, Vuleta G (2010) Lactobionic acid in a natural alkylpolyglucoside-based vehicle: assessing safety and efficacy aspects in comparison to glycolic acid. J Cosmet Dermatol 9:3–10

Tasic-Kostov M, Reichl S, Lukic M, Jaksic I, Savic S (2011a) Does lactobionic acid affect the colloidal structure and skin moisturizing potential of the alkyl polyglucoside-based emulsion systems? Pharmazie 66:862–870

Tasic-Kostov M, Reichl S, Lukic M, Jaksic I, Savic S (2011b) Two alkyl polyglucoside natural surfactants varying in the chain length in stabilization of lactobionic acid containing emulsions: physicochemical characterization and in vitro irritation potential assessment. Riv Ital Sostanze Gr 88(4):256–264

Tasic-Kostov M, Pavlovic D, Lukic M, Jaksic I, Arsic I, Savic S (2012) Lactobionic acid as antioxidant and moisturizing active in alkyl polyglucoside-based topical emulsions: the colloidal structure, stability and efficacy evaluation. Int J Cosmet Sci 34:424–434

Tasic-Kostov M, Vesic S, Savic S (2014) Objective skin performance evaluation: how mild are APGs to the skin? In: Pantelic I (ed) Alkyl polyglucosides: from natural-origin surfactants to prospective delivery systems. Woodhead Publishing, Cambridge, pp 135–161

Tsai J-C, Cheng C-L, Tsai Y-F, Sheu H-M, Chou C-H (2004) Evaluation of in vivo bioequivalence methodology for topical clobetasol 17-propionate based on pharmacodynamic modeling using Chinese skin. J Pharm Sci 93:207–217

US FDA Guidance (1995) Topical dermatologic corticosteroids: in vivo bioequivalence. Available from: http://www.fda.gov/cder/guidance/dd098fn.pdf. 2 June 1995

US FDA Guidance for Industry (1998) Topical dermatological drug product NDAs and ANDAs —In vivo bioavailability, bioequivalence, in vitro release, and associated studies. Draft guidance. Available from: http://www.fda.gov/ohrms/dockets/ac/00/backgrd/3661b1c.pdf

Vucinic-Milankovic N, Savic S, Vuleta G, Vucinic S (2007) The physicochemical characterization and in vitro/in vivo evaluation of natural surfactants-based emulsions as vehicles for diclofenac diethylamine. Drug Dev Ind Pharm 33:221–234

Wiegand C, Hipler U-C (2009) Evaluation of biocompatibility and cytotoxicity using keratinocyte and fibroblast cultures. Skin Pharmacol Physiol 22:74–82

Williams AC, Barry BW (2004) Penetration enhancers. Adv Drug Deliv Rev 56:603–618

Yariv D, Efrat R, Libster D, Aserin A, Garti N (2010) In vitro permeation of diclofenac salts from lyotropic liquid crystalline systems. Colloids Surf B Biointerfaces 78:185–192

Yu RJ, Van Scott EJ (2004) Alpha-hydroxyacids and carboxylic acids. J Cosmet Dermatol 3:76–87

Zgola-Grzeskowiak A, Grzeskowiak T, Franska M, Rzasa A, Lukaszewski Z (2008) Investigations on the biodegradation of alkylpolyglucosides by means of liquid chromatography–electrospray mass spectrometry. Biodegradation 19:635–642

15 Nonionic Surfactants: Polyethylene Glycol (PEG) Ethers and Fatty Acid Esters as Penetration Enhancers

Antonella Casiraghi, Francesca Selmin, Paola Minghetti, Francesco Cilurzo, and Luisa Montanari

Contents

15.1 Structure of Polymer of Ethylene Oxide

Polyethylene glycol (PEG), also known as poly(oxyethylene) (POE) or poly(ethylene oxide) (PEO), is a synthetic polyether available in a range of molecular weights. Materials at molecular weight lower than 100,000 are usually indicated as PEGs, while polymers having molecular weight higher than this value are classified as PEOs. PEGs are manufactured by the polymerisation of ethylene oxide (EO) with water, monoethylene glycol or diethylene glycol, under alkaline conditions (Asmussen 2013).

PEGs and PEG derivatives do not represent definite chemical entities, but they are mixtures of compounds varying in polymer chain lengths. PEGs have the general formula of $H(OCH_2CH_2)_nOH$ where "n" indicates the average number of oxyethylene groups (Fig. 15.1). In this class of compounds, molecular weight is generally ranging from 200 up to 10,000. PEGs at molecular weights lower than 1000 are viscous and colourless liquids at room temperature. PEGs of higher molecular weights are white waxy solids with melting points proportional to their molecular weights to an upper limit of about 67 °C (Bailey and Koleske 1976).

All PEGs are amphiphilic and soluble in water as well as in many organic solvents: the higher the molecular weight, the lower the solubility in

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N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_15

water. Keeping constant the hydrocarbon chain length, PEG derivatives become more hydrophilic at increasing the EO content, as indicated by the number "n" and the hydrophile–lipophile balance (HLB) value.

PEGs are non-volatile, stable and non-hydrolisable compounds, which deteriorate on storage in presence of oxygen.

PEGs are used either as such or in form of their derivatives (Fig. 15.1). Having two terminal primary hydroxyl groups, PEGs can form mono-, di- and polyesters, ethers, amines and acetals. PEGs are also able to react by forming addition compounds or complexes on their ether bridges. PEG derivatives, therefore, include PEG–fatty acid esters (e.g. PEG laurates, dilaurates, stearates and distearates), PEG ethers (e.g. laureths, ceteths, ceteareths, oleths and PEG ethers of glyceryl cocoates), PEG amine ethers (e.g. PEG cocamines), PEG castor oils, PEG propylene glycols and other derivates (e.g. PEG soy sterols, PEG beeswax).

PEG ethers are obtained by the ethoxylation of an alcohol, such as lauryl, cetearyl, cetyl, stearyl or oleyl, with the number of moles of ethylene oxide corresponding to the length desired of the average polyethylene glycol chain. A few examples of trade names include Brij™ formerly known as Volpo™ (Croda International Plc, UK), Kolliphor® formerly known as Eumulgin® (BASF, Germany), Emulgen (Kao Chemicals) and Igepal (Rhodia Operations, France). Similarly, PEG esters are produced by the ethoxylation of fatty acids, that could be saturated or unsaturated straight-chain fatty acids or also with branched-chain. Some common commercial names include Cithrol™ and Myrj™ (Croda International Plc, UK).

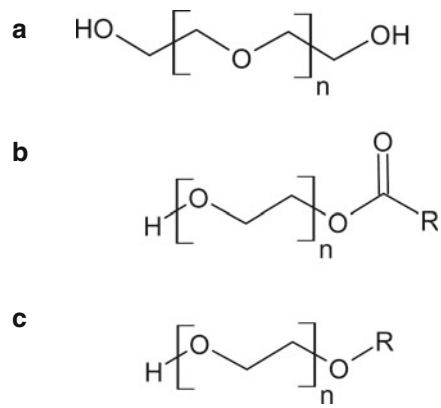

Fig. 15.1 Chemical structure of (**a**) PEG where "*n*" indicates the average number of oxyethylene groups. Having two terminal primary hydroxyl groups, PEGs can form mono-, di- and polyesters, ethers, amines and acetals. PEGs are also able to react by forming addition compounds or complexes on their ether bridges. The chemical structure of (**b**) PEG–fatty acid esters and (**c**) ethers is also simplified

PEGs and their nonionic derivatives find application in food industry and pharmaceutical and biomedical field. As an example, in pharmaceutics, PEGs (also known as Macrogol®, which is the International Nonproprietary Name (INN) for polyethylene glycol) and derivatives are used as excipients for liquid, solid and semisolid dosage forms both in human and veterinary drugs, including parenteral, topical, ophthalmic, oral and rectal formulations.

This family of substances are also widely used in cosmetics as surfactants, cleansing agents, emulsifiers, skin conditioners and humectants due to their safety, as they are not expected to be absorbed into systemic circulation when applied to the skin. PEGs and the corresponding ethers and fatty acid esters produce little or no irritation at ocular or dermal level and have extremely low acute and chronic toxicities. It is generally recognised that nonionic surfactants possess a lower toxicity and skin irritation potential with respect to ionic surfactants (Schaefer and Redelmeier 1996). They are mild on the skin, even at high concentrations in formulations and long-term exposure. On healthy skin, the sensitising potential of these compounds appears to be negligible. PEGs and PEG derivatives of cosmetic grade must not be applied to damaged skin even if the manufacturers are making efforts to remove impurities and/or starting materials such as ethylene oxide and 1,4-dioxane (Fruijtier-Pölloth 2005).

In dermal formulations, this family of nonionic surfactants, known under the trade name of Cithrol™ (Croda International Plc, UK), Kolliphor® formerly known as Cremophor® (BASF, Germany), Myrj™ (Croda International

Plc, UK) formerly known as Crodet™ (Croda International Plc, UK), Brij™ (Croda International Plc, UK), Cromul™ (Croda International Plc, UK) as an example, is mainly used as stabilisers (Bárány et al. 2000), but they also find application as skin penetration enhancers.

The purpose of this chapter is to review the literature data on the use of PEG ethers and fatty acid esters as enhancers of percutaneous absorption and their mechanism of action with a particular insight in the structure–activity relationship.

15.2 The Use of PEG Ethers and Esters as Penetration Enhancers

Nonionic surfactants have the potential to interact with biological membranes, and especially skin, causing an increase in their permeability and the transmembrane transport of solutes (Florence et al. 1984). Breuer suggested two possible mechanisms to describe how nonionic surfactants enhance the rate of transport (Breuer 1979). The former assumes that the surfactants may penetrate into the intercellular regions of stratum corneum, increase fluidity and, at last, solubilise and extract lipid components. In the latter, the penetration of a surfactant into the intercellular matrix followed by interactions and binding with keratin filaments may result in a disruption within the corneocyte. According to this mechanism, nonionic surfactants may enable or enhance diffusion of other molecules or drugs through the skin. However, conflicting data are reported on nonionic surfactant effects on skin penetration. These results can be rationalised considering the self-association properties of the surfactant molecules (French et al. 1993). As skin penetration enhancers, surfactants have direct effects on the skin barrier properties and indirect effects on the thermodynamic activity of the permeant in the vehicle. The thermodynamic activity of a permeant in the vehicle is the driving force for permeant diffusion into the skin. The monomers of the surfactant can penetrate and interact with the skin modifying its barrier properties and, thereby, favouring the easier penetration of permeant into the skin. On the other hand, the micelles having a strong solubilising capacity markedly reduce the thermodynamic activity of the permeant within the vehicle by entrapping the permeant in the micelles and, thereby, decrease its transport rate.

The investigation of penetration enhancing abilities of nonionic surfactants has been mainly focused on five principal series of surfactants, namely, polyoxyethylene derivatives of sorbitan esters, i.e. polysorbates (e.g. Tween®, Croda International Plc, UK; ALKEST® TW, Oxiteno, Brazil); esterified sorbitan, i.e. sorbitan esters (e.g. Span®, Croda International Plc, UK); polyoxyethylene alkyl ethers (e.g. Brij®, Croda International Plc, UK); polyoxyethylene alkylphenols; and nonionic block copolymers based on ethylene oxide and propylene oxide, i.e. poloxamers (e.g. Pluronic®, BASF, Germany) (Attwood and Florence 1983). The penetration enhancing effect of some PEG–fatty acid esters was also verified. Polyoxyethylene alkyl ethers and esters are demonstrated to be more effective enhancers of skin permeation than polysorbates (Walters et al. 1993).

The enhancing ability of surfactants is governed by several factors, including functional groups, hydrocarbon chain length, degree and position of unsaturation, physicochemical properties of permeants and nature of vehicles (Cázares-Delgadillo et al. 2005). In particular, in the case of PEG derivatives, increasing the complexity of the vehicle, the prediction of the absorption enhancing capability seems to be more related to the release from the vehicle itself than the proper combination of the number of ethylene oxide groups and alkyl groups (Casiraghi et al. 2012). The quality and magnitude of the enhancement effect are also dependent on the nature of other constituents in the formulation and the synergistic effects of certain binary mixtures of surfactants (Songkro 2009).

Both the hydrophobic alkyl chain and the hydrophilic ethylene oxide chain demonstrated

some structure–activity behaviour (Walters et al. 1993). As to the polar head group, its influence on the enhancement effect of surfactants was demonstrated, even if the understanding of the structure–enhancing activity relationships is limited. The size of the polar head group is thought to be the main determinant in the enhancement effect in the case of amphiphilic molecules. If it is too large, the molecule cannot insert into the polar regions of the ceramides and, hence, acts as penetration enhancer. If it is small enough and has an appropriate electrostatic charge distribution, it can cause condensation of the ceramides and, thereby, enhanced permeability of the SC (Hadgraft and Walters 1994). Walters stated that the polar head group affects the enhancing activities of surfactants (Walters et al. 1982; Walters 1989). In a study on the influence of a polar functional group on the enhancing activity of Span® 20, sorbitan monolaurate and its ethoxylate derivatives (Tween® 20, PEG-20 sorbitan monolaurate), López et al. evidenced that the nature of the enhancer's head group greatly influenced the impairment of the cutaneous barrier. In particular, pretreatment of the Wistar rat skin with a 1 or 5 % ethanolic solution of Tween® 20 did not cause an increase in the permeability values of a series of compounds with lipophilicities ranging from log Poct = −0.95 to log Poct = −2.33, namely, 5-fluorouracile, antipyrine, 2-phenyl ethanol and 4-phenyl butanol (López et al. 2000). The cutaneous barrier impairment was deeply influenced to the large volume associated with the hydrophilic head group of Tween® 20 consisting of the polyoxyethylene chain length of 20. This structure could impede penetration into the tails of the lipid bilayers in the stratum corneum. Tween®20 exerts its effects by allowing the polar molecule to partition across the barrier more easily. This could be due to the increase in the water content of the stratum corneum. On the contrary, Span®20 enhanced the skin penetration for all compounds, even if linear relationship between enhancement with Span®20 and the lipophilicity of the penetrants was not established (López et al. 2000). Similarly, when physostigmine was loaded in transdermal drug delivery system, the enhancement effect of Span® 20 and Span® 80 (sorbitan monooleate) was higher than that of the corresponding ethoxylated sorbitan esters, Tween® 20 and Tween® 80 (PEG-20 sorbitan monooleate) (Kim et al. 2002).

Many reports highlight the fundamental role of the alkyl chain length in the potency of surfactants as penetration enhancers. Florence noted that a medium-length alkyl chain surfactant may easily penetrate the lipid bilayers in the stratum corneum, because of its proper aqueous solubility and higher critical micelle concentration than a longer alkyl chain surfactant (Florence 1981). By increasing the length of the alkyl chain, one would expect to improve membrane penetration, but the monomer concentration would be reduced, perhaps offsetting the improvement in penetration (Schott 1973; Florence 1981). Other authors underlined the importance of the alkyl chain length in the potency of surfactants as penetration enhancers (Zaslavsky et al. 1978; Walters et al. 1981, 1983). The effects of fatty acids and fatty alcohol series were widely debated as the length and type of the alkyl chain are important in determining the effectiveness of the enhancer (Kanikkannan et al. 2000; Walters et al. 1988; Aungst et al. 1986). Structure–activity studies on unbranched saturated chains evidenced that the optimum chain length is between 12 and 14 carbon atoms (Walters et al. 1988) or, according to Kanikkannan, between 8 and 14 carbons (mostly from 10 to 12 carbons). In the case of unsaturated chains, the most active derivatives exhibited a chain of about 18–20 carbons (Kanikkannan et al. 2000), even if Morimoto and coauthors found the enhancing activity on indomethacin fluxes increased by longer unsaturated fatty acids in the following rank order: C20 > C22 = C18 = C16 > C14 (Morimoto et al. 1996). Finally, the effect of enhancers containing two hydrophobic chains was also evaluated. Their behaviour was similar to those with a single chain, that is, the most active enhancers containing two saturated hydrocarbon chains of the same length were those with 12-carbon chains (Vavrova et al. 2005).

15.2.1 The Effect of the Polyoxyethylene Chain Length in PEG Derivatives

The nature of the enhancer head group influenced the skin penetration enhancement effect also in the case of PEG ethers and PEG–fatty acid esters. Table 15.1 summarises the in vitro experiments carried out in order to elucidate the structure–activity relationship for these types of surfactants.

In recent years, the effect of a homologue series of PEG stearates (PEG-S), differing in oxyethylene content (PEG8-S, PEG12-S, PEG40-S) on in vitro skin permeability of ketoprofen vehicled in plasters, was investigated (Casiraghi et al. 2012). The ketoprofen fluxes obtained from formulations including PEG8-S or PEG12-S were nearly always significantly higher ($p < 0.05$) than that obtained from the control formulation without PEG-S while the addition of PEG40-S never increased ketoprofen flux with respect to the control plasters. To prove the presence of the three types of PEG stearate in hairless mouse skin, an attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) analysis was carried out on the membranes kept in contact with plasters containing 20 % w/w enhancers. Significant shifts in the peak position of the CH asymmetric stretching absorbance at 2917 cm^{-1} and the CH symmetric stretching absorbance at 2850 cm^{-1} were evident when PEG8-S or PEG12-S were used. PEG40-S did not cause any peak shifts, both when loaded in the plaster and in aqueous solution, suggesting that this surfactant did not enter into the stratum corneum, neither modified the conformation of skin lipids (Fig. 15.2).

Also, Bárány, by using the same PEG derivatives of stearic acid or stearyl alcohol, namely, stearic acid, glyceryl stearate, PEG2-S, PEG9-S, PEG40-S, PEG100-S, PEG2-stearyl ether, PEG10-stearyl ether and PEG21-stearyl ether, demonstrated that after application of emulsions containing 5 % emulsifiers, low molecular weight PEG derivatives (i.e. PEG2-S, PEG9-S, PEG2-stearyl ether, PEG10-stearyl ether and PEG21-stearyl ether) were able to damage the human skin barrier. These five emulsifiers significantly increased transepidermal water loss (TEWL) in normal skin. In contrast, higher molecular weight PEG–fatty acid esters, such as PEG40-S, do not have this property. On the basis of these results, it can be assumed that low molecular weight PEG derivatives could be present in the lipid bilayers, increasing TEWL in normal skin and, unexpectedly, decreasing TEWL in damaged skin. A possible consequence is the enhancement in the absorption not only of applied drugs but also of toxic substances into the skin (Bárány et al. 2000).

The incorporation into the stratum corneum of nonionic polyoxyethylene ethers was also evidenced by Shin et al. who investigated the thermal behaviour of rat skin samples and performed histological examinations (Shin et al. 2001). The enhancer's effectiveness, expressed as enhancement ratio (ER), of three types of polyoxyethylene alkyl ether in poloxamer gels was in the following rank order: PEG2-oleyl ether (ER = 2.84), PEG2-stearyl ether (ER = 2.62) and PEG23-lauryl ether (ER = 2.18). The differential thermal analysis (DTA) of the membrane treated with PEG2-oleyl ether showed changes in the membrane due to the presence of this surfactant in the stratum corneum and the consequent decrease in the lipid order (Fig. 15.3). The authors did not carry out a systematic study on membranes treated with the other enhancers (Shin et al. 2001) and, therefore, a definitive conclusion cannot be drawn.

In vivo results obtained in guinea pig on percutaneous absorption of piroxicam from a cataplasm showed that there is a parabolic relation between the piroxicam absorption and the polyoxyethylene length of PEG alkyl ether or ester (Okuyama et al. 1999). The surfactants employed were PEG (n) lauryl ether ($n = 2$, 9 and 21), PEG (n) cetyl ether ($n = 2$, 5.5, 7, 10, 15 and 20), PEG (n) oleyl ether ($n = 2$, 10 and 20) and PEG (n) stearate ($n = 4$, 10 and 25). Each cataplasm was applied to guinea pig and plasma concentrations were determined. The area under the plasma concentration curve (AUC_{0-8}) after the application of cataplasm containing PEG10-cetyl ether and PEG9-lauryl ether showed the largest followed

Table 15.1 Chemical features of polyethylene glycol (PEG) ethers and fatty acid esters investigated as drug permeation enhancers in different vehicles

PEG ethers	Alkyl chain	EO (mol)	Drug	Drug delivery system	Biological membrane	Reference
Polyoxyethylene-20 butanol	Butanol	20	Phenobarbitone Secobarbitone Thiopentone	Solution	Goldfish, in vivo	Walters et al. (1982)
Polyoxyethylene-10 alphanol 79	Alphanol 79	10	Phenobarbitone Secobarbitone Thiopentone	Solution	Goldfish, in vivo	Walters et al. (1982)
Polyoxyethylene-20 alphanol 79	Alphanol 79	20	Phenobarbitone Secobarbitone Thiopentone	Solution	Goldfish, in vivo	Walters et al. (1982)
Polyoxyethylene-10 octyl ether	Octanol (C8)	10	Methyl nicotinate	Solution	Hairless mouse skin	Walters et al. (1988)
Polyoxyethylene-1 [^{14}C] lauryl ether	Lauryl alcohol (C12)	1	–	Surfactant as such	Hairless mouse, in vivo	Nishiyama et al. (1983)
Polyoxyethylene-2 lauryl ether	Lauryl alcohol (C12)	2	Piroxicam	Cataplasm	Guinea pigs, in vivo	Okuyama et al. (1999)
Polyoxyethylene-2 lauryl ether	Lauryl alcohol (C12)	2	Ibuprofen	5 % solution	Rat skin	Park et al. (2000)
Polyoxyethylene-1 [^{14}C] lauryl ether	Lauryl alcohol (C12)	2.6	–	Surfactant as such	Hairless mouse, in vivo	Nishiyama et al. (1983)
Polyoxyethylene-4 lauryl ether	Lauryl alcohol (C12)	4	Phenobarbitone Secobarbitone Thiopentone	Solution	Goldfish, in vivo	Walters (1982)
Polyoxyethylene-4 lauryl ether	Lauryl alcohol (C12)	4	Ibuprofen	5 % solution	Rat skin	Park et al. (2000)
Polyoxyethylene-6.4 [^{14}C] lauryl ether	Lauryl alcohol (C12)	6.4	–	Surfactant as such	Hairless mouse, in vivo	Nishiyama et al. (1983)
Polyoxyethylene-7 [^{14}C] lauryl ether	Lauryl alcohol (C12)	7	–	Surfactant as such	Hairless mouse, in vivo	Nishiyama et al. (1983)
Polyoxyethylene-9 lauryl ether	Lauryl alcohol (C12)	9	Piroxicam	Cataplasm	Guinea pigs, in vivo	Okuyama et al. (1999)
Polyoxyethylene-10 [^{14}C] lauryl ether	Lauryl alcohol (C12)	10	–	Surfactant as such	Hairless mouse, in vivo	Nishiyama et al. (1983)

Polyoxyethylene-10 lauryl ether	Lauryl alcohol (C12)	10	Methyl nicotinate	Solution	Hairless mouse skin	Walters et al. (1988)
Polyoxyethylene-10 lauryl ether	Lauryl alcohol (C12)	10	Phenobarbitone Secobarbitone Thiopentone	Solution	Goldfish, in vivo	Walters et al. (1982)
Polyoxyethylene-21 lauryl ether	Lauryl alcohol (C12)	21	Piroxicam	Cataplasm	Guinea pigs, in vivo	Okuyama et al. (1999)
Polyoxyethylene-23 lauryl ether	Lauryl alcohol (C12)	23	Piroxicam	Poloxamer gel	Full-thickness rat skin	Shin et al. (2001)
Polyoxyethylene-23 lauryl ether	Lauryl alcohol (C12)	23	Phenobarbitone Secobarbitone Thiopentone	Solution	Goldfish, in vivo	Walters et al. (1982)
Polyoxyethylene-23 lauryl ether	Lauryl alcohol (C12)	23	Ibuprofen	5 % solution	Rat skin	Park et al. (2000)
Polyoxyethylene-10 octylphenol ether	Octyl phenol alcohol (C14)	10	Methyl nicotinate	Solution	Hairless mouse skin	Walters et al. (1988)
Polyoxyethylene-7.5 nonylphenol ether	Nonyl phenol alcohol (C15)	7.5	Tenoxicam	Solution	Male guinea pigs	Endo et al. (1996)
Polyoxyethylene-9 nonylphenol ether	Nonyl phenol alcohol (C15)	9	Benzocaine	Saturated solution	Hairless mouse skin	Dalvi and Zatz (1981)
Polyoxyethylene-10 nonylphenol ether	Nonyl phenol alcohol (C15)	10	Methyl nicotinate	Solution	Hairless mouse skin	Walters et al. (1988)
Polyoxyethylene-15 nonylphenol ether	Nonyl phenol alcohol (C15)	15	Benzocaine	Saturated solution	Hairless mouse skin	Dalvi and Zatz (1981)
Polyoxyethylene-30 nonylphenol ether	Nonyl phenol alcohol (C15)	30	Benzocaine	Saturated solution	Hairless mouse skin	Dalvi and Zatz (1981)
Polyoxyethylene-50 nonylphenol ether	Nonyl phenol alcohol (C15)	50	Benzocaine	Saturated solution	Hairless mouse skin	Dalvi and Zatz (1981)
Polyoxyethylene-2 cetyl ether	Cetyl alcohol (C16)	2	Piroxicam	Cataplasm	Guinea pigs, in vivo	Okuyama et al. (1999)
Polyoxyethylene-2 cetyl ether	Cetyl alcohol (C16)	2	Methyl nicotinate	Solution	Hairless mouse skin	Walters et al. (1988)
Polyoxyethylene-2 cetyl ether	Cetyl alcohol (C16)	2	Phenobarbitone Secobarbitone Thiopentone	Solution	Goldfish, in vivo	Walters et al. (1982)

(continued)

Table 15.1 (continued)

PEG ethers	Alkyl chain	EO (mol)	Drug	Drug delivery system	Biological membrane	Reference
Polyoxyethylene-2 cetyl ether	Cetyl alcohol (C16)	2	Ibuprofen	5 % solution	Rat skin	Park et al. (2000)
Polyoxyethylene-5.5 cetyl ether	Cetyl alcohol (C16)	5.5	Piroxicam	Cataplasm	Guinea pigs, in vivo	Okuyama et al. (1999)
Polyoxyethylene-6 cetyl ether	Cetyl alcohol (C16)	6	Methyl nicotinate	Solution	Hairless mouse skin	Walters et al. (1988)
Polyoxyethylene-6 cetyl ether	Cetyl alcohol (C16)	6	Phenobarbitone Secobarbitone Thiopentone	Solution	Goldfish, in vivo	Walters et al. (1982)
Polyoxyethylene-7 cetyl ether	Cetyl alcohol (C16)	7	Piroxicam	Cataplasm	Guinea pigs, in vivo	Okuyama et al. (1999)
Polyoxyethylene-10 cetyl ether	Cetyl alcohol (C16)	10	Piroxicam	Cataplasm	Guinea pigs, in vivo	Okuyama et al. (1999)
Polyoxyethylene-10 cetyl ether	Cetyl alcohol (C16)	10	Methyl nicotinate	Solution	Hairless mouse skin	Walters et al. (1988)
Polyoxyethylene-10 cetyl ether	Cetyl alcohol (C16)	10	Phenobarbitone Secobarbitone Thiopentone	Solution	Goldfish, in vivo	Walters et al. (1982)
Polyoxyethylene-10 cetyl ether	Cetyl alcohol (C16)	10	Ibuprofen	5 % solution	Rat skin	Park et al. (2000)
Polyoxyethylene-14 cetyl ether	Cetyl alcohol (C16)	14	Methyl nicotinate	Solution	Hairless mouse skin	Walters et al. (1988)
Polyoxyethylene-14 cetyl ether	Cetyl alcohol (C16)	14	Phenobarbitone Secobarbitone Thiopentone	Solution	Goldfish, in vivo	Walters et al. (1982)
Polyoxyethylene-15 cetyl ether	Cetyl alcohol (C16)	15	Piroxicam	Cataplasm	Guinea pigs, in vivo	Okuyama et al. (1999)
Polyoxyethylene-20 cetyl ether	Cetyl alcohol (C16)	20	Piroxicam	Cataplasm	Guinea pigs, in vivo	Okuyama et al. (1999)
Polyoxyethylene-20 cetyl ether	Cetyl alcohol (C16)	20	Methyl nicotinate	Solution	Hairless mouse skin	Walters et al. (1988)
Polyoxyethylene-20 cetyl ether	Cetyl alcohol (C16)	20	Phenobarbitone Secobarbitone Thiopentone	Solution	Goldfish, in vivo	Walters et al. (1982)

Polyoxyethylene-20 cetyl ether	Cetyl alcohol (C16)	20	Ibuprofen	5 % solution	Rat skin	Park et al. (2000)
Polyoxyethylene-20 polyoxypropylene −4 cetyl ether	Cetyl alcohol (C16)	20	Tenoxicam	Solution	Male guinea pigs	Endo et al. (1996)
Polyoxyethylene-30 cetyl ether	Cetyl alcohol (C16)	30	Methyl nicotinate	Solution	Hairless mouse skin	Walters (1988)
Polyoxyethylene-45 cetyl ether	Cetyl alcohol (C16)	45	Methyl nicotinate	Solution	Hairless mouse skin	Walters (1988)
Polyoxyethylene-60 cetyl ether	Cetyl alcohol (C16)	60	Methyl nicotinate	Solution	Hairless mouse skin	Walters (1988)
Polyoxyethylene-60 cetyl ether	Cetyl alcohol (C16)	60	Phenobarbitone Secobarbitone Thiopentone	Solution	Goldfish, in vivo	Walters et al. (1982)
Polyoxyethylene-2 stearyl ether	Stearyl alcohol (C18)	2	–	5 % emulsifiers in a water:mineral oil mixture (50:50)	Human normal skin, in vivo	Bárány et al. (2000)
Polyoxyethylene-2 stearyl ether	Stearyl alcohol (C18)	2	Piroxicam	Poloxamer gel	Full-thickness rat skin	Shin et al. (2001)
Polyoxyethylene-2 stearyl ether	Stearyl alcohol (C18)	2	Phenobarbitone Secobarbitone Thiopentone	Solution	Goldfish, in vivo	Walters et al. (1982)
Polyoxyethylene-2 stearyl ether	Stearyl alcohol (C18)	2	Ibuprofen	5 % solution	Rat skin	Park et al. (2000)
Polyoxyethylene-10 stearyl ether	Stearyl alcohol (C18)	10	–	5 % emulsifiers in a water:mineral oil mixture (50:50)	Human normal skin, in vivo	Bárány et al. (2000)
Polyoxyethylene-10 stearyl ether	Stearyl alcohol (C18)	10	Methyl nicotinate	Solution	Hairless mouse skin	Walters et al. (1988)
Polyoxyethylene-10 iso-stearyl ether	Iso-stearyl alcohol (C18)	10	Methyl nicotinate	Solution	Hairless mouse skin	Walters et al. (1988)
Polyoxyethylene-10 stearyl ether	Stearyl alcohol (C18)	10	Phenobarbitone Secobarbitone Thiopentone	Solution	Goldfish, in vivo	Walters et al. (1982)
Polyoxyethylene-10 stearyl ether	Stearyl alcohol (C18)	10	Ibuprofen	5 % solution	Rat skin	Park et al. (2000)

(continued)

Table 15.1 (continued)

PEG ethers	Alkyl chain	EO (mol)	Drug	Drug delivery system	Biological membrane	Reference
Polyoxyethylene-20 stearyl ether	Stearyl alcohol (C18)	20	Phenobarbitone Secobarbitone Thiopentone	Solution	Goldfish, in vivo	Walters et al. (1982)
Polyoxyethylene-20 stearyl ether	Stearyl alcohol (C18)	20	Ibuprofen	5 % solution	Rat skin	Park et al. (2000)
Polyoxyethylene-21 stearyl ether	Stearyl alcohol (C18)	21	–	5 % emulsifiers in a water:mineral oil mixture (50:50)	Human normal skin, in vivo	Bárány et al. (2000)
Polyoxyethylene-100 stearyl ether	Stearyl alcohol (C18)	100	Ibuprofen	5 % solution	Rat skin	Park et al. (2000)
Polyoxyethylene-2 oleyl ether	Oleyl alcohol (C18, C7–C8 unsaturated)	2	Piroxicam	Poloxamer gel	Full-thickness rat skin	Shin et al. (2001)
Polyoxyethylene-2 oleyl ether	Oleyl alcohol (C18, C7–C8 unsaturated)	2	Piroxicam	Cataplasm	Guinea pigs, in vivo	Okuyama et al. (1999)
Polyoxyethylene-2 oleyl ether	Oleyl alcohol (C18, C7–C8 unsaturated)	2	Phenobarbitone Secobarbitone Thiopentone	Solution	Goldfish, in vivo	Walters et al. (1982)
Polyoxyethylene-2 oleyl ether	Oleyl alcohol (C18, C7–C8 unsaturated)	2	Ibuprofen	5 % solution	Rat skin	Park et al. (2000)
Polyoxyethylene-10 oleyl ether	Oleyl alcohol (C18, C7–C8 unsaturated)	10	Methyl nicotinate	Solution	Hairless mouse skin	Walters et al. (1988)
Polyoxyethylene-10 oleyl ether	Oleyl alcohol (C18, C7–C8 unsaturated)	10	Piroxicam	Cataplasm	Guinea pigs, in vivo	Okuyama et al. (1999)
Polyoxyethylene-10 oleyl ether	Oleyl alcohol (C18, C7–C8 unsaturated)	10	Phenobarbitone Secobarbitone Thiopentone	Solution	Goldfish, in vivo	Walters et al. (1982)
Polyoxyethylene-10 oleyl ether	Oleyl alcohol (C18, C7–C8 unsaturated)	10	Tenoxicam	Solution	Male Guinea pigs	Endo et al. (1996)
Polyoxyethylene-20 oleyl ether	Oleyl alcohol (C18, C7–C8 unsaturated)	20	Piroxicam	Cataplasm	Guinea pigs, in vivo	Okuyama et al. (1999)
Polyoxyethylene-20 oleyl ether	Oleyl alcohol (C18, C7–C8 unsaturated)	20	Ibuprofen	5 % solution	Rat skin	Park et al. (2000)

Polyoxyethylene-2 oleyl ether	Oleyl alcohol (C18, C7–C8 unsaturated)	2	Phenobarbitone Secobarbitone Thiopentone	Solution	Goldfish, in vivo	Walters et al. (1982)
Polyoxyethylene-12 cetyl/ stearyl ether	Cetyl alcohol (C16) Stearyl alcohol (C18)	12	Ibuprofen	5 % solution	Rat skin	Park et al. (2000)
Polyoxyethylene-20 cetyl/ stearyl ether	Cetyl alcohol (C16) Stearyl alcohol (C18)	20	Ibuprofen	5 % solution	Rat skin	Park et al. (2000)
Polyoxyethylene-30 cetyl/ stearyl ether	Cetyl alcohol (C16) Stearyl alcohol (C18)	30	Ibuprofen	5 % solution	Rat skin	Park et al. (2000)
Polyoxyethylene-5 cetyl/oleyl ether	Cetyl alcohol (C16) Oleyl alcohol (C18, C7–C8 unsaturated)	5	Ibuprofen	5 % solution	Rat skin	Park et al. (2000)
Polyoxyethylene-10 cetyl/ oleyl ether	Cetyl alcohol (C16) Oleyl alcohol (C18, C7–C8 unsaturated)	10	Ibuprofen	5 % solution	Rat skin	Park et al. (2000)
Polyethylene glycol-8 glyceryl caprylate/caprate	Caprylic/capric acid (C8/C10)	8	Physostigmine	PSA	Hairless mouse skin	Kim et al. (2002)
Polyethylene glycol-8 glyceryl caprylate/caprate	Caprylic/capric acid (C8/C10)	8	Isosorbide dinitrate	PSA	Hairless mouse skin	Myoung et al. (2002)
Polyoxyethylene-8 laurate	Lauric acid (C12)	8	Ketoprofen	Plasters	Hairless mouse skin	Casiraghi et al. (2012)
Polyoxyethylene-8 dilaurate	Lauric acid (C12)	8	Ketoprofen	Plasters	Hairless mouse skin	Casiraghi et al. (2012)
Polyoxyethylene-8 laurate	Lauric acid (C12)	8	Ketoprofen	Saturated solution	Full-thickness hairless mouse skin	Kim and Oh (2011)
Polyoxyethylene-10 laurate	Lauric acid (C12)	10	Ketoprofen	Saturated solution	Full-thickness hairless mouse skin	Kim and Oh (2011)
Polyoxyethylene-20 sorbitan monolaurate	Lauric acid (C12)	20	Physostigmine	PSA	Hairless mouse skins	Kim et al. (2002)
Polyoxyethylene-2 stearate	Stearic acid (C18)	2	–	5 % emulsifiers in a water:mineral oil mixture (50:50)	Human normal skin, in vivo	Bárány et al. (2000)
Polyoxyethylene-4 stearate	Stearic acid (C18)	4	Piroxicam	Cataplasm	Guinea pigs, in vivo	Okuyama et al. (1999)

(continued)

Table 15.1 (continued)

PEG ethers	Alkyl chain	EO (mol)	Drug	Drug delivery system	Biological membrane	Reference
Polyoxyethylene-8 stearate	Stearic acid (C18)	8	Ketoprofen	Plasters	Hairless mouse skin	Casiraghi et al. (2012)
Polyoxyethylene-9 stearate	Stearic acid (C18)	9	–	5 % emulsifiers in a water:mineral oil mixture (50:50)	Human normal skin, in vivo	Bárány et al. (2000)
Polyoxyethylene-10 stearate	Stearic acid (C18)	10	Piroxicam	Cataplasm	Guinea pigs, in vivo	Okuyama et al. (1999)
Polyoxyethylene-10 stearate	Stearic acid (C18)	10	Ketoprofen	Saturated solution	Full-thickness hairless mouse skin	Kim and Oh (2011)
Polyoxyethylene-12 stearate	Stearic acid (C18)	12	Ketoprofen	Plasters	Hairless mouse skin	Casiraghi et al. (2012)
Polyoxyethylene-25 stearate	Stearic acid (C18)	25	Piroxicam	Cataplasm	Guinea pigs, in vivo	Okuyama et al. (1999)
Polyoxyethylene-25 stearate	Stearic acid (C18)	25	Ketoprofen	Saturated solution	Full-thickness hairless mouse skin	Kim and Oh (2011)
Polyoxyethylene-40 stearate	Stearic acid (C18)	40	Ketoprofen	Plasters	Hairless mouse skin	Casiraghi et al. (2012)
Polyoxyethylene-40 stearate	Stearic acid (C18)	40	–	5 % emulsifiers in a water:mineral oil mixture (50:50)	Human normal skin, in vivo	Bárány et al. (2000)
Polyoxyethylene-40 stearate	Stearic acid	40	Phenobarbitone Secobarbitone Thiopentone	Solution	Goldfish, in vivo	Walters et al. (1982)
Polyoxyethylene-45 stearate	Stearic acid (C18)	45	Ketoprofen	Saturated solution	Full-thickness hairless mouse skin	Kim and Oh (2011)
Polyoxyethylene-100 stearate	Stearic acid (C18)	100	–	5 % emulsifiers in a water:mineral oil mixture (50:50)	Human normal skin, in vivo	Bárány et al. (2000)

Polyoxyethylene-2 oleate	Oleic acid (C18, C9-C10 unsaturated)	2	Tenoxicam	Solution	Male guinea pigs	Endo et al. (1996)
Polyoxyethylene-6 oleate	Oleic acid (C18, C9–C10 unsaturated)	6	Tenoxicam	Solution	Male guinea pigs	Endo et al. (1996)
Polyoxyethylene-8 oleate	Oleic acid (C18, C9–C10 unsaturated)	8	Ketoprofen	Plasters	Hairless mouse skin	Casiraghi et al. (2012)
Polyoxyethylene-8 oleate	Oleic acid (C18, C9–C10 unsaturated)	8	Ketoprofen	Saturated solution	Full-thickness hairless mouse skin	Kim and Oh (2011)
Polyoxyethylene-10 oleate	Oleic acid (C18, C9–C10 unsaturated)	10	Ketoprofen	Saturated solution	Full-thickness hairless mouse skin	Kim and Oh (2011)
Polyoxyethylene-10 oleate	Oleic acid (C18, C9–C10 unsaturated)	10	Tenoxicam	Solution	Male guinea pigs	Endo et al. (1996)
Polyoxyethylene-12 oleate	Oleic acid (C18, C9–C10 unsaturated)	12	Ketoprofen	Plasters	Hairless mouse skin	Casiraghi et al. (2012)
Polyoxyethylene-20 sorbitan monooleate	Oleic acid (C18, C9–C10 unsaturated)	20	Physostigmine	PSA	Hairless mouse skins	Kim et al. (2002)
Polyoxyethylene-20 sorbitan monooleate	Oleic acid (C18, C9–C10 unsaturated)	20	Tenoxicam	Solution	Male guinea pigs	Endo et al. (1996)
Polyoxyethylene-8 dioleate	Oleic acid (C18, C9–C10 unsaturated)	8	Ketoprofen	Plasters	Hairless mouse skin	Casiraghi et al. (2012)
Polyoxyethylene-12 dioleate	Oleic acid (C18, C9–C10 unsaturated)	12	Ketoprofen	Plasters	Hairless mouse skins	Casiraghi et al. (2012)
Polyoxyethylene-60 sorbitan tetraoleate	Oleic acid (C18, C9–C10 unsaturated)	60	Tenoxicam	Solution	Male guinea pigs	Endo et al. (1996)
Polyethylene glycol-6 glyceryl monooleate	Oleic acid (C18, C9–C10 unsaturated)	6	Physostigmine	PSA	Hairless mouse skin	Kim et al. (2002)
Polyethylene glycol-6 glyceryl monooleate	Oleic acid (C18, C9–C10 unsaturated)	6	Isosorbide dinitrate	PSA	Hairless mouse skins	Myoung et al. (2002)
Polyethylene glycol-6 glyceryl linoleate	Linoleic acid (C18, C9–C10 and C12–C13 unsaturated)	6	Physostigmine	PSA	Hairless mouse skin	Kim et al. (2002)
Polyethylene glycol-6 glyceryl linoleate	Linoleic acid (C18, C9–C10 and C12–C13 unsaturated)	6	Isosorbide dinitrate	PSA	Hairless mouse skins	Myoung et al. (2002)

(continued)

Table 15.1 (continued)

PEG ethers	Alkyl chain	EO (mol)	Drug	Drug delivery system	Biological membrane	Reference
Polyoxyethylene-12 palm kernel glyceride	Palmitic acid (C16)/stearic acid (C18)/linoleic acid (C18, C9–C10 and C12–C13 unsaturated)	12	Isosorbide dinitrate	PSA	Hairless mouse skins	Myoung et al. (2002)
Polyethylene glycol-20 evening primrose glycerides	Linoleic acid (C18, C9–C10 and C12–C13 unsaturated)/γ -linolenic acid (C17)	20	Physostigmine	PSA	Hairless mouse skin	Kim et al. (2002)
Polyethylene glycol-20 evening primrose glycerides	Linoleic acid (C18, C9–C10 and C12–C13 unsaturated)/γ -linolenic acid (C17)	20	Isosorbide dinitrate	PSA	Hairless mouse skins	Myoung et al. (2002)
Polyoxyethylene-20 almond glyceride	Oleic acid (C18, C9–C10 unsaturated)	20	Physostigmine	PSA	Hairless mouse skins	Kim et al. (2002)
Polyoxyethylene-20 almond glyceride	Oleic acid (C18, C9–C10 unsaturated)	20	Isosorbide dinitrate	PSA	Hairless mouse skins	Myoung et al. (2002)
Polyoxyethylene-30 castor oil	Ricinoleic acid	30	Physostigmine	PSA	Hairless mouse skins	Kim et al. (2002)
Polyoxyethylene-60 almond glyceride	Oleic acid (C18, C9–C10 unsaturated)	60	Physostigmine	PSA	Hairless mouse skin	Kim et al. (2002)
Polyoxyethylene-60 almond glyceride	Oleic acid (C18, C9–C10 unsaturated)	60	Isosorbide dinitrate	PSA	Hairless mouse skins	Myoung et al. (2002)
Polyoxyethylene-60 hydrogenated castor oil	Trihydroxystearic acid	60	Tenoxicam	Solution	Male guinea pigs	Endo et al. (1996)

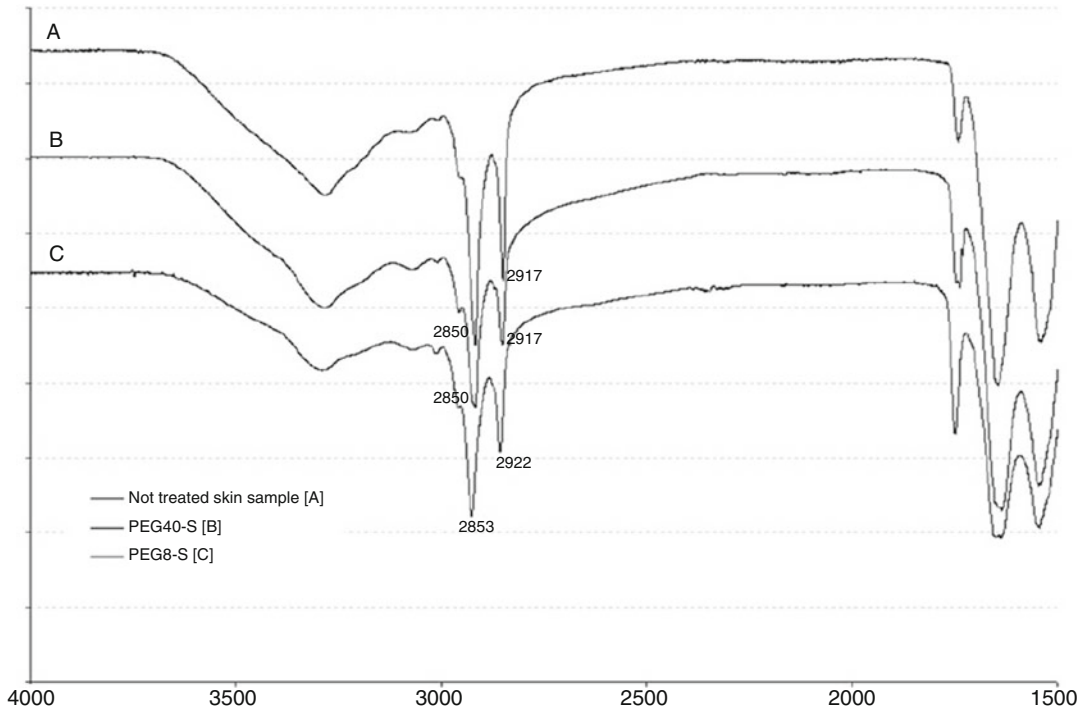

Fig. 15.2 Attenuated total reflectance Fourier transform infrared spectra of full-thickness hairless mouse skin kept in contact with plasters containing 20 % w/w PEG8-S and PEG40-S at the end of skin permeation test (Adapted from Casiraghi et al. (2012))

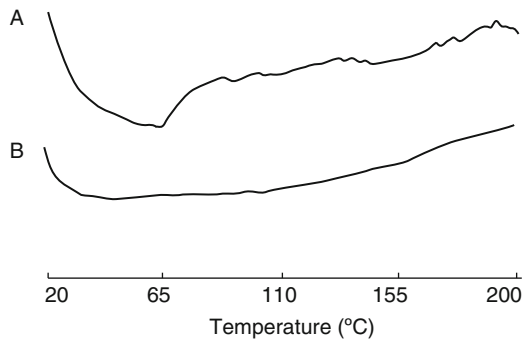

Fig. 15.3 Differential thermal analysis (DTA) curves of the rat stratum corneum pretreated with enhancer: (**a**) control; (**b**) polyoxyethylene-2-oleyl ether (Adapted from Shin et al. (2001))

by PEG7-cetyl ether. The parabolic relation between the ethylene oxide chain length and the AUC_{0-8} of piroxicam revealed the existence of an optimal PEG length to obtain best results in the percutaneous absorption (Fig. 15.4) (Okuyama et al. 1999).

The best enhancing effect of surfactants was measured for the ethylene oxide chain length of 10 in the case of both ether and ester derivatives. The authors concluded that ethylene oxide chain length from 5 to 15 enhanced percutaneous absorption to a greater extent than longer chains (Okuyama et al. 1999).

According to the influence of the polar head group length, Walters and coauthors reported the maximum enhancement effects with PEG10-lauryl ether followed by PEG10-oleyl ether, PEG10-palmitoyl ether and PEG6-palmitoyl ether on the in vitro permeation of methyl nicotinate through hairless mouse skin (Walters et al. 1988). Surfactants having a linear alkyl chain greater than C8 and an ethylene oxide chain length of between 6 and 14 caused significant increases in the flux of methyl nicotinate. Indeed, in the series of surfactants having the constant alkyl chain of 16 and an ethylene oxide chain length from 6 to 14, the increase of the methyl nicotinate permeability coefficient was up to

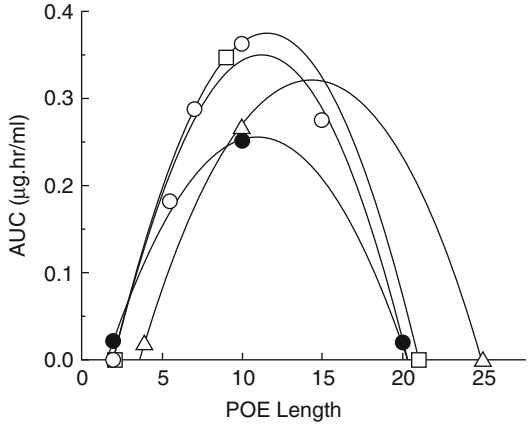

Fig. 15.4 Influence of POE length of nonionic surfactants on percutaneous absorption of piroxicam in vivo in guinea pigs. AUC_{0-8}: area under the concentration–time curve until 8 h after application of cataplasm containing □, POE lauryl ether; ○_, POE cetyl ether; ●, POE oleyl ether; _Δ, POE stearyl ester (Adapted from Okuyama et al. (1999))

50 %, while a minor influence on the permeability coefficient was measured in the case of ethylene oxide chain longer than 20 (Walters et al. 1988). Moreover, the greatest enhancement of barbiturates' absorption in the presence of a series of polyoxyethylene nonionic surfactants, ranging in polyoxyethylene chain length from 2 to 60 and alkyl chain length from 4 to 18, occurred with surfactants with 10–20 ethylene oxide moieties and alkyl chain lengths of C12–C16 (Walters et al. 1982).

Comparing the influence of the ethylene oxide chain length of surfactants having a more complex alkyl chain, the enhancement effect of PEG-20 almond glyceride (Crovol® A40, Croda International PLC, UK) on physostigmine base permeability was significantly higher than that of PEG-60 almond glyceride (Crovol® A70, Croda International PLC, UK), while the enhancement of caprylic/capric triglyceride (Labrafac® CC, Gattefossé, France) was higher than that of PEG-8 glyceryl caprylate/caprate (Labrasol®, Gattefossé, France) (Kim et al. 2002).

On the contrary, Dalvi and Zatz found that benzocaine flux through hairless mouse skin from an aqueous solution system was not affected by differences in either polyoxyethylene chain length of polyoxyethylene nonylphenol ether or surfactant concentration. The concentration of benzocaine not entrapped in micelles was comparable in all solutions, despite the fact that the total benzocaine concentration was different, due to the differences in the surfactant concentration and polyoxyethylene chain length (i.e. EO 9, EO 15, EO 30, EO 50) (Dalvi and Zatz 1981). Apparently, the percutaneous penetration of benzocaine decreased as the ethylene oxide chain length increased. This can be explained by benzocaine solubilisation due to micellar entrapment. As the hydrophilic chain length increased, the amount of benzocaine solubilised in micelles increased and the amount of free drug was reduced. The lower free drug concentration could be responsible for the reduced rate of penetration (Dalvi and Zatz 1981).

An unexpected result was reported by Kim and Oh in an investigation on the effect of polyoxyethylene alkyl ester nonionic surfactants on percutaneous permeation enhancement of ketoprofen used as a model drug (Kim and Oh 2011). The results demonstrated that all surfactants enhanced the percutaneous absorption, irrespective of their molecular weight. Surprisingly, 1 and 5 % PEG45-S, which has the longest polyethylene oxide chain, provided a ketoprofen-permeated amount comparable to those obtained using a 5 % PEG25-S solution. According to the overall results commented above, one may expect that it could be more difficult for the surfactant to penetrate into the stratum corneum lipids and, therefore, to increase the drug flux, as the length of the polyethylene oxide chain increases. In this case, the mechanism involved in the enhancement effect was elucidated by impedance measurements and solubility of ketoprofen in a propylene glycol:water (2:1) mixture. Impedance data allowed to evidence quite different enhancement mechanism for PEG10-oleate and PEG45-S. When skin was immersed in propylene glycol:water (2:1) solution (control), the resistance (Re) decreased slowly over time and it reached 32 % of the initial value after 24 h. When skin was immersed in propylene glycol:water (2:1) solution containing

PEG10-oleate, the Re rapidly decreased and after 24 h, the Re was only 5 % of the initial value. However, when PEG45-S was used, the decrease rate of the Re over time was much slower than that observed for PEG10-oleate and was similar to control skin. Contrary to electrical resistance, the capacitance (Ca) increased in all cases. For control skin, the Ca slowly increased reaching 144 % of initial value. The addition of PEG10-oleate caused a faster increase of the Ca up to twofold after 24 h. However, when PEG45-S was used, the Ca increased very slowly and it increased only up to 125 % of the initial value after 24 h. The large change in Re and Ca of the skin when PEG45-S was applied suggested that PEG10-oleate was absorbed into the stratum corneum, disrupting the lipid structure of the inter-corneocyte domain and the current conducting pathways. The variation of Re and Ca of the skin in the presence of PEG45-S was minimal and similar to the values obtained for the control experiment, suggesting the possibility that PEG45-S may have not penetrated into the stratum corneum (Kim and Oh 2011). This is highly probable, because PEG45-S, having the molecular weight of about 2300, is not expected to diffuse into the stratum corneum, as confirmed by other authors (Casiraghi et al. 2012; Bárány et al. 2000). The measured electrical Re and Ca of the skin treated with PEG45-S makes it difficult to provide a simple explanation for the found skin permeation results. Compared to the propylene glycol:water solution used as control, the permeated amount of ketoprofen increased nearly twofold after treating by a 1 % PEG45-S solution. The permeated amount for PEG45-S was comparable to that of PEG10-oleate, indicating that the ability of these surfactants to enhance the skin absorption was similar, despite the slower decrease in the electrical Re of the skin induced by PEG45-S, which is known to be inversely related to the skin permeability. These results suggest that PEG45-S could be a potential candidate for a skin penetration enhancer of high molecular weight, which may cause less skin irritation and systemic side effects than the smaller surfactant molecules.

15.2.2 The Effect of the Hydrophobic Chain in PEG Derivatives

The importance of the alkyl chain for the potency of penetration enhancers was extensively reported in literature for fatty acids and alcohols (Kanikkannan et al. 2000).

In the case of PEG derivatives (Table 15.1), experimental results obtained by using plasters containing ketoprofen and nonionic surfactants differing in fatty acid and number of alkyl chain length showed that the drug penetration into the excised hairless mouse skin and the partitioning behaviour from the vehicle resulted not so strongly dependent on the hydrophobic portion (i.e. alkyl chain length and number of chains) of the surfactant structure. Nevertheless, it appears that the presence of a double bond in the alkyl chain could lead to more efficient enhancers as the fluidity of the stratum corneum is altered. Keeping constant the number of C atoms of the hydrophobic chain, polar head group and the loaded concentration of nonionic surfactants in the plasters, PEG8-monooleate influenced significantly the ketoprofen skin permeation with respect to fluxes obtained by using PEG8-monolaurate or PEG8-S, while the differences between fluxes obtained by using the PEG derivatives with the two saturated fatty acids were not significant (Casiraghi et al. 2012). At a constant ethylene oxide chain length, the trend of the ketoprofen fluxes from plasters containing PEG8 was always unsaturated C18 > saturated C12, even if the difference between fluxes obtained by using unsaturated C18 (i.e. PEG8-oleate) and saturated C12 (i.e. PEG8-laurate) fatty acid was significant only at the highest concentration and in the case of the monoesters. The opposite trend was observed for PEG12-monoester. In general, the enhancer potency, expressed as ER values, of the laurate and stearate PEG esters was lower than those of the oleate PEG esters (Casiraghi et al. 2012). The enhancer activity was influenced by the bond saturation. Indeed, the unsaturated long-chain fatty acids (C18) showed a greater enhancing activity than the analogous saturated fatty acids (Aungst 1995). The same results were obtained also in a series of polyoxyethylene alkyl

ethers (Park et al. 2000). PEG2-oleyl ether showed a significantly higher enhancing effect compared to PEG2-stearyl ether containing the same alkyl chain length. This effect could be explained by the double bond of oleyl ether (Park et al. 2000). The presence of cis-double bonds in fatty acids and alcohols has been shown to increase considerably their enhancement potential when compared with the unsaturated trans-double bond counterparts (Aungst et al. 1986; Cooper 1984). The effects of the double bond in the alkyl chain were evaluated on the permeation of physostigmine from transdermal patches. At 24 h, the derivatives of linoleate with two double bonds, namely, PEG-20 evening primrose glycerides (Crovol™ EP40, Croda International Plc, UK) and PEG-6 glyceryl linoleate (Labrafil®2609, Gattefossé, France), showed higher enhancement in the drug permeability than the derivatives of oleate having one double bond, namely, PEG-20 almond glycerides (Crovol™ A40, Croda International Plc, UK) and PEG-6 glyceryl monooleate (Labrafil®1944, Gattefossé, France). It was speculated that the presence of at least a double bond in the alkyl chain could lead to more efficient enhancement effects than the PEG derivatives having saturated fatty acid. Indeed, oleic acid, linoleic acid and linolenic acid are non-linear fatty acids, due to the presence of cis-double bond(s). Due to this structural property, the insertion of oleic acid into the lipid domain of the stratum corneum is thought to open up channels for diffusion and alter the lipid's fluidity (Kim et al. 2002).

Data on the comparison between corresponding one and two chain-containing enhancers are limited. Casiraghi studied the effect of the corresponding one and two hydrophobic chain-containing enhancers. Only in few cases significant differences were measured in the ketoprofen flux values by doubling the same hydrophobic chain, namely, in plasters containing PEG8-monolaurate vs. PEG8-dilaurate ($p = 0.02$) and plasters containing PEG12-monooleate vs. PEG12-dioleate ($p = 0.02$). In these cases, there was no trend seen in the ranking order among the fluxes obtained from analogues differing in the number of hydrophobic chains, as the order of fluxes from plasters was PEG12-monolaurate > PEG12-dilaurate and PEG12-monooleate < PEG12-dioleate. This confirms that the length and number of the alkyl chains seemed to have less influence on the enhancing ability of the PEG derivatives when the hydrophobic chain is combined with a relatively short ethylene oxide chain (Casiraghi et al. 2012).

15.2.3 The Influence of the Hydrophile–Lipophile Balance (HLB)

The hydrophile–lipophile balance (HLB) of a surfactant is a measure of the degree to which it is hydrophilic or lipophilic, due to the combination of the polyoxyethylene group and the hydrophobic chain. This value could be considered as a predictor of the penetration enhancing ability of a surfactant, even if in literature conflicting conclusions are reported on this topic.

Park and coauthors suggested that the HLB alone is not a reliable predictor of the penetration enhancing ability, but the size and shape of both the alkyl chain and the polar group (e.g. ethylene oxide chain) influence the penetration enhancing ability. They concluded that the non-ionic surfactants were effective in enhancing the ibuprofen flux if they contained an ethylene oxide chain containing 2–5 ethylene oxide moieties and an alkyl chain length of C16–C18 and had HLB value of 7–9 (Park et al. 2000).

On the other hand, Kim concluded that the more lipophilic the surfactant is, the higher the permeation rate within the same group of surfactants is. In the esters of sorbitol and fatty acid, the enhancement effect of Span® 80 (C18, HLB = 4.3) was higher than that of Span® 20 (C12, HLB = 8.6). Similarly, the enhancement effect of Tween® 80 (C18, HLB = 15) was higher than that of Tween® 20 (C12, HLB = 16.7). Of course, this was also a comparison between surfactants having an unsaturated C18 or a saturated C12 alkyl chain. In the caprylic/capric glycerides series, the enhancing ability of caprylic/capric triglyceride (Labrafac® CC, Gattefossé,

France, HLB = 1) was the highest, followed by propylene glycol caprylate/caprate (Labrafac® PG, Gattefossé, France, HLB = 2) and PEG-8 glyceryl caprylate/caprate (Labrasol®, Gattefossé, France, HLB = 14) (Kim et al. 2002). The preferential use of low HLB value surfactants was indicated also by Myoung and Choi (Myoung and Choi 2002) and Furuishi and coauthors (Furuishi et al. 2007). Nevertheless, Myoung and Choi suggest that moderate lipophilicity may be more effective than the extreme lipophilicity, even if there is wide variability in correlation between the isosorbide dinitrate flux and the HLB of the surfactants. PEG-20 almond glyceride (Crovol™ A 40, Croda International Plc, UK HLB = 10) showed the most potent enhancing effect on the isosorbide dinitrate permeability, followed by PEG-6 linoleyl glyceryl ester (Labrafil 1944, Gattefossé, France, HLB = 3), PEG-6 oleyl glyceryl ester (Labrafil®2609, Gattefossé, France, HLB > 4) and PEG-20 linoleyl/linolenyl glyceryl ester (Crovol™ EP 40, Croda International Plc, UK, HLB not reported). PEG-60 oleyl glyceryl ester (Crovol™ A 70, Croda International Plc, UK, HLB = 15) on the other hand provided the lowest drug flux. Although the alkyl chain length of the glyceryl esters tested was held at 18, it was not possible to find any trend between the EO chain length and the measured flux. However, when fluxes and HLB were fitted to second-order polynomial, the correlation between the HLB of the surfactants and the isosorbide dinitrate flux was found. The relationship between the HLB values of the selected surfactants and the measured isosorbide dinitrate flux showed a decrease of flux at HLB values greater than 12. Moreover, it was shown that the maximum drug flux was obtained when the HLB value of the surfactant was about 7 (Myoung and Choi 2002). Furuishi et al. investigated the percutaneous absorption of pentazocine from the isopropyl myristate solution system containing glyceryl monocaprylate (HLB = 7.2), which is a type of glycerol ester of fatty acid. They revealed that the HLB value of about 8 seemed optimal for the percutaneous enhancing effect of pentazocine (Furuishi et al. 2007). High skin permeability of tenoxicam through guinea pig skin from a 10 % propylene glycol:water solution in the presence of hydrophobic surfactants was obtained also by Endo and coauthors (Endo et al. 1996). They investigated the effect of 14 types of nonionic surfactants, including PEG-oleyl ether, on the in vitro drug permeation. The change in the ethylene oxide chain length of the PEG-monooleate showed that drug permeation through skin tended to decrease with the increase of hydrophilicity of the surfactants from 4.5 (PEG2-monooleate), 8.5 (PEG6-monooleate) and 11.0 (PEG10-monooleate). These data may be confirmed by the fact that also PEG-lauryl ether showing low HLB value penetrated the skin at a higher rate than the more hydrophilic PEG-lauryl ether with a longer ethylene oxide chain length and hence a higher HLB value (Nishiyama et al. 1983).

In the case of PEG-S series, the less hydrophilic the PEG-S (PEG8-S:HLB = 11.7; PEG12-S:HLB = 13.5), the higher the enhancement effect on the drug permeation. Also when PEG esters with oleic or lauric fatty acid was used to enhance the skin permeation of ketoprofen, the best results were achieved with derivatives of HLB values from 10 to 14. More hydrophilic surfactants resulted effective in enhancing the permeation of drugs having the tendency to escape best from a more complex vehicle, i.e. from a lipophilic matrix of a plaster having a relatively low content of water-soluble acrylic monomers (Casiraghi et al. 2012).

The proper combination of alkyl groups and the number of ethylene oxide groups in this family of molecules could be fundamental in determining the enhancement effect of the surfactant, but relevant is also the complexity of the vehicle and/or dosage form, the release rate of the enhancer from the matrix and the consequent ability to penetrate into the skin.

Conclusion

A systematic review of literature data evidenced that polyoxyethylene alkyl ethers and esters can act as skin permeation enhancers, even if the permeated drug amounts were not remarkably increased using these PEG derivatives.

Generally speaking, their enhancing ability is governed by many factors; some of them are related to their molecular structure (e.g. functional groups, hydrocarbon chain length, degree and position of unsaturation), while others are related to the overall composition of the formulation (e.g. physicochemical properties of the permeants) and the type of vehicles (e.g. simple solutions of solvents and surfactants or more complex polymeric matrices). The experimental results reported in this chapter revealed that the oxyethylene chain length of surfactants appeared to have more influence than the fatty acid and alkyl chain length on their ability to enhance the penetration and/or permeation of a drug. The contribution of both moieties in the structure–activity relationship and HLB values is whereas controversial since some relations were found only in some studies and not always, there was a good sound of correlation. The HLB value was successfully exploited to predict the penetration enhancing capability of PEG derivatives in solution; meanwhile only when were present, it resulted quite difficult as the complexity of the vehicle increased.

References

Asmussen C (2013) Chemische charakterisierung von alkylethoxylaten. http://ublibsprod.ub.tu-berlin.de/F/LIVRV5X9HJUPN4SLC6VE4NRGTTQXSUJD29VV2D7J8VG2Q2457M-18153?func=full-set-set&set_number=002801&set_entry=000001&format=999. Accessed 9 Jan 2013

Attwood D, Florence AT (1983) Surfactant systems: their chemistry, pharmacy and biology. Chapman and Hall Ltd, London

Aungst B (1995) Fatty acids as skin permeation enhancers. In: Smith EW, Maibach HI (eds) Percutaneous penetration enhancers. CRC Press, Boca Raton, pp 277–287

Aungst BJ, Rogers NJ, Shefter E (1986) Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactants, sulfoxides and amides. Int J Pharm 33:225–234

Bailey FE, Koleske JV (1976) Poly(ethylene oxide). Academic Press Inc, New York

Bárány E, Lindberg M, Lodén M (2000) Unexpected skin barrier influence from nonionic emulsifiers. Int J Pharm 195:189–195

Breuer MM (1979) The interaction between surfactants and keratinous tissues. J Soc Cosmet Chem 30:41–64

Casiraghi A, Grigoli M, Cilurzo F, Gennari CGM, Rossoni G, Minghetti P (2012) The influence of the polar head and the hydrophobic chain on the skin penetration enhancement effect of poly(ethylene glycol) derivatives. AAPS PharmSciTech 13:247–253

Cázares-Delgadillo J, Naik A, Kalia YN, Quintanar-Guerrero D, Ganem-Quintanar A (2005) Skin permeation enhancement by sucrose esters: a pH dependent phenomenon. Int J Pharm 297:204–212

Cooper ER (1984) Increased skin permeability for lipophilic molecules. J Pharm Sci 73:1153–1156

Dalvi UG, Zatz JL (1981) Effects of nonionic surfactants on penetration of dissolved benzocaine through hairless mouse skin. J Soc Cosmet Chem 32:87–94

Endo M, Yamamoto T, Ijuin T (1996) Effect of non ionic surfactants on the percutaneous absorption of tenoxicam. Chem Pharm Bull 44:865–867

Florence AT (1981) Surfactant interactions with biomembranes and drug absorption. Pure Appl Chem 53:2057–2068

Florence AT, Tucker IG, Walters KA (1984) Interactions of non-ionic alkyl and aryl ethers with membranes and other biological systems. In: Rosen MJ (ed) Structure: performance relationships in surfactants, ACS symposium series., pp 189–207

French EJ, Pouton CW, Walters KA (1993) Mechanism and prediction of non-ionic surfactant effects on skin permeability. In: Walters KA, Hadgraft J (eds) Pharmaceutical skin penetration enhancement. Marcel Dekker, New York, pp 113–143

Fruijtier-Pölloth C (2005) Safety assessment on polyethylene glycols (PEGs) and their derivatives as used in cosmetic products. Toxicology 214:1–38

Furuishi T, Oda S, Saito H, Fukami T, Suzuki T, Tomono K (2007) Effect of permeation enhancers on the in vitro percutaneous absorption of pentazocine. Biol Pharm Bull 30(7):1350–1353

Hadgraft J, Walters KA (1994) Skin penetration enhancement. J Derm Treat 5:43–47

Kanikkannan N, Kandimalla K, Lamba SS, Singh M (2000) Structure-activity relationship of chemical penetration enhancers in transdermal drug delivery. Curr Med Chem 7:593–608

Kim HS, Oh SY (2011) Effect of polyoxyethylene alkyl esters on permeation enhancement and impedance of skin. Biomol Ther 19:109–117

Kim JH, Lee CH, Choi HK (2002) Transdermal delivery of physostigmine: effects of enhancers and pressure-sensitive adhesives. Drug Dev Ind Pharm 28:833–839

López A, Llinares F, Cortell C, Herráez M (2000) Comparative enhancer effects of Span®20 with Tween®20 and Azone® on the in vitro percutaneous penetration of compounds with different lipophilicities. Int J Pharm 202:133–140

Morimoto K, Tojima H, Haruta T, Suzuki M, Kakemi M (1996) Enhancing effects of unsaturated fatty acids with various structures on the permeation of indomethacin through rat skin. J Pharm Pharmacol 48:1133–1137

Myoung Y, Choi HK (2002) Effects of vehicles and pressure sensitive adhesives on the penetration of isosorbide dinitrate across the hairless mouse skin. Drug Deliv 9:121–126

Nishiyama T, Iwata Y, Nakajima K, Mitsui T (1983) In vivo percutaneous absorption of polyoxyethylene lauryl ether surfactants in hairless mice. J Soc Cosmet Chem 34:263–271

Okuyama H, Ikeda Y, Kasai S, Imamori K, Takayama K, Nagai T (1999) Influence of non-ionic surfactants, pH and propylene glycol on percutaneous absorption of piroxicam from cataplasm. Int J Pharm 186:141–148

Park ES, Chang SY, Hahn M, Chi SC (2000) Enhancing effect of polyoxyethylene alkyl ethers on the skin permeation of ibuprofen. Int J Pharm 209:109–119

Schaefer H, Redelmeier TE (1996) Strategies to alter skin barrier properties. In: Schaefer H, Redelmeier TE (eds) Skin barrier: principles of percutaneous absorption. Karger, Basel, p 169

Schott H (1973) Effect of chain length in homologous series of anionic surfactants on irritant action and toxicity. J Pharm Sci 62:341–343

Shin SC, Cho CW, Oh IJ (2001) Effects of non-ionic surfactants as permeation enhancers towards piroxicam from the poloxamer gel through rat skins. Int J Pharm 222:199–203

Songkro S (2009) An overview of skin penetration enhancers: penetration enhancing activity, skin irritation potential and mechanism of action. Songklanakarin J Sci Technol 31:299–321

Vavrova K, Zbytovska J, Hrabalek A (2005) Amphiphilic transdermal permeation enhancers: structure-activity relationships. Curr Med Chem 12:2273–2291

Walters KA (1989) Penetration enhancers and their use in transdermal therapeutic systems. In: Hadgraft J, Guy RH (eds) Transdermal drug delivery. Marcel Dekker, New York, pp 197–246

Walters KA, Dugard PH, Florence AT (1981) Non-ionic surfactants and gastric mucosal transport of paraquat. J Pharm Pharmacol 33:207–213

Walters KA, Florence AT, Dugard PH (1982) Non-ionic surfactants and the membrane transport of barbiturates in goldfish. Int J Pharm 10:153–163

Walters KA, Florence AT, Dugard PH (1983) Interaction of polyoxyethylene alkyl ethers with cholesterol monolayers. J Colloid Interface Sci 89:584–587

Walters KA, Walker M, Olejnik O (1988) Non-ionic surfactant effects on hairless mouse skin permeability characteristics. J Pharm Pharmacol 40:525–529

Walters KA, Bialik W, Brain KR (1993) The effects of surfactants on penetration across the skin. Int J Cosmet Sci 15:260–271

Zaslavsky BY, Ossipov NN, Krivitch VS, Baholdina LP, Rogozhin SV (1978) Action of surface-active-substances on biological membranes. II. Haemolytic activity of non-ionic surfactants. Biochim Biophys Acta 507:1–7

Sucrose Esters as Transdermal Permeation Enhancers

16

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16.1 Introduction

Although transdermal drug delivery provides significant advantages in relation to conventional routes, the barrier function of the skin severely limits its use for the administration of drugs. One alternative to increase the range of drugs that can be released effectively through the skin is the use of permeation promoters (also known as penetration or permeation enhancers) (Williams and Barry 2004). The permeation enhancers are defined as chemicals which themselves are pharmacologically inert but may partition into and interact with the stratum corneum (SC) when added to a transdermal formulation, reducing the resistance of the skin to the transport of drugs (Chantasart and Li 2012). Permeation promoters enhance the ability of the skin to absorb a drug by altering the lipid domains of the SC and the protein elements of the tissue, or by increasing the partitioning of the drug within the SC (Marjukka Suhonen et al. 1999). Additionally, permeation promoters can change the hydration level of the skin, e.g., if the promoter is lipophilic, it can form a layer on the surface of the skin, reducing the evaporation of the endogenous water (Papp 2012).

Some of the desirable properties for a permeation enhancer, described by Williams and Barry (2004), are the following: It should be pharmacologically inert, nontoxic, non-irritating, and

N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_16

non-allergenic. It should act quickly, with a predictable activity, reproducible, and reversible. Additionally, they should be compatible with excipients and drugs for appropriate formulation into diverse topical preparations.

Some of the most studied chemical absorption enhancers are ionic and nonionic surfactants. The main problem with a formulation containing absorption enhancers is generally related to their potential of causing irritation in the skin membrane and mucosal tissue, limiting its clinical application. Cationic and anionic surfactants are reported as more potent enhancers than nonionic surfactants but also are considered more toxic and may damage the barrier at relatively low concentrations (Ganem-Quintanar et al. 1998). In this context, sucrose fatty acid esters (SEs) exhibit some of the characteristics of an ideal enhancer. This chapter deals with the utility of SEs as promising skin penetration enhancers for topical and transdermal drug delivery.

16.2 Properties of Sucrose Fatty Acid Esters

Although a significant number of publications on SEs appeared from the mid-1950s, a marked increase of studies in the last two decades reflects a renewed interest for these compounds. These studies have been motivated by their properties such as biodegradability, biocompatibility, low toxicity, and its origin from renewable resources.

SEs are nonionic surfactants consisting of sucrose as hydrophilic group and fatty acids as lipophilic group. The fatty acids are attached to sucrose, a disaccharide consisting of two monosaccharides, glucose, and fructose. These esters are a class of nonionic detergents that are nontoxic, skin compatible, biodegradable, and nonpolluting. Sucrose monoesters were approved in Japan since 1959 for the use as food additives, subsequently have been approved as nonionic surfactants and emulsifiers in food products in other countries, and are listed in Japanese Pharmaceutical Excipients and the European Pharmacopeia (Okamoto et al. 2011). Furthermore, some esters of sucrose such as monolaurate inhibit the growth of *Escherichia coli* and other bacteria. SE derivatives can be used in the food, cosmetic, and pharmaceutical industries. They are also used as antimicrobial, as plasticizers, and as antistatic agents in plastics (Polat and Linhardt 2001). The surface activity described for commercial mixtures of sucrose esters (mixtures of mono-, di-, tri-, and higher esters) is similar to those of alkyl glucosides, a family of detergents commonly used in membrane solubilization studies.

The main advantage of these compounds is their overall metabolism and biodegradability in the body via initial ester hydrolysis. It has been reported that unsubstituted sucrose esters degrade very quickly in the body (Csizmazia 2011).

Other advantages of SEs are that they are formed via an esterification reaction, and their synthesis is not dependent on the petrochemical industry and can be made from renewable resources (at least the polar part) (Cruces et al. 2001; Höller 2008; Toro et al. 2009). Sucrose is one of the most abundant organic products in the world, and it is available with high purity and low cost. Sucrose is synthesized by most green plants and is absorbed by most organisms. It is an early product of photosynthesis, and plants such as sugarcane and beet serve as the main storage of saccharides. In other plants, sucrose is converted to starch, inulin, or levan for carbohydrate storage. In addition to having the advantage of low cost, sucrose is a readily available material with few problems of transportation and storage. Compared with oil, sucrose has the advantage of being a renewable resource with low environmental impact (Polat and Linhardt 2001).

Sucrose is a nonreducing disaccharide containing nine chiral centers (Fig. 16.1). The eight hydroxyl groups numbered as shown in the figure include three primary hydroxyl groups (at carbon 6, 1′, and 6′) and five secondary hydroxyl groups (at carbons 2, 3, 4, 3′, and 4′). Sucrose is hydrolyzed at extreme acidic conditions but is reasonably stable in the presence of strong bases. Therefore, the preparation of derivatives of sucrose is restricted to neutral or basic conditions.

Partially substituted sucrose derivatives are very difficult to isolate due to the formation of

mixtures as a result of the multiplicity of hydroxyl groups present. In theory, the esterification with equimolar amounts of reagents could give eight possible sucrose monoesters. However, the differential reactivity of primary and secondary hydroxyl groups varies slightly. In practice, the primary hydroxyl groups react preferentially. Of the three primary hydroxyl groups, those of carbons 6 and 6′ are generally more reactive than the hydroxyl group of carbon 1′ (Polat and Linhardt 2001; Huang et al. 2010).

Synthesis of SEs can be performed both chemically and biochemically. The industrial processes are not selective. All of them provide mixtures of compounds with different degrees of esterification and/or acylation positions. The reaction product of equimolar derivatization is contaminated with small amounts of other monoesters, as well as esters di- and trisubstituted, and the composition varies according to the reaction conditions. The derivatives with three or more fatty acid residues are hydrophobic and have limited applications. As developing methods require highly selective preparation and purification methods (Cruces et al. 2001; Polat and Linhardt 2001; Huang et al. 2010), an alternative regioselective synthesis of esters of carbohydrates involves the use of enzymes in organic solvents. Forty percent of esterification (conversion of sucrose to monoester) was obtained using lipases from *Byssochlamys fulva* NTG9 and *Candida antarctica*. With the lipase of *Mucor miehei*, a 25 % yield was obtained, mainly of ester 6-O-acyl sucrose (Polat and Linhardt 2001).

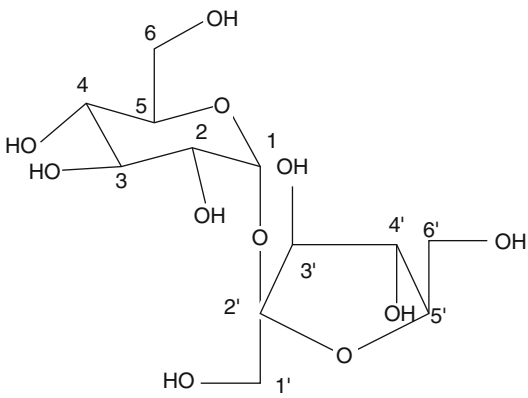

Fig. 16.1 General structure of sucrose

As already mentioned, it is difficult to achieve the monoacylation of sucrose, so due to several commercial routes for the synthesis of sucrose esters, the final product is generally a mixture of monoesters (in greater proportion), di- and higher esters, sucrose unreacted, and triglycerides. As sucrose has a total of eight hydroxyl groups, compounds ranging from sucrose mono- to octa-fatty acid esters can be produced. Therefore, research has lately focused on searching different synthetic routes with higher regioselectivity to provide sucrose monoesters. The general structure of sucrose monoesters is shown in Fig. 16.2.

The free hydroxyl groups of sucrose react with aliphatic or aromatic acids to produce SE. The substituents most widely used are fatty acids.

Fatty acids, commonly used in SEs for pharmaceutical applications are lauric, myristic, palmitic, stearic, oleic, behenic, and erucic acids. The hydrocarbon chains of the fatty acids can be saturated (e.g., capric, lauric, myristic, palmitic, and stearic acids) or unsaturated (e.g., oleic, linoleic, and palmitoleic acids). The amphiphilic behavior of SEs is the result of the presence of free hydroxyl groups, hydrophilic in nature, and hydrophobic hydrocarbon chains. The lipophilicity increases as the degree of esterification increases; for example, SEs with high monoester content are more hydrophilic, whereas a high esterification degree results in lipophilic SEs (Szűts and Szabó-Révész 2012). The nature of the fatty acid ester group has also influence on the hydrophilic-lipophilic balance (HLB) value, e.g., glycerin, sorbitan, or propylene glycol fatty acid esters are more oil soluble (i.e., low HLB value) than polysorbate fatty acid esters with a higher HLB value. Therefore, based on these two properties, SEs offer a wide range of HLB values.

This balance can be modulated by varying (1) the length of the alkyl chain of the acyl group, (2) the number of ester groups per molecule, and (3) the degree of unsaturation of the alkyl chain. Therefore, there are water-soluble esters (high HLB) and oil soluble esters (low HLB). The

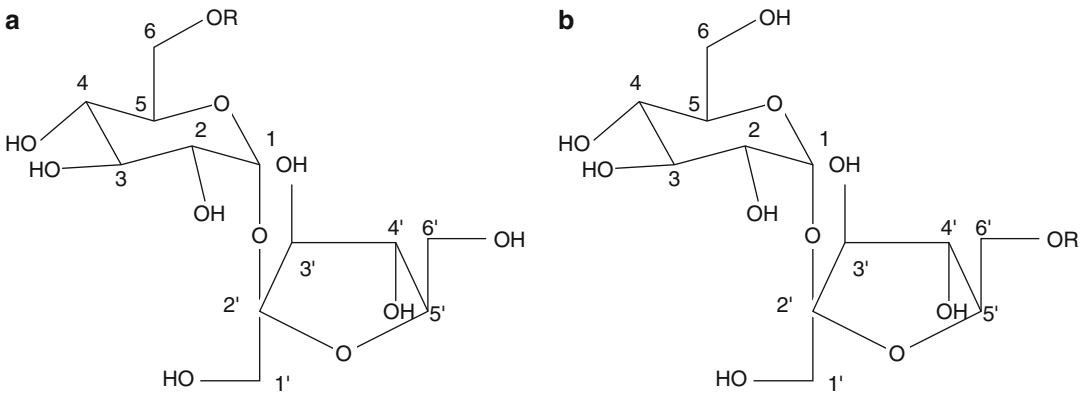

Fig. 16.2 Chemical structure of sucrose monoesters (*R* fatty acid). (**a**) Substitution in carbon 6 and (**b**) substitution in carbon 6′

mono-, di-, and tri-esters are used as emulsifiers, especially esters with fatty acids containing 12 or more carbon atoms (Cruces et al. 2001). Table 16.1 summarizes the HLB values and type of fatty acid in commercial sucrose esters.

Besides SEs listed in Table 16.1, sucrose cocoate has been recently reported to be included in nasal and ocular systems. Sucrose cocoate is a mixture of fatty acid sucrose esters, and it is obtained by the chemical esterification of coconut oil with sucrose. Coconut oil contains fatty acid chains of different lengths, mainly 12 carbons. Sucrose cocoate has been widely used as an excipient in cosmetic and dermatological products. With an HLB value of 15, sucrose cocoate is used not only for their surfactant properties but also as a skin emollient and moisturizer (Ahsan et al. 2003).

Sucrose esters with different HLB values can accomplish different tasks such as emulsifying and solubilizing agents, as lubricants in solid dosage forms, and as permeation enhancers. Szűts and Szabó-Révész (2012) reviewed the main applications of SEs in drug delivery systems. Table 16.2 shows the recommended HLB of SEs for different applications.

At the critical micelle concentration (CMC), surfactant molecules aggregate to form micelles. The value of CMC is of practical importance, since it is the concentration of surfactant required to solubilize hydrophobic molecules in water. The CMC value of some sucrose esters has been determined by means of a calorimetric method. In general, they are lower than those of other commercially available surfactants. The value of

Table 16.1 Characteristics of some commercially available SEs

Fatty acid	C-atom number of fatty acid chain	Degree of unsaturation	Monoester (%)	HLB
Lauric acid	12	0	1	1
			30	5
			70	16
			80	15
Myristic acid	14	0	80	16
Palmitic acid	16	0	1	1
			70	15
			80	16
Stearic acid	18	0	20	3
			30	5
			40	7
			50	9
			55	11
			70	15
			75	16
Oleic acid	18	1	1	1
			70	15
Behenic acid	22	0	20	3
Erucic acid	22	1	0	1
			2	2

Adapted from Szűts and Szabó-Révész (2012) and Mitsubishi-Kagaku Foods Corporation

Table 16.2 Sucrose ester's recommended HLB values for different pharmaceutical applications

Application	Recommended HLB
Emulsification	Low to high
Solubilization	High
Microemulsion formation	Mid to high
Dispersion of solid particles	High
Improvement of fat crystallization	Low (saturated SE)
Tablet lubricant	Low to mid
Dissolution improvement	High
Controlled/sustained release	Low to mid
Absorption/penetration enhancement	High (especially SE with C12–C14 fatty acids)

Modified from Szűts and Szabó-Révész (2012) and Mitsubishi-Kagaku Foods Corporation

CMC decreases with increasing the length of the acyl chain. Although the 6-*O*-stearoyl and 1′-*O*-stearoyl sucrose derivatives with more than 14 carbon acyl chains are expected to be the most efficient surfactants, these compounds (with more than 14 carbon acyl chains) are generally water insoluble. The introduction of a polar sulfo group in these SEs increases their water solubility (Polat and Linhardt 2001).

The properties of SEs are determined by the nature of the esterified fatty acid and by the degree of esterification of the sucrose molecule. Depending on the composition, SEs exist as solids (e.g., sucrose stearate, HLB values from 1 to 16), waxy materials (e.g., sucrose laurate, HLB values from 1 to 16), or liquids (e.g., sucrose oleate, HLB values from 1 to 15). The HLB values depend on the ester composition, i.e., the higher the monoester content, the higher the HLB. The solubility of monoesters in water is good, while di- and higher esters are not water-soluble. Moreover, the shorter the fatty acid chain, the higher is the HLB and the better the water solubility. Depending on the degree of esterification, SEs decrease the surface tension of water (31–38 dyn/cm). The increase in hydrophobic chain length changes the interfacial tension, while the decrease in the proportion of monoester relies on changes in the solubility and hydrophobicity or hydrophilicity of monoesters, diesters, and triesters (Yanke et al. 2004), i.e., the lower degree of esterification (related to having more monoesters), the lower the surface tension. They also exhibit different solubilizing abilities and foaming properties depending on HLB, monoester content, but mainly on fatty acid type (Husband et al. 1998; Soultani et al. 2003), e.g., sucrose stearate (HLBs 15 and 16, monoester content 70 and 75 %) has a foaming property of 11 and 12 mm, respectively; sucrose oleate (HLB 15, monoester content 70 %) has a foaming property of 14 mm; sucrose palmitate (HLBs 15 and 16, monoester content 70 and 80 %) has a foaming property of 15 and 24 mm, respectively; and sucrose laurate (HLB 15, monoester content 70 %) has a foaming property of 177 mm (Technical information from Mitsubishi-Kagaku Foods Corporation 2012).

Melting temperature depends on the degree of esterification, and usually, a higher degree of esterification results in lower melting temperature. The lipophilic SEs have characteristic melting points, while SEs with higher HLB values merely soften during heating. They can be heated up to 180 °C without harmful effects on their properties. They suffer decomposition at around 230 °C (Szűts et al. 2007; Technical information from Mitsubishi-Kagaku Foods Corporation 2012).

16.3 Skin Permeability Enhancing Properties of Sucrose Fatty Acid Esters

Percutaneous permeation involves several steps: (1) drug release from a vehicle, (2) partitioning into the SC, and (3) diffusion through the SC to reach the deeper layers of the skin (more aqueous in nature). These processes depend on the physicochemical properties of the drug itself combined with the influence of the vehicle to alter the drug penetration profile (Cázares-Delgadillo et al. 2005).

Methods to enhance or promote absorption by using formulation additives have therefore been extensively studied. Surfactants are often used as emulsifiers in formulations for dermal applications. They are also added to solubilize lipophilic

drugs in formulations. The improvement of drug solubility can be achieved, for example, by the formation of micelles by the molecules of surfactant present in the formulation. Further, the surfactants can act as penetration enhancers. Promoting drug penetration or permeation through the skin is closely related to the nature of the surfactant used in the formulation. Many penetration enhancers are capable of being inserted between the alkyl chains of the lipid bilayers in the SC, altering lipid bilayers' packaging, increasing fluidity, and subsequently, leading to easy drug diffusion (Calderilla-Fajardo et al. 2006).

Another way to explain the effect of the surfactants as penetration promoters is their interaction with keratin. Typically, cationic surfactants are more effective as percutaneous penetration enhancers than the anionic or nonionic compounds. However, the penetration-enhancing effect of the surfactant is related to its potential skin irritation. Therefore, in formulations for dermal application, mostly nonionic surfactants are used since these tend to be safer. The surfactants with low skin-irritating potential are those with a structure similar to the SC lipids. The problem with this kind of surfactants is their low penetration-enhancing effect, due to their integration into the SC bilayers instead of solubilization of the SC lipids (Trommer and Neubert 2006).

Among nonionic surfactants, SEs have been shown to temporarily alter the membrane barrier properties. They can insert their hydrocarbon chain between the lipophilic tails of SC lipids, allowing sucrose cycle to interact with the polar head groups of SC lipids. Interaction with these groups can modify the hydrogen bonds and ionic forces, distributing the hydration spheres of the SC lipids, thus resulting in alteration of the head group domain, fluidizing the lipids of the SC. It has also been reported that sucrose esters are able to extract lipids from the SC (Höller 2008).

There are only few literature data about the applicability of SEs as penetration enhancers through the skin or mucosa till now. These compounds have been used as absorption promoters through different tissues; sucrose laurate or sucrose stearate enhances nasal mucosal absorption of peptides, such as calcitonin and insulin (Ahsan et al. 2003), and sucrose laurate increases the permeation of lidocaine hydrochloride through excised pig palate and cheek (Ganem-Quintanar et al. 1998). It has been found that sucrose esters with HLB values of 11–16 have a higher promoter effect through oral mucosa in rats. Examples of sucrose esters as skin penetration enhancers are listed in Tables 16.3 and 16.4.

The percutaneous absorption of bromhexine through rat skin was examined *in vitro* and *in vivo* by Ogiso et al. (1991) using sucrose stearate (F-160 DK-ester: a blend of mono:di:triester 72:23:5, with HLB value of 15) as enhancer. The ointment formulation containing this SE showed the best penetration profile of bromhexine compared with Plastibase and macrogol formulations. Furthermore, a synergic effect was observed when SE was combined with 1-dodecylazacycloheptan-2-one (laurocapram; Azone®) or lauric acid.

Lerk and Sucker (1993) evaluated the irritating potential of sucrose laurate in normal guinea pig model and skin permeation of cyclosporine A formulated in a sucrose laurate hydrogel and found that sucrose laurate has some intermediate skin permeability enhancing properties, without irritating effects, measured by the inhibition of contact sensitivity skin reaction to dinitrofluorobenzene.

Vermeire et al. (1996) studied the penetration-enhancing capacity of sucrose laurate (type L-1695, a Ryoto Sugar Ester® with an HLB value of 16 and a monoester content of 80 %) for estradiol formulated in a hydrogel, evaluating the effect of single and multiple applications. Rabbits were used as animal model. Two sucrose laurate concentrations (5 and 15 % w/w) were tested to evaluate the concentration effect. After a single application, AUCs were significantly higher when administering the gel containing 15 % sucrose laurate, compared with the Oestrogel® (Piette International Laboratoria NV, Drogenbos, Belgium) containing 30 % ethanol. There was no significant difference for Oestrogel® and the 5 % sucrose laurate gel. The results obtained for the 15 % sucrose laurate gel were significantly higher than for the other gels (Oestrogel® and 5 %

Table 16.3 Sucrose esters as penetration enhancers in drug delivery systems

Model drug or molecule	Sugar esters	Formulation	Model membrane	Reference
Bromhexine	Sucrose stearate	Solution	Rat skin, *in vitro* and *in vivo*	Ogiso et al. (1991)
Cyclosporine A	Sucrose laurate	Hydrogel	Rat skin, *in vivo*	Lerk (1993)
Estradiol	Sucrose laurate (type L-1695)	Gel	Rabbits' back shaved *in vivo*	Vermeire et al. (1996)
Niflumic acid	Sucrose monolaurate sucrose dilaurate	Microemulsion	Human skin, *in vivo*	Bolzinger et al. (1998)
Hydrocortisone	Sucrose laurate L-595 and L-1695	Microemulsion	Human skin, *in vivo*	Lehmann et al. (2001)
Apomorphine	Sucrose laurate, L-595	Elastic vesicles	Human skin, *in vitro*	Li et al. (2001)
Pergolide	Sucrose laurate, L-595	Elastic and rigid vesicles	Human skin, *in vitro*	Honeywell-Nguyen et al. (2002a)
Lidocaine	Sucrose laurate, L-595	Elastic and rigid vesicles	Human skin, *in vitro*	Honeywell-Nguyen et al. (2002b)
4-Hydroxybenzonitrile	Sucrose oleate Sucrose laureate	Solution in Transcutol®	Human skin *in vivo*	Ayala-Bravo et al. (2003)
Lidocaine Ketoprofen	J-1216 J-1205 Lauryl chain	Sugar esters in propylene glycol	Hairless mouse skin, *in vitro*	Okamoto et al. (2005)
Lidocaine hydrochloride	Sucrose oleate Sucrose laureate	Solution in Transcutol®	Porcine ear skin *in vitro*	Cázares-Delgadillo et al. (2005)
Octyl methoxycinnamate	Sucrose laurate	Nanoemulsion and nanocapsules	Human skin, *in vivo*	Calderilla-Fajardo et al. (2006)
Metoprolol tartrate	Sucrose stearate (S-370, S-970, S-1670) Sucrose oleate (O-1570) Sucrose palmitate (P-1670) Sucrose laurate (L-1695) Sucrose myristate (M-1695)	Patch; Eudragit® NE 30D (20 % w/w)	Sartorius impregnated membrane, *in vitro*	Csóka et al. (2007)
Diclofenac	Sucrose monolaurate, L-1695	O/W-like microstructure	Rat skin, *in vitro*	Spernath et al. (2008)
Sodium diclofenac	Sucrose erucate	Solid-in-oil nanosuspension	Pig dorsal skin, *in vitro*	Piao et al. (2008)
Enhanced green fluorescent protein and horseradish peroxidase	Sucrose erucate	Solid-in-oil nanodispersion	Stratum corneum of Yucatan micro pig skin, *in vitro*	Tahara et al. (2008)
Sulfadiazine sodium salt	Sucrose cocoate	Niosomes	Rabbit ear skin, *in vitro*	Muzzalupo et al. (2011)
Timolol maleate	Sucrose laurate	Matrix controlled transdermal patch	Hairless rat skin, *in vivo*	El-Laithy (2009)
Propofol	L595, L1695, O1570, and S1570	Solution	Rat skin *in vitro, in vivo*	Yamato et al. (2009)
Hydroquinone and octadecenedioic acid	Sucrose cocoate	Emulsion	Human skin, *in vitro*	Otto et al. (2010)

(continued)

Table 16.3 (continued)

Model drug or molecule	Sugar esters	Formulation	Model membrane	Reference
Progesterone	Sucrose stearate	Nanoemulsion	Porcine abdominal skin, *in vitro*	Klang et al. (2010)
Diclofenac and sulfadiazine	Sucrose cocoate	Niosomes	Rabbit ear skin, *in vitro*	Tavano et al. (2010)
Lidocaine	Sucrose laurate, J-1216	Aqueous vehicle at different pH	Mouse skin, *in vitro*	Okamoto et al. (2011)
Ibuprofen	Sucrose laurate	Hydrogel	Synthetic membrane, *in vitro* Human skin, *in vitro*	Csizmazia et al. (2011)
Flufenamic acid, diclofenac acid, and curcumin	Sucrose stearate	Macroemulsions and nanoemulsions	Porcine abdominal skin, *in vitro*	Klang et al. (2011)
Vinpocetine	Sucrose laurate, L-1965	Proniosomal system	Rat skin, *in vitro*	El-Laithy et al. (2011)
Ibuprofen	Sucrose laurate (D-1216)	Hydrogel	Hairless mice *in vivo*	Csizmazia et al. (2012)
Methotrexate	Sucrose erucate	Solid-in-oil (S/O) nanocarrier	Pig skin, *in vitro*	Yang et al. (2012)
Catalase	S-370, S-970, L-1695, M-1695, P-1670, and S-1670	Niosomes	Rat skin	Abdel-Mageed et al. (2012)

sucrose laurate gel). Therefore, they assumed that sucrose laurate enhanced the skin penetration of non-solubilized estradiol after the single application. However, multiple applications led to a significant increase in estradiol bioavailability for the gel containing 30 % ethanol (Oestrogel®) and a significant decrease in estradiol bioavailability for the 5 and 15 % sucrose laurate gels. Histological evaluation of the untreated and treated skin biopsies showed a significantly higher incidence of infiltrate for all treated skin biopsies in comparison with the untreated ones. A significant increase in skinfold thickness (indicating some skin irritation potential) was seen for the skin biopsies treated with the gel containing 15 % sucrose laurate (Vermeire et al. 1996). This phenomenon was also described by Lerk (1991), who was unable to measure skinfold thickness after the application of a gel with 10 % sucrose laurate, due to the formation of incrustations. Although sucrose laurate has been defined as a skin moisturizer and an increase of skin hydration enhances the skin permeability, the main mechanism of penetration enhancement is related to the disruption ability of this surfactant on SC lipids, which dissolves and extracts lipids.

The effects of SEs /diethylene glycol monoethyl ether (Transcutol®, Gattefossé, Saint-Priest, France) on the permeation of lidocaine hydrochloride (LC), a charged molecule, were investigated as a function of vehicle pH. The authors found that sucrose laureate enhanced the penetration of the ionized form of lidocaine (12-fold higher flux than control), whereas sucrose oleate was more effective in promoting the permeation of the molecule in its nonionic form. They concluded that the structural properties of SEs and the degree of ionization of the drug are important characteristics affecting the transdermal flux of lidocaine (Cázares-Delgadillo et al. 2005).

Okamoto et al. (2005) selected six sugar esters with HLB values of 5–16 and evaluated their effect on the *in vitro* percutaneous permeation of lidocaine (pKa = 7.9) and ketoprofen (pKa = 4.0) as a model basic and acidic drug, respectively.

Table 16.4 Sucrose esters as skin penetration enhancers in the patent literature

Drug	Sucrose ester	Claim	Reference
Tetracycline HCl	Sucrose monooleate	Combination of decyl methyl sulfoxide and sucrose ester increases penetration depth of drug	Smith (1977)
Hydromorphone	Sucrose monolaurate Sucrose coconut fatty ester	Higher drug flux was obtained with sucrose esters	Cheng et al. (1988)
Progesterone and estradiol ester	Sucrose monolaurate	Increased permeation	Gale et al. (1988)
	Sucrose laurate, L595 and L-1695	Elastic vesicles as a vector system of controlled release and depth transport of drug through the skin	Bouwstra (1998)
Buprenorphine HCl	Sucrose cocoate	Dual permeation enhancer: sucrose cocoate and methyl laurate	Chang (1990)
Octyl methoxycinnamate Lidocaine	Sucrose laurate Sucrose oleate	Use of sucrose fatty acid ester in low concentration to promote penetration of a pharmaceutical or cosmetic active	Quintanar-Guerrero et al. (2005)
Clotrimazole Terbinafine	Sucrose stearate, SP70	Antifungal pharmaceutical foam carriers are non-irritating and nondrying	Tamarkin et al. (2008)
Methotrexate	Sucrose stearate/ distearate	Topical local administration of the methotrexate containing hydrogel permits smaller total doses of methotrexate to be administered, thus providing effective treatment of rheumatoid arthritis in affected joints with reduced total patient exposure of drug and therefore reduced likelihood of unwanted side effects	Maggio et al. (2009)
Zolmitriptan	Sucrose palmitate, P1670	Transdermal system for the systemic administration of antimigraine including SEs as enhancer	Hoo-Kyun et al. (2010)

The drugs were dissolved in buffer solutions with different pH values, and the effect of SEs on the percutaneous permeation of ionized and unionized drug species was evaluated, using the pretreatment technique, i.e., treatment of the skin for 2 h, with SEs, before applying drug solutions. The skin pretreated with J-1216 (sucrose laurate, HLB = 16 with 81 % monoester content) showed a significantly increased permeability coefficient for lidocaine at pH 6 (lidocaine is almost fully ionized at this pH), while the effect of the other sugar esters was not significant.

The permeation rate from the LC suspensions at pH 10, in which most of the lidocaine was unionized, was not affected by J-1216 and J-1205 (sucrose laurate, HLB = 5 with 32 % monoester content). This can be attributed to decreased LC thermodynamic activity in the solutions. J-1205, which was not reported earlier as a penetration enhancer, increased twofold the permeability coefficient of lidocaine applied in combination with propylene glycol. In the case of ketoprofen (KP), which was 99.1 % ionized at pH 6 and 98.9 % unionized at pH 2, it showed a modest increase in the permeability coefficient with the sugar esters (J-1216 and J-1205). As in the case of lidocaine, J-1216 did not increase the permeation of the ionized form of KP. When dissolved in propylene glycol at 1.5 %, J-1205 increased significantly the unionized KP permeation rate. KP permeation from a 5 % KP solution in propylene glycol, added with J-1205, was much faster than that from an aqueous KP suspension (pH 3.8), suggesting that the combination of J-1205 and propylene glycol would be a potent vehicle for transdermal formulations. J-1216 and J-1205, having lauryl chain(s) as the lipophilic moiety, act as effective percutaneous absorption enhancers. These two studies suggest that the effect of SEs depended on the pH of the aqueous vehicles.

Csóka et al. (2007) also examined the applicability of a series of Ryoto Sugar Esters® with different fatty acid kinds (stearic (S), oleic (O), palmitic (P), lauric (L), and myristic (M)), with a range of HLB values and monoester content S-370 (HLB = 3, monoester content = 20 %), S-970 (HLB = 9, monoester content = 50 %), S-1670 (HLB = 16, monoester content = 75 %), O-1570 (HLB = 15, monoester content = 70 %), P-1670 (HLB = 16, monoester content = 80 %), L-1695 (HLB = 16, monoester content = 80 %), and M-1695 (HLB = 16, monoester content = not reported), as drug delivery agents in a matrix-type transdermal therapeutic system containing Eudragit® NE (ethyl acrylate and methyl methacrylate copolymer dispersion, Röhm Pharma Polymers, Germany). The results showed that both the HLB value of SEs and the length of fatty acid chains affect drug (metoprolol tartrate) release. SEs of shorter fatty acid chain increased the release more than SEs of longer fatty acid chains. The amount of drug released depends on both HLB value and C number in the fatty acid chain. In comparison with the sample prepared without SEs, samples with SEs with a C18 number increased the amount of drug released by five- to sixfold, while it was increased up to eight- to tenfold in the case of SEs with lower C number and almost the same HLB values (HLB = 15–16). They also investigated the *in vitro* absorption using a Sartorius resorption model apparatus and found that all evaluated SEs increased drug absorption but the highest differences were found with SE containing lauric acid (C = 12, HLB = 16), which increased the amount of absorbed metoprolol tartrate by more than sixfold. Using SEs of the same HLB value, SEs with shorter fatty acid chains increased the absorption better than SEs with longer fatty acid chains.

El-Laithy (2009) investigated the possibility of matrix controlled transdermal patch based on SEs as penetration and absorption enhancers containing timolol maleate (TM). The influence of fatty acid type, chain length, and HLB on the *in vitro* drug release, as well as its permeation across hairless rat skin, was studied and compared in order to select a patch formulation for clinical performance. The results indicated that among different SEs tested, sucrose laurate (1.5 % w/w) significantly increased the amount of TM released from the patch and its permeation across rat skin. The total drug permeated and flux values were approximately fivefold greater compared to the SEs' free patch. The *in vivo* study was carried out using a nonblinded, randomized, and crossover design in 12 healthy volunteers to compare the patch to an oral aqueous solution, both containing 30 mg TM. The absorption was much slower and extended over a longer period in the case of TM SE transdermal patch. The developed patch was well tolerated by the subjects with only moderate skin irritation, which was recovered in 24 h after patch removal.

To increase the transdermal delivery of propofol, several penetration enhancers were studied by Yamato et al. (2009). Four kinds of SEs, namely, L-595 (HLB = 5, monoester content = 30 %), L-1695 (HLB = 16, monoester content = 80 %), O-1570 (HLB = 15, monoester content = 70 %), and S-1570 (HLB = 15, monoester content = 70 %), both alone and in combination with other enhancers, such as propylene glycol, isopropyl myristate, macrogol 400 MG, l-menthol, d-limonene, oleic acid, and stearic acid, were investigated. The *in vitro* transdermal permeation experiments were performed with propofol solutions containing 1 % (w/w) SEs. All SEs evaluated increase the transdermal permeation of propofol compared with propofol alone. There was a synergic effect of the combined use of propylene glycol (PG) and SEs, showing a greater enhancing effect than PG alone. The highest enhancing effect was observed with high HLB value SEs (O-1570, S-1570, and L-1695) when they are combined with a large amount of PG (30 %, w/w). High values of permeation parameters were observed with the combined use of propylene glycol and menthol, sucrose fatty acid esters, or sodium dodecyl sulfate. However, the combination of PG and SEs was not suitable for transdermal delivery of propofol *in vivo*.

Okamoto et al. (2011) examined the effect of J-1216, a sucrose laurate with HLB value of 16, on the *in vitro* percutaneous permeation of LC in aqueous vehicle at different pH values (6.0, 7.0, 8.0, and 10.0) with or without 1.5 % SE. In the

absence of SE, the permeation of LC increased with increasing the pH of the solution, a fact that may be explained by the pH-partition theory. SE increased the drug permeation at pH 6.0 and 7.0 but decreased it at higher pH value. The percutaneous absorption enhancers affect not only the permeability of the skin but also the thermodynamic properties of drugs in the vehicle. SEs form micelles in water and in organic solvents if they are formulated at a concentration above its CMC. It was observed that the interaction of the SE micelles and the drug would affect the drug penetration into the skin. J-1216 micelles interacted predominantly with the unionized form of the drug, leading to micellar solubilization of LC, which diminished the activity of LC in the solution and therefore decreased its permeation through the skin. J-1216 increased the permeation rate of LC at low pH value, increasing the permeability coefficient of the ionized drug (for low LC concentration). However, at high pH values, J-1216 decreases the permeation rate of LC from a diluted solution by entrapping unionized LC and decreasing the free unionized LC. The enhancing effect of J-1216 on the LC permeation rate for saturated LC solutions at low pH was remarkable; however, J-1216 had almost no effect at high pH.

Csizmazia et al. (2011) compared the *ex vivo* skin permeation of ibuprofen (IBU) formulated in a hydrogel containing Transcutol® or sucrose laurate in order to improve the permeation through the skin. The gel containing sucrose laurate increased IBU permeation through the skin notably (2.15-fold) compared to the enhancer free gel, and the Transcutol® containing gel did not enhance the IBU permeation showing an overall transdermal permeation decrease. Moreover, the lag time was shorter in the case of IBU-SE gel compared to the SE-free gel.

16.4 Mechanism of Action of Sucrose Fatty Acid Esters

All types of SEs tested have shown to increase drug release. This effect and the *in vitro* drug absorption depend on the HLB value and the number of carbon atoms of the fatty acid chain. Structural parameters such as chain length, degree and position of unsaturation, and the nature of the substituent can influence the ability of surfactants to act as permeation promoters. It is well accepted that a C12 linear alkyl chain maximizes the effect of a surfactant on membrane permeability. It has been proposed that the hydrocarbon chain of laurate sucrose is able to be inserted between the lipophilic tails of the SC lipids, allowing the polar sucrose ring to interact with the polar head groups of the lipids (Calderilla-Fajardo et al. 2006).

The Fourier transform infrared (FTIR) spectroscopic measurement by attenuated total reflectance (ATR) method is a powerful tool for studying the biophysical structure of the SC at the molecular level, characterizing its lipid, protein, and water content, and for examining the penetration pathway and the biochemical modifications of the SC induced by the interaction of penetration enhancers with the SC barrier. The big advantage is that this technique enables the elucidation of the extent and mechanism of percutaneous penetration enhancement *in vivo* (Csizmazia et al. 2012).

Ayala-Bravo et al. (2003) evaluated the effects of sucrose oleate (O-1570, HLB: 15) and sucrose laureate (L-1695, HLB: 16) in water or in Transcutol® on the *in vivo* percutaneous penetration of a model permeant, 4-hydroxybenzonitrile. The effects were monitored by ATR/FTIR in combination with tape stripping. They found that treatment of the skin with 2 % SE in Transcutol® significantly promotes the *in vivo* percutaneous penetration of the molecule relative to control. Infrared spectroscopy study revealed two important features: (1) a shift of the CH_2 asymmetric and symmetric stretching bands of the lipid methylene groups to higher wavenumbers (related to lipid fluidization) and (2) a decrease of peak height and area of these bands (lipid extraction). These results showed that the combination of SEs and Transcutol® can temporarily alter the SC barrier properties, promoting drug penetration. This effect was more marked for sucrose laureate. However, this phenomenon was only observed for SEs in Transcutol®. Despite the

alterations observed in the FTIR spectra, transepidermal water loss (TEWL) kept unaltered.

In another study, the effect of sucrose laureate on the structure of hairless mice skin was evaluated by means of FTIR spectroscopy combined with tape-stripping method, in order to examine whether SEs modify the special lipid bilayer structure of the SC and the protein conformation in the corneocytes. The most characteristic bond of the surfactant assigned to the ester group can be seen at 1728 cm^{-1}. The hydrocarbon chains of lipids give asymmetric and symmetric CH_2 stretching vibrations at 2920 and 2850 cm^{-1}, respectively. Any extraction of the lipids by the enhancers results in a decrease of peak height and area. They found that SC took up the lipophilic hydrocarbon-based components of the preparations, so the absorbance peak near 2850 cm^{-1} increased significantly. Some enhancers may fluidize the SC lipids, which can be noted from the shift of CH_2 stretching peaks to higher wavenumbers (conversion of trans to gauche conformation) and an increase in peak width. Minimal shifts were observed in both cases. The absorption bands at around 1650 cm^{-1} (amide I) and 1550 cm^{-1} (amide II) are typical protein bands which arise mainly from C=O stretching and N-H bending vibrations. These frequencies are sensitive to the conformation of proteins present in the SC. They found that the amide I peak at 1670 cm^{-1} did not shift due to the treatment, so the preparations caused only minimal changes in the protein structure. It was also found that the upper layers of the SC were the most hydrated and that the water content decreased with the number of tape strips. Examining the penetration of ibuprofen (IBU) through SC *in vivo*, it could be assessed that the IBU-SE (sucrose laurate, HLB = 16, monoester content = 80 %) gel treatment attained higher water and IBU content in each layer compared to the IBU gel control treatment, in spite of the same drug content in both preparations. Thus, it can be established that both preparations caused only minimal modification in the lipid and the protein structure, promoting skin hydration and therefore also the penetration of IBU. Although the hydration degree and penetration were more intense in the case of the IBU-SE gel treatment, it did not cause greater alterations in the SC structure than the IBU gel. It has been proven that SEs act as effective and non-irritating hydration enhancers as well as skin penetration enhancers for IBU (Csizmazia et al. 2012). Vučinić-Milanković et al. (2007) have also demonstrated the valuable contribution of this class of surfactants to increase skin hydration, which enables a penetration-enhancing effect. In this sense, a slight increase in skin hydration and possible perturbation of the skin barrier was observed for disaccharide mixed emulsifier (sorbitan stearate and sucrose cocoate), while samples based on monosaccharide mixed emulsifier (cetearyl alcohol and cetearyl glycoside) produced less fluctuation in transepidermal water loss (TEWL) values and higher skin moisturizing.

16.5 Colloidal Drug Delivery Systems with SEs

Colloidal carriers are receiving great attention from researchers because they appear as promising systems for transdermal drug delivery. The common characteristics of all colloidal systems are the submicron particle size. Colloidal preparations have shown to improve drug delivery across membranes by mediating a more direct and prolonged contact of the carrier than that achieved with a single molecule dispersed in a solution.

Among these systems, microemulsions (MEs) are transparent fine dispersions of oil and water droplets stabilized by surfactants. MEs are isotropic and thermodynamically stable liquids with ultralow interfacial tension, large interfacial area, and the capacity to solubilize both hydrophilic and hydrophobic compounds (Thevenin et al. 1996; Peltola et al. 2003; Chen et al. 2006). Several reports about the pharmaceutical use of ME, particularly in the transdermal drug delivery field, are available in the literature (Kreilgaard et al. 2000; Podlogar et al. 2005).

Bolzinger et al. (1998) prepared a bicontinuous SE microemulsion as a drug carrier system to release niflumic acid into the skin. The ME

system, containing a blend of sucrose monolaurate (3.2 %) and sucrose dilaurate (14.8 %), was compared with the commercially available Nifluril® ointment (UPSA Laboratories, USA). Their results showed that ME containing 1 % niflumic acid was as effective as Nifluril® ointment containing 3 % niflumic acid.

Different mechanisms have been proposed to explain the advantages of ME for dermal and transdermal use. However, although components of ME can act as permeation enhancers, surfactants and co-surfactants included in high concentration can be toxic and bioincompatible. Therefore, a formulation of ME in combination with mild surfactants of natural origin such as SEs to improve the transport of drugs through the skin and reduce skin injuries would be desirable. Lehmann et al. (2001) performed an *in vivo* human study with a ME containing a blend of sucrose laurates L-595 and L-1695. The authors found a pronounced dehydration and increased TEWL, as evidence of an impaired barrier, and therefore an increased penetration of hydrocortisone through the SC.

Another interesting group of vehicles for drug delivery are nanoemulsions. They may be defined as oil in water emulsions with mean particle size diameters between 20 and 200 nm. Due to their lipophilic interior, nanoemulsions are more suitable for the transport of hydrophobic compounds. The large surface area of the emulsion system allows rapid penetration of actives (Gutiérrez et al. 2008). SEs have been successfully employed as co-surfactants in nanoemulsions, particularly sucrose stearate, that has shown to exert positive effects on stability and skin permeation of lecithin-based nanoemulsions (Klang et al. 2010).

The incorporation of sucrose laurate into colloidal suspensions (nanoemulsions and nanocapsules) enhanced the *in vivo* penetration of octyl methoxycinnamate (OMC) into the skin, predominantly in nanoemulsions. The enhancement was the result of the combination of several factors such as droplet size, enhancer type, and system's nature (nanocapsules, nanoemulsions, or emulsion). A twofold increase in OMC skin deposition was observed with the nanoemulsion containing sucrose laureate when compared to the control. The effect of sucrose oleate was also evaluated. Although nanoemulsion with sucrose oleate showed a slight increase in the OMC amount penetrated, the effect was not as marked as with the nanoemulsion including sucrose laurate. The enhancing effect was attributed to the interaction of SEs with the lipids of the SC. In the case of nanocapsules, the exact mechanism is not fully understood, although it is possible that the polymeric matrix of nanocapsules decreases the release of OMC molecules. In this case, the rigidity of the particles is a factor to be taken into account, because it could hinder particle penetration (Calderilla-Fajardo et al. 2006).

Micellar and microemulsion systems are excellent potential vehicles for delivery of drugs because of their high solubilization capacity and improved transmembrane bioavailability. The concentrates undergo phase transitions from reverse micelles to swollen reverse micelles, via the bicontinuous transitional mesophase, into inverted O/W microstructures. Skin penetration of sodium diclofenac from the inverted bicontinuous mesophase and from the O/W-like microstructure was shown to be higher than that measured from the W/O-like droplets, especially when the micellar system contains the SE L-1695 and hexaglycerol laurate in 1:1 ratio (Spernath et al. 2008).

Sodium diclofenac permeation through the skin was increased (3.8-fold) compared to the control, when formulated in solid-in-oil nanosuspension with a complex formed between sodium diclofenac and sucrose erucate with HLB value of 1 (ER-190) and 2 (ER-290) (Piao et al. 2008).

The transdermal permeation of hydroquinone and octadecenedioic acid increased with emulsions possessing lamellar gel-like structure in the water phase. The emulsions having this structure were prepared from a blend of sorbitan stearate and sucrose cocoate (Arlatone™ Dioic DCA, Uniqema, Gouda, The Netherlands) as surfactants. The increase in skin permeation of drugs could be explained by an enhanced partitioning of drugs into the upper layers of the SC. Moreover, the surfactants and their aggregates (e.g., micelles and liquid crystalline phases) can affect the

solubility properties of the drug in the formulation and, hence, modify the thermodynamic activity of the permeant in the emulsion, with the consequence of an enhanced penetration (Otto et al. 2010).

Klang et al. (2010) evaluated the permeation of progesterone from lecithin-based nanoemulsions added with sucrose stearate and cyclodextrins as stabilizing agents. Sucrose stearate was found to enhance progesterone skin permeation. The combined use of SEs and cyclodextrins led to the increased skin permeation of the drug, irrespective of the type of cyclodextrin, acting in a synergic way.

Klang et al. (2011) used sucrose stearate (S-970, 5 % w/w) of intermediate lipophilicity to produce lipid-based drug delivery systems (macroemulsions and nanoemulsions) for the dermal application of three model drugs: flufenamic acid, diclofenac acid, and curcumin. The *in vitro* skin permeation study of the macroemulsion and nanoemulsion with 5 % w/w of sucrose stearate (S-970) was performed in porcine abdominal skin, and *in vitro* tape-stripping experiments were carried out in porcine ear skin. No differences between the formulations (macroemulsion or nanoemulsion) were found as to skin permeation rates, leading to highly similar release profiles in all three cases. No effect of particle size was observed. The transdermal penetration of drugs was mainly governed by the excipients and the type of drug, and the same was found with tape stripping. The highest transdermal permeation and skin penetration depth was achieved for diclofenac acid.

Sucrose erucate was used to prepare a solid-in-oil (S/O) nanodispersion for the transcutaneous delivery of different model proteins: fluorescein isothiocyanate (FITC)-labeled insulin (MW ca. 6 kDa), enhanced green fluorescent protein (EGFP, MW ca. 27 kDa), and horseradish peroxidase (HRP, MW ca. 40 kDa). Permeation was performed through the SC of Yucatan micro pig skin *in vitro* by forming an S/O nanodispersion. The EGFP and HRP permeated into the skin in a functional form (Tahara et al. 2008).

The permeation of the hydrophilic drug methotrexate (MTX) was enhanced by using the S/O nanocarrier due the lipophilic characteristics and nanosize of the carriers that promoted the fusion of the nanocarriers with the lipids of the skin. The S/O nanocarrier is a reverse micellar system, in which the hydrophilic drug is encapsulated in a layer formed by a nonionic sugar ester surfactant with low HLB such as sucrose erucate (HLB = 1) dispersed in an oil phase. The transdermal delivery of S/O nanocarrier is dependent on the release capability of MTX from the carrier, and this ability is related to the amount of sucrose erucate in the system. The dermis is the rate-limiting step for the transdermal drug delivery using S/O nanocarriers rather than the transport through the SC, and as the S/O nanocarrier is lipophilic, it penetrates more easily the lipophilic stratum corneum lipid barrier, with a slow release of the hydrophilic drug into the hydrophilic tissue (Yang et al. 2012).

In recent decades, drug delivery systems based on vesicles have emerged as a good option for transdermal delivery. Nonionic surfactants such as SEs can form lamellar phases at a high water concentration. The closed lamellar phase structure corresponds to the typical formation of the vesicles named niosomes. It has been reported that transdermal delivery of various drugs is enhanced by encapsulation into niosomes. The SC intercellular lipids may change drastically, becoming more permeable, by treatment with nonionic surfactants in the form of niosomes acting as penetration enhancers. Niosomes can act as a reservoir for drugs, controlling their release and enhancing their skin penetration (Kumar and Rajeshwarrao 2011; Muzzalupo et al. 2011).

Muzzalupo et al. (2011) evaluated the promoting effect of sucrose cocoate (TEGOSOFT LSE 65 K®, A.C.E.F. s.p.a., Italy) on skin permeation of sulfadiazine sodium salt. The nonionic surfactant was used both as component of niosomal systems or in the form of sub-micellar solution. No percutaneous enhancement was achieved by using sub-micellar solution containing free drug or when performing a pretreatment with empty niosomes or surfactant sub-micellar solution. Only the direct treatment of the skin with loaded niosomes gave a relevant increase on the percuta-

neous permeation of the drug. Low percutaneous permeation effects of sucrose ester as a submicellar solution can be attributed to the hydrogen-bond-donating groups of these molecules, as well as to their inter-lipid hydrogen bonding, that stabilizes the lipid bilayer of SC and suppresses the percutaneous permeation of the drug.

Ding et al. (2008) developed stable diphtheria toxoid-containing elastic vesicles with high loading efficiency, as a potential candidate for transcutaneous immunization studies, overcoming the inability of diphtheria toxoid to penetrate the skin when applied alone. The elastic vesicles were composed of sucrose laurate (L-595) and the micelle-forming surfactant octaoxyethylene-laurate ester (PEG-8-L). The authors proposed this system as a potential candidate for transcutaneous immunization studies looking for a more efficient vaccination.

Tavano et al. (2010) used sucrose cocoate to obtain niosomes as permeation enhancers for transdermal drug delivery of two model drugs (diclofenac and sulfadiazine). The *in vitro* drug permeation was significantly higher for drug-loaded niosomes than for the free drug solutions, indicating the favorable interaction of SE niosomes with the skin.

An *in vivo* study of the topical application of the antioxidant enzyme catalase encapsulated in biocompatible flexible SE nanovesicles showed a significant effect on wound healing diminishing the oxidative injury to the rat skin in a thermal injury model and observing a decrease in lesion size compared to untreated and control groups. The SEs S-370, S-970, L-1695, M-1695, P-1670, and S-1670 were used (Abdel-Mageed et al. 2012).

A novel sustained release proniosomal system was designed using SEs as nonionic surfactants for transdermal vinpocetine delivery. The proniosomes were converted into niosomes upon water hydration in the skin following topical application under occlusive conditions. The amount of drug permeated through the skin from proniosomal formulation containing sucrose laurate (L-1965, with short fatty acid chain length and high HLB) as permeation and absorption enhancer was quite high after 48 h under occlusive conditions, maintaining for 48 h an effective therapeutic concentration (El-Laithy et al. 2011).

16.6 Potential Skin Irritation of Sucrose Esters

In dermal formulations, nonionic surfactants are usually used, because they tend to be widely regarded as safe (Trommer and Neubert 2006). SEs have been reported as nonionic surfactants well tolerated by the skin.

Epidermal damage caused by irritants originated the recruitment of epidermal cells from the inactive stage into the mitotic cycle by the activation of the phosphoinositol cycle and the release of inflammatory mediators such as prostaglandin E2 (PGE2), leading to epidermal proliferation which is manifested by the increase in skinfold thickness. Skin swelling and thickening are main signs of skin irritation and inflammation (Fang et al. 2003). In order to evaluate the potential irritation effect of SE proniosomes, El-Laithy et al. (2011) measured skin conductivity and skinfold thickening by histopathological evaluation in albino rabbits. The proniosomes of sucrose laurate (L-1965) did not increase significantly the thickness compared to epidermis swelling caused by proniosomes of Tween 80 (polyoxyethylene (20) sorbitan monooleate; HLB = 15, Oxford Chemicals, Mumbai, India) after 48 h application. The application of proniosomes of L-1965 for 7 days led only to a mild hyperkeratosis (thickening of the SC) with no observed signs of inflammation. Lerk et al. (1993) reported the complete absence of sucrose laurate irritating effect. However, in some investigations, the histological evaluation of skin biopsies of the skin treated with 15 % w/w sucrose laurate increased skinfold thickness (Vermeire et al. 1996). These could be attributed to a high SEs concentration, as demonstrated by Kürti et al. (2012) who found that effects of SEs on nasal epithelial permeability were dose dependent. Microemulsions with sucrose laurate (L-595 and L-1695) showed no *in vitro* irritability in the hen's egg test on chorioallantoic membranes (Lehmann et al. 2001).

16.7 Perspectives

As was clearly shown in this chapter, SEs share characteristics that make them excipients for topical and transdermal formulations, not only for their emulsifying and solubilizing properties but also for their ability to enhance dermal/transdermal penetration. Furthermore, all of these sucrose-based surfactants display exceptional surface activity; are renewable materials, biodegradable, and environmentally safe; and have proven potential commercial value. However, it must be taken into account that the effects of SEs can vary depending on drug properties, nature of SEs, animal models used in experiments, the appropriate selection of the vehicle, and characteristics of the drug delivery system. These relatively innocuous nonionic surfactant molecules deserve further investigation as potential candidates for inclusion into transdermal formulations as penetration enhancers.

References

Abdel-Mageed HM, El-Laithy HM, Mahran LG, Fahmy AS, Mader K, Mohamed SA (2012) Development of novel flexible sugar ester vesicles as carrier systems for the antioxidant enzyme catalase for wound healing applications. Process Biochem 47:1155–1162

Ahsan F, Arnold JJ, Meezan E, Pillion DJ (2003) Sucrose cocoate, a component of cosmetic preparations, enhances nasal and ocular peptide absorption. Int J Pharm 251:195–203

Ayala-Bravo H, Quintanar-Guerrero D, Naik A, Kalia NY, Cornejo-Bravo JM, Ganem-Quintanar A (2003) Effects of sucrose oleate and sucrose laureate on *in vivo* human stratum corneum permeability. Pharm Res 47:1267–1273

Bolzinger MA, Thevenin, Carduner C, Poelman MC (1998) Bicontinuous sucrose ester microemulsion: a new vehicle for topical delivery of niflumic acid. Int J Pharm 176:39–45

Bouwstra JA (1998) Inventor; Rijksuniversiteit Leiden assignee. Phospholipid-and cholesterol-free aqueous composition for topical application to the skin. US Patent US 5,830,499

Calderilla-Fajardo SB, Cázares-Delgadillo J, Villalobos-García R, Quintanar-Guerrero D, Ganem-Quintanar A (2006) Influence of sucrose esters on the *in vivo* percutaneous penetration of octyl methoxycinnamate formulated in nanocapsules, nanoemulsion, and emulsion. Drug Dev Ind Pharm 32:107–113

Cázares-Delgadillo J, Naik A, Kalia YN, Quintanar-Guerrero D, Ganem-Quintanar A (2005) Skin permeation enhancement by sucrose esters: a pH-dependent phenomenon. Int J Pharm 297:204–212

Chang Y (1990) Inventor; Paco Pharmaceutical Services, Inc, assignee. Transdermal drug delivery device containing a dual permeation enhancer. US Patent US 4,956,171 A

Chantasart D, Li SK (2012) Structure enhancement relationship of chemical penetration enhancers in drug transport across the stratum corneum. Pharmaceutics 4:71–92

Chen H, Chang X, Du D, Li J, Xu H, Yang X (2006) Microemulsion-based hydrogel formulation of ibuprofen for topical delivery. Int J Pharm 315:52–58

Cheng YL, Gale RM, Sugihara E, Sanders HF (1988) Inventors; ALZA Corporation, assignee. Transdermal delivery devices containing sucrose coconut fatty esters as skin permeation enhancers. European Patent EP 280413 A1

Cruces MA, Plou FJ, Ferrer M, Bernabé M, Ballesteros A (2001) Improved synthesis of sucrose fatty acid monoesters. J Am Oil Chem Soc 78(5):541–546

Csizmazia E (2011) Drug permeation study through biological membrane barriers. PhD thesis, University of Szeged, Szeged

Csizmazia E, Erős G, Berkesi O, Berkó S, Szabó-Révész P, Csányi E (2011) Penetration enhancer effect of sucrose laurate and transcutol on ibuprofen. J Drug Deliv Sci Technol 21(5):411–415

Csizmazia E, Erős G, Berkesi O, Berkó S, Szabó-Révész P, Csányi E (2012) Ibuprofen penetration enhance by sucrose ester examined by ATR-FTIR *in vivo*. Pharm Dev Technol 17(1):125–128

Csóka G, Marton S, Zelko R, Otomo N, Antal I (2007) Application of sucrose fatty acid esters in transdermal therapeutic systems. Eur J Pharm Biopharm 65:233–237

Ding Z, Bivas-Benita M, Hirschberg H, Kersten GFA, Jiskoot W, Bouwstra JA (2008) Preparation and characterization of diphtheria toxoid-loaded elastic vesicles for transcutaneous immunization. J Drug Target 16(7–8):555–563

El-Laithy HM (2009) Novel transdermal delivery of Timolol maleate using sugar esters: preclinical and clinical studies. Eur J Pharm Biopharm 72(1):239–245

El-Laithy HM, Shoukry O, Mahran LG (2011) Novel sugar esters proniosomes for transdermal delivery of vinpocetine: preclinical and clinical studies. Eur J Pharm Biopharm 77:43–55

Fang JY, Hwang TL, Fang CL, Chiu HC (2003) *In vitro* and *in vivo* evaluations of the efficacy and safety of skin permeation enhancers using flurbiprofen as a model drug. Int J Pharm 255:153–166

Gale RM, Nelson M, Enscore DJ, Cheng YL, Nedberge DE, Libicki SB (1988) Inventors; ALZA Corporation, assignee. Transdermal delivery system and permeation enhancers for administration of progesterone and/or estradiol esters. European Patent EP 279977 A2

Ganem-Quintanar A, Quintanar-Guerrero D, Falson-Rieg F, Buri P (1998) Ex vivo oral mucosal permeation of lidocaine hydrochloride with sucrose fatty acid esters as absorption enhancers. Int J Pharm 173:203–210

Gutiérrez JM, González C, Maestro A, Solè I, Pey CM, Nolla J (2008) Nano-emulsions: new applications and optimization of their preparation. Curr Opin Colloid Interface Sci 13(4):245–251

Huang D, Jiang X, Zhu H, Fu X, Zhong K, Gao W (2010) Improved synthesis of sucrose fatty acid monoesters under ultrasonic irradiation. Ultrason Sonochem 17:352–355

Höller S (2008) Cubic gels, microemulsions and nanoemulsions for optimized skin penetration/permeation of active molecules and improved chemical stability. PhD thesis, Universität Wien, Wien

Honeywell-Nguyen PL, Frederik PM, Bomans PHH, Junginger HE, Bouwstra JA (2002a) Transdermal delivery of pergolide from surfactant-based elastic and rigid vesicles: characterization and *in vitro* transport studies. Pharm Res 19(7):991–997

Honeywell-Nguyen PL, Van den Bussche MH, Junginger HE, Bouwstra JA (2002b) The effect of surfactant-based elastic and rigid vesicles on the penetration of lidocaine across human skin. STP Pharma Sci 12(4):257–262

Hoo-Kyun C, Je-Phil R (2010) Inventors; Transdermal triptan delivery system. Patent application publication. US Patent 20,100,209,484 A1

Husband FA, Sarney DB, Barnard MJ, Wilde PJ (1998) Comparison of foaming and interfacial properties of pure sucrose monolaurates, dilaurate and commercial preparations. Food Hydrocoll 12:237–244

Klang V, Matsko N, Zimmermann AM, Vojnikovic E, Valenta C (2010) Enhancement of stability and skin permeation by sucrose stearate and cyclodextrins in progesterone nanoemulsions. Int J Pharm 393:152–160

Klang V, Schwarz JC, Matsko N, Rezvani E, El-Hagin N, Wirth M, Valenta C (2011) Semi-solid sucrose stearate-based emulsions as dermal drug delivery systems. Pharmaceutics 3:275–306. http://www.mdpi.com/1999-4923/3/2/275

Kreilgaard M, Pedersen EJ, Jaroszewski JW (2000) NMR characterisation and transdermal drug delivery potential of microemulsion systems. J Control Release 69:421–433

Kumar GP, Rajeshwarrao P (2011) Nonionic surfactant vesicular systems for effective drug delivery-an overview. Acta Pharmaceutica Sinica B 1(4):208–219

Kürti L, Veszelka S, Bocsik A, Khue Dung NT, Ózsvári B, Puskás LG, Kittel A, Szabó-Révész P, Deli MA (2012) The effect of sucrose esters on a culture model of the nasal barrier. Toxicol In Vitro 26:445–454

Lehmann L, Keipert S, Gloor M (2001) Effects of microemulsions on the stratum corneum and hydrocortisone penetration. Eur J Pharm Biopharm 52:129–136

Lerk PC, Sucker H (1993) Application of sucrose laurate in topical preparations of cyclosporine A. Int J Pharm 92(1–3):203–210

Lerk PC (1991) Characterisation and pharmaceutical application of new polyoxyethyleneglycol free surfactants in particular sucrose laurate. PhD Thesis, Basel, Switzerland

Lerk PC, Sucker H (1993) Application of sucrose laurate in topical preparations of cyclosporine A. Int J Pharm 92: 203-210.

Li GL, Danhof M, Bouwstra JA (2001) Effect of elastic liquid-state vesicle on apomorphine iontophoresis transport through human skin *in vitro*. Pharm Res 18(11):1627–1630

Maggio ET, Meezan E, Pillion DJ, Morgan SL, Baggott JE (2009) Inventors; Aegis Therapeutics, Llc, assignee. Controlled release stable, self-assembling, biocompatible and biodegradable therapeutic hydrogel formulations. Patent Application WO 2009029543 A1

Marjukka Suhonen T, Bouwsra JA, Urtti A (1999) Chemical enhancement of percutaneous absorption in relation to stratum corneum structural alterations. J Control Release 59:149–161

Mitsubishi-Kagaku Foods Corporation [Internet] (2012) Ryoto Sugar Ester Technical Information. SURFHOPE™SE COSME; [cited 2012 Dec 13]. Available from: http://www.mfc.co.jp/english/se_cosme/secosme.htm

Muzzalupo R, Tavano L, Cassano R, Trombino S, Ferrarelli T, Picci N (2011) A new approach for the evaluation of niosomes as effective transdermal drug delivery systems. Eur J Pharm Biopharm 79:28–35

Ogiso T, Iwaki M, Tsuji S (1991) Percutaneous absorption of bromhexine in rats. Chem Pharm Bull 39(6):1609–1611

Okamoto H, Sakai T, Danjo K (2005) Effect of sucrose fatty acid esters on transdermal permeation of lidocaine and ketoprofen. Biol Pharm Bull 28(9):1689–1694

Okamoto H, Sakai T, Tokuyama C, Danjo K (2011) Sugar ester J-1216 enhances percutaneous permeation of ionized lidocaine. J Pharm Sci 100(10):4482–4490

Otto A, Wiechers JW, Kelly CL, Dederen JC, Hadgraft J, du Plessis J (2010) Effect of emulsifiers and their liquid crystalline structures in emulsions on dermal and transdermal delivery of hydroquinone, salicylic acid and octadecenedioic acid. Skin Pharmacol Physiol 23:273–282

Papp J (2012) Preparation and evaluation of metoprolol tartrate patches containing different polymer components. PhD thesis, University of Semmelweis, Budapest

Peltola S, Saarinen-Savolainen P, Kiesvaara J, Suhonen TM, Urtti A (2003) Microemulsions for topical delivery of estradiol. Int J Pharm 254:99–107

Piao H, Kamiya N, Hirata A, Fijiii T, Goto M (2008) A novel solid-in-oil nanosuspension for transdermal delivery of diclofenac sodium. Pharm Res 25(4):896–901

Podlogar F, Bester Rogac M, Gasperlin M (2005) The effect of internal structure of selected water-Tween 40-Imwitor-IPM microemulsions on ketoprofene release. Int J Pharm 302:68–77

Polat T, Linhardt RJ (2001) Syntheses and applications of sucrose-based esters. J Surfact Deterg 4(4):415–421

Quintanar-Guerrero D, Ganem-Rondero FA, Cazares-Delgadillo J, Ayala-Bravo HA (2005) Inventors; FES-Cuautitlan UNAM, assignee. Sucrose-fatty acid ester-based drug penetration promoter. PCT Int. Appl. WO 2005079855

Smith DE (1977) Inventor; The Procter and Gamble Company, assignee. Dermatological compositions. US Patent US 4,046,886

Spernath A, Aserin A, Sintov AC, Garti N (2008) Phosphatidylcholine embedded micellar systems: enhanced permeability through rat skin. J Colloid Interface Sci 318:421–429

Soultani S, Ognier S, Engasser JM, Ghoul M (2003) Comparative study of some surface active properties of fructose esters and commercial sucrose esters. Colloids Surf A 227:35–44

Tamarkin D, Friedman D, Meir E (2008) Inventors, Foamix Ltd. assignee. Alcohol-free cosmetic and pharmaceutical foam carrier comprising hydrophobic solvent such as mineral/silicone/vegetable oil, triglycerides, isopropyl myristate, octyl dodecanol, foam adjuvant agent, surfactant and gelling agent. US Patent Appl. US 20,080,031,907

Tahara Y, Honda S, Kamiya N, Piao H, Hirata A, Hayakawa E, Fujii T, Goto M (2008) A solid-in-oil nanodispersion for transcutaneous protein delivery. J Control Release 131(1):14–18

Tavano L, Muzzalupo R, Cassano R, Trombino S, Ferrarelli T, Picci N (2010) New sucrose cocoate based vesicles: preparation characterization and skin permeation studies. Colloids Surf B Biointerfaces 75:319–322

Toro C, Sanchez SA, Zanocco A, Lemp E, Gratton E, Gunther G (2009) Solubilization of lipid bilayers by myristyl sucrose ester: effect of cholesterol and phospholipid head group size. Chem Phys Lipids 157(2):104–112

Thevenin MA, Grossiord JL, Poelman MC (1996) Sucrose esters/cosurfactant microemulsion systems for transdermal delivery: assessment of bicontinuous structures. Int J Pharm 137:177–186

Trommer H, Neubert RHH (2006) Overcoming the stratum corneum: the modulation of skin penetration. Skin Pharmacol Physiol 19:106–121

Szűts A, Pallagi E, Regdon G, Aigner Z, Szabó-Révész P (2007) Study of thermal behaviour of sugar esters. Int J Pharm 336:199–207

Szűts A, Szabó-Révész P (2012) Sucrose esters as natural surfactants in drug delivery systems—a mini-review. Int J Pharm 433:1–9

Vermeire A, De Muynck C, Vandenbossche G, Eechaute W, Geerts ML, Remon JP (1996) Sucrose laurate gels as a percutaneous delivery system for oestradiol in rabbits. J Pharm Pharmacol 48:463–467

Vučinić-Milanković M, Savić S, Vuleta G, Vučinić S (2007) The physicochemical characterization and *in vitro*/*in vivo* evaluation of natural surfactants-based emulsions as vehicles for diclofenac diethylamine. Drug Dev Ind Pharm 33:221–234

Williams AC, Barry BW (2004) Penetration enhancers. Adv Drug Deliv Rev 56:603–618

Yamato K, Takahashi Y, Akiyama H, Tsuji K, Onishi H, Machida Y (2009) Effect of penetration enhancers on transdermal delivery of propofol. Biol Pharm Bull 32(4):677–683

Yang F, Kamiya N, Goto M (2012) Transdermal delivery of the anti-rheumatic agent methotrexate using a solid-in-oil Nanocarrier. Eur J Pharm Biopharm 82:158–163

Yanke L, Shufen Z, Jinzong Y, Qinghui W (2004) Relationship of solubility parameters to interfacial properties of sucrose esters. Colloids Surf A 248:127–133

Pyrrolidones as Penetration Enhancers

17

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Contents

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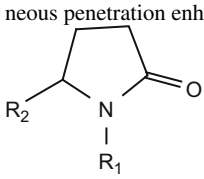
17.1 Introduction

Normal skin is capable of maintaining a reasonably constant level of hydration even when large changes in environmental humidity occur. This is due to the presence of a group of compounds (i.e., pyrrolidone carboxylic acid, free amino acids, lactates, and urea), collectively known as natural moisturizing factor (NMF). NMF can make up to 10 % corneocyte dry weight and these materials can absorb water extensively. In case of severe disease conditions like psoriasis, ichthyosis vulgaris, and flaking, the NMF is appearing to be absent (Walters 1987; Walters and Roberts 2002). Pyrrolidone carboxylic acid, the principal humectant in the NMF, has been shown to increase the water-binding capacity of stratum corneum (SC). Similar to this structure, several pyrrolidones (Table 17.1) were developed and tested for their potential as penetration enhancers.

Early studies investigated various pyrrolidones such as (1) *N*-substituted alkylated (C_1–C_{12}) derivatives, (2) 4-carboxy *N*-substituted alkylated (C_1, C_6, C_{12}) derivatives (3), methoxycarbonyl *N*-substituted alkylated (C_1, C_6, C_{12}) derivatives (4), and oleyl, decyl, and dodecyl esters of pyroglutamic acid. Their lipophilicities widely vary according to the substituted moieties. The percutaneous absorption behavior and localization in the SC of the enhancer itself,

N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_17

Table 17.1 Structures of pyrrolidones used as percutaneous penetration enhancers



Name of pyrrolidone	$-R_1$	$-R_2$
2-pyrrolidone (2P)	–H	–H
N-methyl-2-pyrrolidone (NMP)	$-CH_3$	–H
1-ethyl-2-pyrrolidone (EP)	$-C_2H_5$	–H
5-methyl-2-pyrrolidone (5MP)	–H	$-CH_3$
1,5-dimethyl-2-pyrrolidone (DMP)	$-CH_3$	$-CH_3$
2-pyrrolidone-5-carboxylic acid (2PC)	–H	–COOH
N-hexyl-2-pyrrolidone (NHP)	$-(CH_2)_5CH_3$	–H
N-octyl-2-pyrrolidone (NHP)	$-(CH_2)_7CH_3$	–H
N-lauryl-2-pyrrolidone (NLP)	$-(CH_2)_{11}CH_3$	–H

according to its physicochemical property, can be considered as the most important factors controlling the penetration-enhancing activity, as well as the side effects (Sasaki et al. 1995).

17.2 Pyrrolidone Derivatives

Lower alkylated *N*-substituted-2-pyrrolidones were found to be promising enhancers for anticancer agents, antibiotics, steroids, fungicidal agents, and hormones (Barry 1983). *N*-substituted alkyl (C_1, C_6, C_{12}) derivatives of pyrrolidone and their respective 4-carboxy or 4-methoxycarbonyl substituted derivatives were studied for their penetration enhancement effects on transdermal delivery of phenol red (Sasaki et al. 1988). These derivatives possess various lipophilicities due to the introduction of various functional groups. The C_1 derivative significantly increased the penetration of phenol red through isolated rat skin compared to phenol red alone. Although C_6 and C_{12} derivatives demonstrated better skin penetration of phenol red than C_1, the lag time increased with increase in the carbon chain length from C_1 to C_{12}. C_1 and C_6 derivatives showed high permeation of the enhancer itself into the receptor, while C_{12} derivative was hardly detected in the receptor (Sasaki et al. 1988). Introduction of the 4-carboxy or the 4-methoxycarbonyl group into *N*-alkyl-2-pyrrolidone reduced the enhancers' permeation through the skin and decreased its promoting effect.

It was also reported that *N*-alkyl-2-pyrrolidones promoted the penetration and skin accumulation of 5-fluorouracil, aminopyrine, flurbiprofen, triamcinolone acetonide, indomethacin, and sulfaguanidine (Sasaki et al. 1990a; Sasaki et al. 1991). Yoneto et al. (1995) studied penetration enhancement effects of a series of C2–C8 alkyl pyrrolidones on β-estradiol, corticosterone, and hydrocortisone penetration through the hairless mouse skin in vitro. A nearly semilogarithmic linear relationship was obtained between the enhancement potency and the carbon number of the alkyl chain; there was about an approximate 3.5-fold increase in the enhancement potency per 1-alkyl-2-pyrrolidone methylene group. It was found that the enhancement potencies of the 1-alkyl-2-pyrrolidones were essentially the same as those *n*-alkanols when compared at the same carbon numbers of the alkyl groups. This result suggests that the enhancer action resides entirely in the alkyl group, and the nature of the polar head group may not be intrinsically important in transdermal enhancement of the lipoidal pathway within a class of permeation enhancers (Yoneto et al. 1995; Warner et al. 2001; Kim et al. 1992).

The C_{12} derivative of pyrrolidone showed an increase of threefold higher skin permeation for melatonin through the porcine skin than the C_1 derivative (Rachakonda et al. 2008). In a different study, the C_{12} derivative was also shown to be very effective for transdermal penetration of hydrocortisone in experiments conducted in vitro in the hairless mouse skin; the C_{12} derivative showed an about 42-fold higher skin permeation and fivefold higher skin retention of hydrocortisone compared to the vehicle (propylene glycol) alone (Godwin et al. 1997). However, the lower alkyl derivatives of NMP were not effective in increasing the skin permeation of hydrocortisone. The low lipophilicity of these enhancers was the reason for the lack

of the skin permeation enhancement effect for a highly lipophilic drug such as hydrocortisone. However, the content of hydrocortisone in the skin was substantially higher due to the use of hydrophilic compounds as compared to more lipophilic compounds. The alkyl derivatives rather showed the ability to accumulate the drug in the skin, which can be used for a dermal application or for a gradual drug release from the skin to blood capillaries (Godwin et al. 1997). *N*-substituted 2-pyrrolidones were ineffective in increasing the permeation of verapamil, a lipophilic drug, through hairless mouse skin (Shah et al. 1992). The general conclusion was that the permeation enhancement by the lower alkyl derivatives of pyrrolidone was greater for hydrophilic drugs than for lipophilic drugs (Barry and Williams 1995).

Pyroglutamic acid (PGA) and its derivatives are used as moisturizers in cosmetics. Oleyl, decyl, and dodecyl esters of PGA were reported to enhance the permeation of enalapril and clonidine across shed snake skin (Santos and Baker 1993). Lambert et al. (1993) demonstrated the usefulness of a biodegradable transdermal penetration enhancer, decanoic acid ester of *N*-(2-hydroxyethyl)-2-pyrrolidone (HEP-Dec). The ester linkage was readily cleaved by hydrolytic enzymes in plasma and the skin. This enhancer increased the permeation of hydrocortisone through mouse skin by three orders of magnitude compared to saline (control) and showed a much lower irritation potential than traditional penetration enhancers (Azone, oleyl alcohol). *N*-ethyl pyrrolidinone ester prodrug of ketoprofen was more effective than ketoprofen itself (Bonina et al. 2003). Many of the abovementioned pyrrolidone derivatives as penetration enhancers did not appear in the recent literature. The toxicity and skin irritancy of these enhancers (Sasaki et al. 1995) may be the reason that these enhancers were not studied further in recent years.

17.3 *N*-Methyl-2-Pyrrolidone

N-methyl-2-pyrrolidone (NMP) is also known as 1-methyl-2-pyrrolidone and 1-methyl-2-pyrrolidinone. NMP has been a widely studied skin penetration enhancer in the recent years. It is a colorless hygroscopic liquid with a mild amine odor. It is a basic and polar compound with high stability. It has the following physicochemical properties: molecular mass, 99.13; density, 1.028 g/cm^3; melting point, 23–24.4 °C; boiling point, 202 °C; log octanol/water partition coefficient, −0.38; and viscosity (cP), 1.80. The substance is completely miscible with water. It is highly soluble in lower alcohols, lower ketones, ether, ethyl acetate, chloroform, and benzene and moderately soluble in aliphatic hydrocarbons. It is a biodegradable solvent; therefore, environmental contamination considerations are fewer in its industrial applications (Chow and Ng 1983). It is used in different fields and is considered a safe solvent (Strickley 2004).

NMP has been approved by the US Food and Drug Administration (FDA) for its use as a cosolvent in parenteral dosage forms and as an excipient in periodontal drug delivery systems. It is used as a pharmaceutical aid as a vehicle and cosolvent, complexing agent, and co-surfactant (Uch et al. 1999; Bachhav et al. 2006). NMP was employed as a solubilizer for several drugs such as itraconazole, hydrocortisone, florfenicol, carvedilol, clonazepam, diazepam, lamotrigine, phenobarbital, pioglitazone, estrogen, and griseofulvin (Reviewed in Jouyban et al. 2010).

NMP is used as an effective percutaneous penetration enhancer (Reviewed in Godavarthy et al. 2009; Rachakonda et al. 2008). NMP used at 5 % concentration in a gel and solution as a penetration enhancer for the permeation of bupranolol across rat skin provided 1.5- and 2.4-fold higher permeation rates, respectively, compared to formulations without NMP (Babu and Pandit 2005a, b). The skin permeation of Spantide II across hairless rat skin was studied in vitro using NMP (10 % wt/vol) as an enhancer. NMP increased dermal levels of Spantide II by 3.5- and 2-fold for the solution and gel, respectively, as compared to the formulations without NMP. No permeation of Spantide II through intact and stripped skin into receptor phase was detected (Kikwai et al. 2005). Recently, NMP has been used in a nanoemulsion system for the

transdermal delivery of granisetron hydrochloride. NMP used at 15 % concentration in the emulsion provided a 4.1-fold higher drug permeation across rat skin compared to the control emulsion without NMP (Zheng et al. 2010). The percutaneous absorption of a peptide drug, luteinizing hormone-releasing hormone (LHRH), was found to be enhanced by NMP (neat solvent)-treated porcine epidermis (Bhatia and Singh 1997). Several examples of drugs and their topical and transdermal formulations containing NMP as a penetration enhancer are represented in Table 17.2.

Early studies have shown that NMP increases the transport of mannitol and hydrocortisone (Barry and Bennett 1987), steroids (Bennett et al. 1984; Barry et al. 1984), caffeine (Southwell and Barry 1983), ibuprofen and flurbiprofen (Akhter and Barry 1985), and acetylsalicylic acid (Southwell and Barry 1984). NMP has been found to accelerate the permeation of mefenamic acid across rabbit skin in vivo (Naito et al. 1985), but it did not increase the permeation of naloxone, in vitro (Aungst et al. 1986), and metronidazole (Mollgaard et al. 1988) through human skin, in vitro. However, NMP in combination with a nonpolar cosolvent isopropyl myristate (IPM) enhanced the permeation of metronidazole (Hoelgaard et al. 1988). NMP and its combination with other solvents such as IPM are extensively researched in recent years as penetration enhancers (Table 17.2). Several reports have documented the synergistic enhancement effect of a mixture of NMP and IPM. A binary mixture of NMP:IPM (75:25) significantly improved lidocaine flux across human skin showing an enhancement of 25-fold over 100 % IPM and fourfold over 100 % NMP (Lee et al. 2006). NMP: Menthol and NMP:IPM (4:1, 3:2, 1:1, 2:3, 1:4) binary mixtures have also been documented to show synergistic enhancement effect on the transdermal delivery of formoterol fumarate (Kakubari et al. 2006). NMP, a small molecule, shows a high propensity for forming combinations with other penetration enhancers which have synergistic effects on increasing skin permeability (Karande and Mitragotri 2009).

17.4 Penetration Enhancement Mechanisms

17.4.1 Influence of Composition of the Formulation

The concentration of a penetration enhancer in a formulation markedly influences percutaneous absorption of drugs. A decrease in the concentration of alkyl pyrrolidones in the formulation was found to decrease the flux and skin accumulation, and to prolong the lag time for a steady-state penetration of phenol red (Sasaki et al. 1990b). This reduced enhancing effect of a penetration enhancer can be explained by a decrease in the penetration and skin accumulation of the enhancer. The flux of lidocaine across human skin was low at an NMP concentration between 0 and 50 % in the formulation. However, as the NMP concentration increased above 50 %, the enhancement properties of NMP increased significantly. The flux of lidocaine was 170-fold higher at 100 % NMP as compared to 50 % NMP concentration in water. The enhanced lidocaine flux correlated well with the transdermal flux of NMP itself (Lee et al. 2005).

The vehicle in a topical formulation is an important factor which controls the penetration of not only the drug but also the enhancer itself. Skin pretreatment with C_1, C_6, and C_{12} alkyl pyrrolidones for 5 h shortened the lag time for the penetration of phenol red (Sasaki et al. 1990b). NMP in an aqueous vehicle showed a low enhancing effect on the transdermal penetration of phenol red and indomethacin as compared to NMP in IPM vehicle (Sasaki et al. 1990c). This inactivity of NMP was explained by the poor penetration of NMP from the aqueous vehicle through the skin. Both C_6 and C_{12} alkyl pyrrolidones in an aqueous vehicle showed a high enhancing effect for the penetration of indomethacin. In contrast, pretreatment of rat skin in vitro with 10 % NMP solution in water for 3 h increased the flux of bupranolol by threefold as compared to the control alone (skin treated with water alone) (Babu and Pandit 2005b).

Some vehicles can penetrate the SC and have also the role of a penetration enhancer. Sasaki

Table 17.2 NMP as percutaneous penetration enhancer for various drugs

Drug	NMP	Skin	Comments	Reference
Lidocaine HCl	1:1 NMP:water	Human cadaver	Fourfold higher flux versus water (no NMP)	Lee et al. (2005)
Prilocaine HCl	1:1 NMP:water	Human cadaver	2.5-fold higher flux versus water (no NMP)	Lee et al. (2005)
Spantide II	10 % NMP in ethanol:water (1:1)	Hairless rat	3.5-fold higher skin levels versus control (no NMP)	Kikwai et al. (2005)
Formoterol Fumarate	1:1 = cineole:NMP 1:1 = menthol:NMP	Rat	Both cineole and menthol show 22-fold higher flux versus 100 % NMP	Kakubari et al. (2006)
Lidocaine HCl	10 % NMP + 10%oleyl alcohol as a dual enhancer system in IPM based o/w microemulsion	Human cadaver	30-fold higher flux versus water	Lee et al. (2003)
Diltiazem HCl	10 % NMP + 10% oleyl alcohol as a dual enhancer system in IPM based o/w microemulsion	Human cadaver	520 times enhanced permeation versus water	Lee et al. (2003)
Estradiol	3:7 = NMP:soybean oil	Yucatan micropig	25-fold higher permeation versus soybean oil	Koizumi et al. (2004)
Flurbiprofen	5 % NMP in IPM	Rat	Sixfold higher permeation versus IPM only	Ma et al. (2010)
Atenolol	5 % NMP in 3:1 = ethanol :water system	Porcine	2.5-fold higher flux versus vehicle alone	Nair et al. (2009)
Bupranolol	5 % IPM:5 % NMP in a gel base	Rabbit	3.6 times higher flux versus the gel with no enhancers	Ogiso et al. (2001)
Granisetron HCl	10 % NMP in a nanoemulsion base	Rat	4.1-fold higher permeation compared to the emulsion with no NMP	Zheng et al. (2010)
Griseofulvin	10 % NMP in a hydrogel base	Mice	3.5-fold higher flux versus hydrogel with no NMP	Shishu and Aggarwal (2006)
Zidovudine	10 % NMP in 20/80 = ethanol/IPM mixture	Pig	2.4-fold higher flux versus the vehicle with no NMP	Suwanpidokkul et al. (2004)

et al. (1990b) found that NMP promoted the rapid penetration of *N*-lauryl (C_{12}) pyrrolidone (NLP) into the skin, and potentiated the enhancing effect of NLP on the penetration of phenol red and 5-fluorouracil through the skin. The effect of the combination of IPM and NMP was investigated for the transdermal delivery of the anesthetic drug lidocaine across pig and human skin in vitro. The binary system improved drug transport. At 2 % of lidocaine dose, this synergistic enhancement peaked when IPM:NMP was used at the ratio 1:3 with a fourfold enhancement in the permeation rate over a 100 % NMP and over 25-fold increase over 100 % IPM (Lee et al. 2006). The enhancers increased each other's penetration into the skin and improved their enhancing activity. Therefore, the combination of vehicles acts synergistically rather than additively.

17.4.2 Cotransportation of Enhancer and Drug and Interaction of Enhancer with Skin Components

The SC is made up of dense layers of keratinized cells which are surrounded by a lipid matrix. According to the lipophilicity of the penetrant, it follows the polar route and/or the nonpolar route for the permeation across the SC (Williams and Barry 2012; Barry 1987). Pyrrolidones enhance drug permeation through the polar route of the skin by increasing the diffusivity, and reduce the passage through the nonpolar route by decreasing diffusivity and partitioning (Southwell and Barry 1983). The ability of skin-permeable solvent molecules to influence the cotransport of drugs and enhancers has been previously reported (Cross et al. 2001). In a binary system of NMP and water, the NMP transport across the skin was minimal up to the concentration of 50 % NMP. Between 50 and 100 % MNP concentration in water, NMP flux across skin increases from 1 to 18 mg/cm^2/h. Lidocaine was also cotransported with NMP. As NMP concentration increased from 50 to 100 % NMP, lidocaine free base flux increased from 1.7 to 290 mg/cm^2/h (Lee et al. 2005). The mechanism by which NMP increases the flux of lidocaine transport was explained by hydrogen bonding. The carbonyl oxygen in NMP can form a hydrogen bond with the amine group of lidocaine (Lee et al. 2005, 2006). Formation of a lipophilic ion pair of an amide enhancer with an anionic penetrant might cause the strong solubilizing capacity of pyrrolidones. However, *N*-alkyl-2-pyrrolidones showed a great enhancing effect on the penetration of even nonionic drugs (Sasaki et al. 1990d). El-Hinnawi and Najib (1987) reported that the nitrogen of the pyrrolidone ring interacted with carbonyl groups of drugs through hydrogen bonding. Hydrogen bonding may contribute to the solubilizing capacity of pyrrolidones.

At high concentrations, NMP was able to interact with the SC lipids, increasing their fluidity. Also, NMP was reported to increase the water content in the SC, by determining skin conductance with an impedance meter (Sasaki et al. 1990e). Pyrrolidones exert their direct influence on the aqueous regions between the polar lipid head groups of the bilayer. These enhancers penetrate into this region of tissue in such amounts that they alter the solubilizing ability of this site, thereby promoting drug partition into skin, which subsequently results in increased flux of the penetrant (Barry 1987).

17.5 Skin Irritation and Sensitization

Skin irritation tests in New Zealand white rabbits ($n=6$) exposed to 0.5 ml of C_1 C_{10} alkyl pyrrolidones (neat solvents) were performed with 24 h occlusion of the exposed sites and then examined for skin reactions. Very slight or no erythema was observed for C_1, C_2 and C_3 derivatives. However, C6, C8, and C10 derivatives produced severe irritation within 24 h application (Ansell and Fowler 1988).

Aqueous solutions of NMP were tested for primary skin irritation in ten male albino guinea pigs. Twenty-four hours after application, slight erythema was observed in two guinea pigs treated with the 50 % NMP solution (in water) and in no erythema with the 5 % solution ($n=10$). After 48 h, no effects were registered (Becci et al. 1982).

Sensitization potential tests, defined as the increase of response at challenge after a series of four intradermal injections (0.1 ml of 1 % NMP in 0.9 % saline solution; one injection per week), were performed in ten male albino guinea pigs. Two weeks after the intradermal injections, the animals were exposed to aqueous solutions of NMP. About 0.05 ml each of a 5 % and a 50 % solution were applied and lightly rubbed in to the shaved intact shoulder skin. Nine guinea pigs that did not have intradermal injections of NMP were used as control animals. No sensitization was found when the animals were examined after 24 and 48 h. After 24 h, there was slight erythema at the 50 % solution test sites in six out of ten challenged guinea pigs and in four out of nine controls. No effects were observed when animals were examined after 48 h. The 5 % NMP solution

caused no irritation (E.I. du Pont de Nemours and Company 1976). Dermal exposures of NMP in rats and humans showed no serious dermatological effects (Payan et al. 2003; Akrill et al. 2002).

Conclusion

Pyrrolodine derivatives were widely researched as percutaneous penetration enhancers in the past years. However, these did not appear much in the literature in the past decade. The toxicity and skin irritancy of these enhancers may be the reason that these enhancers were not studied further. *N*-methyl-2-pyrrolidone (NMP) has been approved by the US-FDA for its use as a co-solvent and an excipient in parenteral and other drug delivery systems. NMP and its combination with other solvents such as isopropyl myristate (IPM) are researched in recent years for their synergistic enhancements in the percutaneous absorption of drugs. Pyrrolidones exert their direct influence on the aqueous regions between the polar lipid head groups of the intercellular lipid bilayers in the SC. These enhancers penetrate into this region of tissue in such amounts that they alter the solubilizing ability of this site, thereby promoting drug partition into skin, which subsequently results in their increased flux. NMP is also known to form hydrogen bonds with basic drugs and cotransport the penetrant across the skin.

References

Akhter SA, Barry BW (1985) Absorption through human skin of ibuprofen and flurbiprofen; effect of dose variation, deposited drug films, occlusion and the penetration enhancer N-methyl-2-pyrrolidone. J Pharm Pharmacol 37(1):27–37

Akrill P, Cocker J, Dixon S (2002) Dermal exposure to aqueous solutions of N-methyl pyrrolidone. Toxicol Lett 134:265–269

Ansell JM, Fowler JA (1988) The acute oral toxicity and primary ocular and dermal irritation of selected N-alkyl-2-pyrrolidones. Food Chem Toxicol 26:475–479

Aungst BJ, Rogers NJ, Shefter E (1986) Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactants, sulfoxides and amides. Int J Pharm 33:225–234

Babu RJ, Pandit JK (2005a) Effect of penetration enhancers on the release and skin permeation of bupranolol from reservoir-type transdermal delivery systems. Int J Pharm 288(2):325–334

Babu RJ, Pandit JK (2005b) Effect of penetration enhancers on the transdermal delivery of bupranolol through rat skin. Drug Deliv 12(3):165–169

Bachhav YG, Date AA, Patravale VB (2006) Exploring the potential of N-methyl pyrrolidone as a cosurfactant in the microemulsion systems. Int J Pharm 326: 186–189

Barry BW (1983) Properties that influence percutaneous absorption. In: Dermatological formulations percutaneous absorption. Marcel Dekker, New York, pp 127–233

Barry BW (1987) Mode of action of penetration enhancers in human skin. J Control Release 6(1):85–97

Barry BW, Bennett SL (1987) Effect of penetration enhancers on the permeation of mannitol, hydrocortisone and progesterone through human skin. J Pharm Pharmacol 39:535–546

Barry BW, Williams AC (1995) Permeation enhancement through skin. In: Swarbrick J, Boylon JC (eds) Encyclopedia of pharmaceutical technology, vol 12. Marcel Dekker Inc, New York, pp 449–493

Barry BW, Southwell D, Woodford R (1984) Optimization of bioavailability of topical steroids: penetration enhancers under occlusion. J Invest Dermatol 82:49–52

Becci PJ, Knickerbocker MJ, Reagan EL, Parent RA, Llewellyn WB (1982) Teratogenicity study of N-methylpyrrolidone after dermal application to Sprague-Dawley rats. Fundam Appl Toxicol 2:73–76

Bennett SL, Barry BW, Woodford R (1984) Optimization of bioavailability of topical steroids: non-occluded penetration enhancers under thermodynamic control. J Pharm Pharmacol 37:298–304

Bhatia KS, Singh J (1997) Percutaneous absorption of LHRH through porcine skin: effect of N-Methyl-2-pyrrolidone and isopropyl myristate. Drug Dev Ind Pharm 23:1111–1114

Bonina F, Santagati NA, Puglia C (2003) Ketoprofen 1-alkylazacycloalkan-2-one esters as dermal prodrugs: in vivo and in vitro evaluations. Drug Dev Ind Pharm 29(2):181–190

Chow ST, Ng TL (1983) The biodegradation of N-methyl-2-pyrrolidone in water by sewage bacteria. Water Res 17(1):117–118

Cross SE, Pugh WJ, Hadgraft J, Roberts MS (2001) Probing the effect of vehicles on topical delivery: understanding the basic relationship between solvent and solute penetration using silicone membranes. Pharm Res 18:999–1005

E.I. du Pont de Nemours and Company, Wilmington, DE (1976) Primary skin irritation and sensitization test on guinea pigs. Haskell Laboratory Report No. 307–776

El-Hinnawi MA, Najib NM (1987) Ibuprofen-polyvinylpyrrolidone dispersions. Proton nuclear magnetic resonance and infrared studies. Int J Pharm 37:175–177

Godavarthy SS, Yerramsetty KM, Rachakonda VK, Neely BJ, Madihally SV, Robinson RL Jr, Gasem KA (2009) Design of improved permeation enhancers for transdermal drug delivery. J Pharm Sci 98:4085–4099

Godwin DA, Michniak BB, Player MR, Sowell JW (1997) Transdermal and dermal enhancing activity of pyrrolidinones in hairless mouse skin. Int J Pharm 155:241–250

Hoelgaard A, Mollgaard B, Baker E (1988) Effect of N-methyl pyrrolidone and polar lipids on percutaneous drug transport. Int J Pharm 43:233–240

Jouyban A, Fakhree MAA, Shayanfar A (2010) Review of pharmaceutical applications of N-ethyl-2-pyrrolidone. J Pharm Sci 13(4):524–535

Kakubari I, Nakamura N, Takayasu T, Yamauchi H, Takayama S, Takayama K (2006) Effects of solvents on skin permeation of formoterol fumarate. Biol Pharm Bull 29(1):146–149

Karande P, Mitragotri S (2009) Enhancement of transdermal drug delivery via synergistic action of chemicals. Biochim Biophys Acta 1788(11):2362–2373

Kikwai L, Babu RJ, Prado R, Kolot A, Armstrong CA, Ansel JC et al (2005) In vitro and in vivo evaluation of topical formulations of Spantide II. AAPS PharmSciTech 6(4):E565–E572

Kim YH, Ghanem AH, Mahmoud H, Higuchi WI (1992) Short chain alkanols as transport enhancers for lipophilic and polar/ionic permeants in hairless mouse skin: mechanism (s) of action. Int J Pharm 80(1):17–31

Koizumi A, Fujii M, Kondoh M, Watanabe Y (2004) Effect of N-methyl-2-pyrrolidone on skin permeation of estradiol. Eur J Pharm Biopharm 57:473–478

Lambert WJ, Kudla RJ, Holland JM, Curry JT (1993) A biodegradable transdermal penetration enhancer based on N-(2-hydroxyethyl)-2-pyrrolidone. I. Synthesis and characterization. Int J Pharm 95:181–192

Lee PJ, Langer R, Shastri VP (2003) Novel microemulsion enhancer formulation for simultaneous transdermal delivery of hydrophilic and hydrophobic drugs. Pharm Res 20:264–269

Lee PJ, Langer R, Shastri VP (2005) Role of n-methyl pyrrolidone in the enhancement of aqueous phase transdermal transport. J Pharm Sci 94:912–917

Lee PJ, Ahmad N, Langer R, Mitragotri S, Prasad Shastri V (2006) Evaluation of chemical enhancers in the transdermal delivery of lidocaine. Int J Pharm 308:33–39

Ma X, Fang L, Guo JP, Zhao N, He ZG (2010) Effect of counter-ions and penetration enhancers on the skin permeation of flurbiprofen. J Pharm Sci 99: 1826–1837

Mollgaard B, Hoelgaard A, Baker E (1988) Vehicle effects on topical drug delivery: effect of N-methyl pyrrolidone, polar lipids and Azone on percutaneous drug transport. Proc Int Symp Controlled Rel Bioact Mater 15:209

Nair A, Reddy C, Jacob S (2009) Delivery of a classical antihypertensive agent through the skin by chemical enhancers and iontophoresis. Skin Res Technol 15:187–194

Naito SI, Nakamori S, Awataguchi M, Nakayama T, Tominaga H (1985) Observations on and pharmacokinetic discussion of percutaneous absorption of mefenamic acid. Int J Pharm 24:127–147

Ogiso T, Hata T, Iwaki M, Tanino T (2001) Transdermal absorption of bupranolol in rabbit skin in vitro and in vivo. Biol Pharm Bull 24:588–591

Payan JP, Boudry I, Beydon D, Fabry JP, Grandclaude MC, Ferrari E et al (2003) Toxicokinetics and metabolism of N-[(14)C]N-methyl-2-pyrrolidone in male Sprague-Dawley rats: in vivo and in vitro percutaneous absorption. Drug Metab Dispos 31:659–669

Rachakonda VK, Yerramsetty KM, Madihally SV, Robinson RL, Gasem KA (2008) Screening of chemical penetration enhancers for transdermal drug delivery using electrical resistance of skin. Pharm Res 25:2697–2704

Santos GC, Baker RW (1993) Transdermal enhancer patent literature. J Control ReIease 25:1–20

Sasaki H, Kojima M, Mori Y, Nakamura J, Shibasakl J (1988) Enhancing effects of pyrrolidone derivatives on transdermal drug delivery. I. Int J Pharm 44:15–24

Sasaki H, Kojima M, Nakamura J, Shlhasaki J (1990a) Enhancing effect of pyrrolidone derivatives on the transdermal penetration of sulfaguanidine, aminopyrine and sudan Ill. J Pharmacobiodyn 13:200–205

Sasaki H, Kojima M, Morl Y, Nakamura J, Shlbasakl J (1990b) Enhancing effect pyrrolidone derivatives on transdermal drug delivery. II. Effect of application concentration and pretreatment of enhancer. Int J Pharm 60:177–183

Sasaki H, Kojima M, Nakamura J, Shlbasaki J (1990c) Enhancing effect of pyrrolidone derivatives on transdermal penetration of phenolsulfonphthalein and indomethacin from aqueous vehicle. Chem Pharm Bull 38:797–799

Sasaki H, Kojima M, Nakamura J, Shibasaki J (1990d) Enhancing effect of combining two pyrrolidone vehicles on transdermal drug delivery. J Pharm Pharmacol 42:196–199

Sasaki H, Kishikawa M, Nakamura J, Shibasaki J (1990e) Effect of pyrrolidone derivatives on lipid membrane and protein conformation as transdermal penetration enhancer. J Pharmacobiodyn 13:468–474

Sasaki H, Kojima M, Morl Y, Nakamura J, ShlbaSaki J (1991) Enhancing effect 0 pyrrolidone derivatives on transdermal penetration of 5-fluorouracil, triamcinolone acetonide indomethacin, and flurbiprofen. J Pharm Sci 80:533–538

Sasaki H, Nishida K, Nakamura J (1995) Pyrrolidones as penetration enhancers. In: Maibach HI, Smith EW (eds) Percutaneous penetration enhancers. CRC Press, Boca Raton, pp 211–220

Shah HS, Tojo K, Chien YW (1992) Enhancement of in vitro skin permeation of verapamil. Drug Dev Ind Pharm 18:1461–1476

Shishu, Aggarwal N (2006) Preparation of hydrogels of griseofulvin for dermal application. Int J Pharm 326:20–24

Southwell D, Barry BW (1983) Penetration enhancers for human skin: mode of action of 2-pyrrolidone and dimethylformamide on partition and diffusion of

model compounds, water, n-alkanols and caffeine. J Invest Dermatol 80:507–514

Southwell D, Barry BW (1984) Penetration enhancement in human skin: effect of 2-pyrrolidone, dimethylformamide and increased hydration on finite dose permeation of aspirin and caffeine. Int J Pharm 22:291–298

Strickley RG (2004) Solubilizing excipients in oral and injectable formulations. Pharm Res 21(2):201–230

Suwanpidokkul N, Thongnopnua P, Umprayn K (2004) Transdermal Delivery of Zidovudine (AZT): the effects of vehicles, enhancers, and polymer membranes on permeation across cadaver pig skin. AAPS PharmSciTech 5(3):1–8

Uch AS, Hesse U, Dressman JB (1999) Use of 1-methylpyrrolidone as a solubilizing agent for determining the uptake of poorly soluble drugs. Pharm Res 16:968–971

Walters KA (1987) Penetration enhancers and their use in transdermal therapeutic systems. In: Hadgraft J, Guy RH (eds) Transdermal drug delivery, developmental issues and research initiatives. Marcel Dekker Inc, New York, pp 197–246

Walters KA, Roberts MS (2002) The structure and function of skin. In: Walters KA (ed) Dermatological and topical formulations. Marcel and Dekker Inc, New York, pp 1–42

Warner KS, Li SK, Higuchi WI (2001) Influences of alkyl group chain length and polar head group on chemical skin permeation enhancement. J Pharm Sci 90: 1143–1153

Williams AC, Barry BW (2012) Penetration enhancers. Adv Drug Deliv Rev 64:128–137

Yoneto K, Ghanem AH, Higuchi WI, Peck KD, Li SK (1995) Mechanistic studies of the 1-alkyl-2-pyrrolidones as skin permeation enhancers. J Pharm Sci 84:312–317

Zheng WW, Zhao L, Wei YM, Ye Y, Xiao SH (2010) Preparation and the in vitro evaluation of nanoemulsion system for the transdermal delivery of granisetron hydrochloride. Chem Pharm Bull 58(8):1015–1019

Urea and Derivatives as Penetration Enhancers

18

Adrian C. Williams

Contents

18.1 Introduction

Urea (also called carbamide) has a long history of use in agriculture, manufacturing processes and medicine. Naturally produced through the urea cycle from oxidation of amino acids, urine contains approximately 10 g/L urea together with creatinine, ions and other organic and inorganic materials including enzymes (e.g. urokinase). Both urine and urea are valuable fertilisers due to the high nitrogen content of urea, and indeed approximately 90 % of the world urea production is for nitrogen-releasing fertilisers. Urine was used over 9000 years ago for tanning, softening animal hides and used to help remove hairs. Indeed, such was the value of urine in the tanning industry that the Roman emperor Nero imposed a tax on it and "piss pots" were located on street corners, where human urine could be collected for use in tanneries. The medicinal benefits of urine can also be traced to ancient history; Aztecs and ancient Egyptians practised urotherapy, using one's own urine to maintain health, to treat illness or to enhance beauty. In the seventeenth century, aristocratic French women reportedly bathed in urine to beautify their skin and Mexican farmers prepare poultices for broken bones by having a child urinate into a bowl of powdered charred corn; the mixture is made into a paste and applied to the skin (Gardner 1999). Such reports illustrate the use of urea not only as an active

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N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_18

pharmaceutical ingredient but also its effects on the properties and function of skin.

Urea was first discovered by Herman Boerhaave, who held chairs in Chemistry and Botany at the University of Leiden, as he studied evaporates of urine. Subsequently, the French chemist Hilaire Marin Rouelle crystallised urea from dog urine. In 1828, Friedrich Wöhler at the Polytechnic School in Berlin synthesised urea from silver isocyanate and ammonium chloride; this was the first time that an organic material was synthesised from inorganic materials and the "Wöhler synthesis" is regarded as a foundation stone in organic chemistry. The discovery prompted Wöhler to write to his mentor, the Swedish chemist Jöns Jacob Berzelius:

> I cannot, so to say, hold my chemical water and must tell you that I can make urea without thereby needing to have kidneys, or anyhow, an animal, be it human or dog. Ammonium cyanate is urea.

18.2 Urea: An Emollient

Urea is a hydrating agent (a hydrotrope) used in the treatment of scaling conditions such as psoriasis, ichthyosis and other hyper-keratotic skin conditions. Numerous emollient creams and lotions contain urea, typically at between 5 and 10 %, and it is also combined with lactic acid (British National Formulary 2012). Creams and gels with 40 % urea are available as keratolytic emollients for nails and skin and to soften hyperkeratotic skin areas. Additionally, urea (typically 10 %) is included in various corticosteroid preparations (e.g. in 1 % hydrocortisone creams).

Water within skin has been described according to three "forms" (Bettinger et al. 1995). Primary water, amounting to approximately 5 % of the tissue dry weight, is tightly bound to the stratum corneum and cannot be removed even when the tissue is maintained in a dry (0 % relative humidity, RH) environment. This tightly bound water remains as the skin ages and is near invariant in healthy and diseased (e.g. psoriatic) skin. Secondary water is more weakly bound within skin, and exposing stratum corneum to 70 % RH leads to approximately 30 % (of dry weight) secondary water. Exposure to higher humidity (100 % RH) leads to free water within the stratum corneum and can generate water contents between 200 and 500 % (of dry weight). Natural Moisturising Factor (NMF) is an endogenous breakdown product from filaggrin hydrolysis and is a hygroscopic mixture including amino acids, pyrrolidone carboxylic acid, lactic acid and urea (Cler and Fourtanier 1981). NMF plays a significant role in maintaining free water within the stratum corneum (Visscher et al. 2003) and indeed NMF generation together with other processes such as corneocyte maturation, desquamation and lipid biosynthesis regulate stratum corneum hydration. Stratum corneum has been described as having a "biosensory function", responding to external humidity to maintain optimal water content (Rawlings and Hardy 2004).

Whilst only a relatively minor component of NMF (approximately 7 %), urea is an effective hydrotrope and is highly water soluble (approximately 1.08 g/mL at 20 °C). It has been shown that urea fits well into the hydrogen bond network of water (Soper et al. 2003) where it can substitute for a water dimer. Urea can reform the six hydrogen bonds that existed between the water dimer and adjacent water molecules, with one water molecule in the solvation shell sharing two hydrogen bonds with urea (Rezus and Bakker 2006), as illustrated in Fig. 18.1. The keratolytic and osmotic effects of urea on human epidermal membranes were reported over 35 years ago (Hellgren and Larsson 1974). The study concluded that the increased water-binding capacity of urea-treated tissue resulted from osmotic effects. Urea was shown to increase the free-water content of the stratum corneum rather than influencing the bound "primary" or "secondary" water that is associated with keratin and other biomolecules in the tissue (Bettinger and Maibach 1997).

Applied in a water-in-oil vehicle, urea alone or in combination with ammonium lactate produced significant stratum corneum hydration and improved barrier function when compared to the vehicle alone in human volunteers in vivo (Gloor et al. 2001). However, it has been

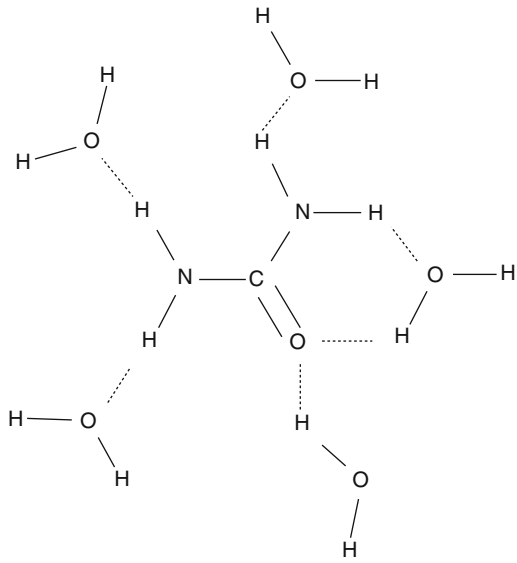

Fig. 18.1 Illustration of the solvation shell around a urea molecule showing hydrogen bond interactions with water molecules (Adapted from Rezus and Bakker 2006)

reported that this effect may compromise the skin barrier to exogenous chemical permeation (Brand et al. 2007). Interestingly, despite its clear keratolytic properties, treatment with 10 % urea for up to 6 h did not alter the amount of stratum corneum removed following tape stripping, in contrast to the other known keratolytic agents such as 2 % salicylic acid (Loden et al. 1995). In a subsequent in vivo study using healthy volunteers, Loden demonstrated that multiple treatments with urea-containing moisturisers decreased transepidermal water loss (TEWL) and reduced irritation reactions to sodium lauryl sulphate (SLS) challenge, illustrating *increases* in normal skin barrier function (Loden 1996). Interestingly, it has recently been reported that a 10 % urea-containing cream applied to hairless rat skin in vivo not only decreased TEWL but also inhibited cutaneous arterial sympathetic nerve activity whilst elevating cutaneous blood flow (Horii et al. 2011). In addition, there are numerous reports describing the benefits of urea-containing moisturisers to improve skin barrier function for disorders such as atopic dermatitis (e.g. Loden et al. 1999) or ichthyosis (Grice et al. 1973).

Considering the disparate reports on the influence of urea on normal skin barrier properties, Savic et al. explored the influence of different emulsion vehicles on the effects of urea in human volunteers (Savic et al. 2004). From assessments of TEWL, electrical capacitance and subsequent SLS irritation, the authors show that formulation variables (such as the use of nonionic ethoxylated emulsifiers or sugar-based emulsifiers) had significant impact on urea release and delivery, moisturisation and barrier function of skin; it appears likely that these variables account for some of the above literature discrepancies.

18.3 Urea: An Enhancer (?)

Considering both the kertolytic actions and the ability of urea to increase the water content of the stratum corneum, it would be expected that urea would act as a strong permeation promoter. Early investigations of enhancer activities explored the role of urea in facilitating transdermal delivery of hydrocortisone through human volunteer's skin, assessed by the vasoconstrictor assay (Feldman and Maibach 1974; Barry and Woodford 1976; Woodford and Barry 1984). Urea (included at 10 % in most formulations) was shown to significantly enhance steroid delivery and bioavailability, typically generating 2–3-fold enhancements. Likewise, incorporating urea into a 1 % hydrocortisone preparation increased drug penetration 2–3-fold into all three skin layers (stratum corneum, epidermis, dermis) (Wohlrab 1984). More recently, tape-stripping human volunteers followed by Fourier transform infrared spectroscopy were used to monitor terbinafine penetration from a 50:50 ethanol:isopropyl myristate formulation containing 1 % urea. Unlike an oleic acid-containing formulation, urea did not improve terbinafine penetration into the stratum corneum (Alberti et al. 2001).

Other workers have explored the enhancement activity of urea from differing formulations and vehicles using a variety of animal skin membranes. It is well recognised that enhancer activities vary for different skin membranes with typically hairless mouse and other rodent skin

affected to a significantly greater extent than human skin (Lu et al. 1992; Williams 2003). Additionally, enhancer activities can be modified by the vehicle from which they are applied, for example, where a solvent such as propylene glycol (PG) can partition into the stratum corneum and provide an environment into which the enhancer itself can more readily partition. Further, as with transdermal delivery of an active pharmaceutical ingredient, the degree of saturation (or more accurately the fraction of maximal thermodynamic activity) can also influence delivery of the enhancer into the stratum corneum and hence affect its activity.

Vehicle composition influenced the enhancement activity of urea towards ketoprofen through excised rat skin (Kim et al. 1993). The drug permeability coefficient was enhanced 8- to 50-fold with greatest effects seen when delivered from an aqueous vehicle and, surprisingly, less pronounced effects when PG or PG:ethanol:water systems were employed. Similarly, Chi et al. reported that addition of urea to a PG vehicle *decreased* permeation of flurbiprofen through excised rat skin (Chi et al. 1995); flurbiprofen flux (from a 1 % formulation in a PG vehicle) was 22.7 μg/cm^2/h, but when 5 % urea was added, this fell to 8.1 μg/cm^2/h and to only 3.2 μg/cm^2/h when 10 % urea was used in the formulation. Interestingly, the authors report that urea increased the diffusion coefficient of the drug in the skin 1.8- and 2-fold with 5 % and 10 % urea, respectively, but this was confounded by a 5- and 17-fold decrease in the partitioning of the drug when using 5 and 10 % urea, respectively. In contrast, progesterone diffusion *increased* 2.5-fold using 10 % urea in PG (as compared to PG alone) on both porcine and rat skins (Valenta and Wedenig 1997). Applied to human cadaver skin, a 10 % solution of urea in PG had no effect on naloxone flux (Aungst et al. 1986). In our own work, urea saturated in a range of vehicles (PG, dimethyl isosorbide and liquid paraffin) was ineffective in promoting 5-fluorouracil permeation through human cadaver skin (Williams and Barry 1989).

The influence of solvent/co-enhancers on urea permeation-enhancing activity is complex. The study reported by Chi illustrated that when urea was used in combination with oleic acid, flurbiprofen enhancement was greater than using oleic acid alone (Chi et al. 1995). In contrast, oleic acid enhanced dihydroergotamine flux over 200-fold through rabbit skin, whereas 6 % urea in the formulation was ineffective (Niazy 1991). Again using rat skin, urea was an ineffective enhancer for indomethacin, but when combined with fatty alcohols such as 1-octanol or 1-decanol in an aqueous gel, some mild enhancement activity was recorded (Nishihata et al. 1990).

Using hairless mouse skin, 10 % urea in an aqueous solution had no influence on permeation or skin accumulation of the zwitterion baclofen (Sznitowska et al. 1996) and only a marginal dose-dependent enhancement of hydrocortisone acetate (Bentley et al. 1997). In contrast, 10 % urea was a potent enhancer of diclofenac diethylamine through horse skin (Ferrante et al. 2010).

Urea has been a component of more complex transdermal delivery systems and at 15 % w/w was reported to effectively enhance glipizide delivery from an ointment containing the drug in a β-cyclodextrin complex along with PG and oleic acid (Ammar et al. 2006). It has been included in Eudragit and ethyl cellulose/polyvinylpyrrolidone patches containing budesonide (Amgaokar et al. 2011). A recent study explored the combination of magnetophoresis with chemical enhancers in a hydroxymethyl cellulose gel containing lidocaine hydrochloride; urea (5 %) acted additively to magnetophoresis in the excised rat skin and provided ~4-fold further elevation in drug delivery (Sammeta et al. 2011).

From the above it is clear that urea is, at best, a mild penetration enhancer whose potency is affected by the formulation from which it is applied. With the disparate reports on its potency, the mechanisms by which urea acts are unclear. Kim et al. suggested that urea forms hydrophilic channels through excised skin (Kim et al. 1993) which were reported to account for the enhancement of relatively small molecules such as ketoprofen (mw 254) and hydrocortisone (mw 362) and the lack of enhancement for the larger molecule dihydroergotamine (mw 680) (Godwin et al. 1998). Urea did not affect the lag time in studies using horse skin (Ferrante

et al. 2010) suggesting that diffusivity was not altered and gold nanoparticle penetration into the stratum corneum was not enhanced by urea (but was by dimethylsulphoxide), suggesting that urea did not affect intercellular lipid domains (Labouta et al. 2012).

In contrast, urea effects on stratum corneum lipids have been reported; from temperature-dependent small-angle X-ray diffraction measurements, urea caused a concentration-dependent shift in model stratum corneum lipid phase transitions (Zbytovska et al. 2009). Beastall et al. suggested that urea lowers intercellular lipid phase transitions and so they are fluidised at room temperature, thus explaining the reduced time to onset of erythema caused by hexyl nicotinate when combined with urea (Beastall et al. 1986). A reduced lag time was reported for haloperidol permeation through rat skin after urea treatment, though this was attributed to its binding with keratin rather than alterations to lipid organisation (Vaddi et al. 2001). Further, urea delivery into the stratum corneum can be facilitated by vehicle selection or the use of co-enhancers such as alkanols (Nishihata et al. 1990).

18.4 Urea: Chemically Modified

As urea itself possesses only marginal penetration-enhancing activity, attempts have been made to synthesis analogues containing more potent-enhancing moieties. A series of cyclic ureas (Fig. 18.2) was described as putative skin penetration enhancers (Higuchi and Pogany 1987; Wong et al. 1988). When evaluated from ointments containing indomethacin, several of the new materials showed greater enhancement capabilities that Azone the positive control (Wong et al. 1989) in promoting delivery through shed snake and hairless mouse skin. These urea derivatives were shown to be well tolerated when injected into mice, and the enhancers were biodegradable. Subsequently, dodecyl 2-(N,N,-dimethylamino)propionate was prepared as a biodegradable enhancer that increased transport of indomethacin, clonidine and hydrocortisone across shed snake skin

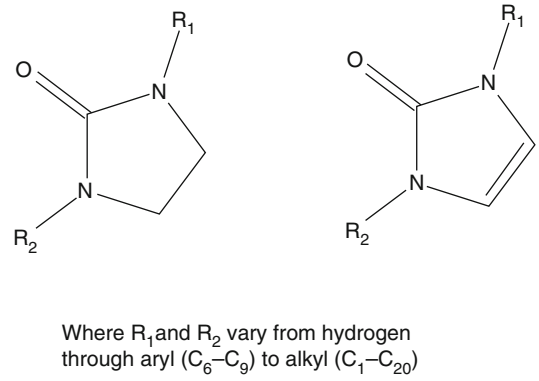

Fig. 18.2 Chemical structures of a series of cyclic ureas developed as potential skin penetration enhancers (Redrawn from Higuchi and Pogany 1987)

(Buyuktimkin et al. 1993) and N-adamantyl n-alkanamides were used as cyclic urea analogues to enhance salicylic acid delivery through rabbit skin in vivo (Han et al. 1995); delivered from petrolatum ointment, enhancement activity correlated with the length of the alkyl chain on the derivatives.

Seeking to exploit the keratolytic activity of urea with the enhancing properties of fatty acids, a series of alkyl and aryl urea analogues (Fig. 18.3) were evaluated as permeation enhancers for saturated solutions of 5-fluorouracil through human epidermal membranes in vitro (Williams and Barry 1989). Interestingly, when the urea analogues were applied saturated in liquid paraffin, none of the urea derivatives, nor urea itself or the vehicle alone, promoted 5-fluorouracil flux, but employing dimethylisosorbide as the vehicle doubled the drug permeability coefficient, with urea alone again ineffective. When applied in propylene glycol, the modified ureas provided sixfold increases in the drug permeability coefficient, whereas the vehicle alone, or urea in propylene glycol, were again ineffective enhancers. Propylene glycol was also shown to be the most effective vehicle for delivering dodecylcarbonylpentylurea as an enhancer of theophylline flux through excised human skin (Hrabalek et al. 1997). As with the reports described above, the enhancing potential of modified ureas is clearly sensitive to the vehicle from which they are applied.

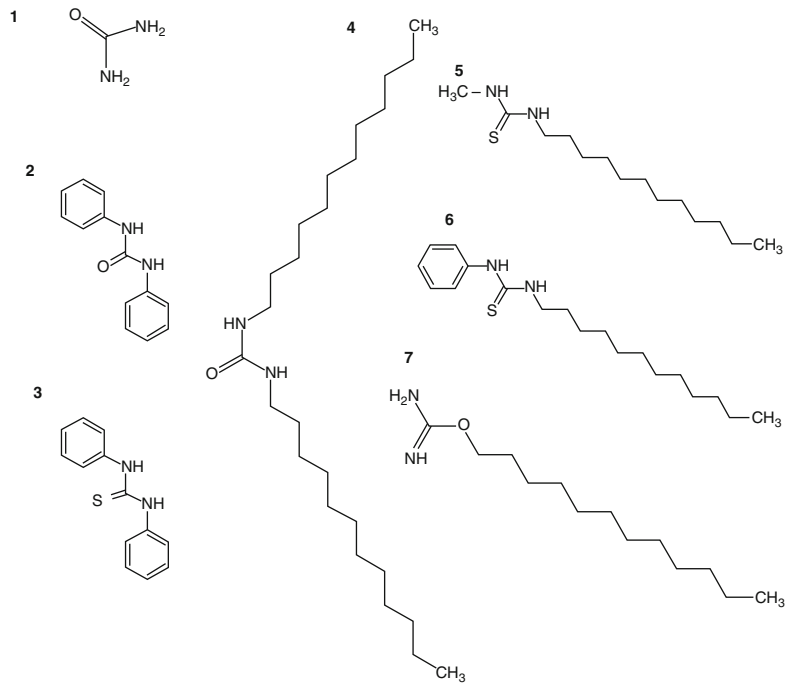

Fig. 18.3 Chemical structures of some urea compounds synthesised as penetration enhancers; (**1**) urea; (**2**) 1,3-diphenylurea; (**3**) 1,3-diphenylthiourea; (**4**) 1,3-didodecylurea; (**5**) 1-dodecyl-3-methylthiourea; (**6**) 1-dodecyl-3-phenylthiourea; (**7**) 1-dodecylurea (Williams and Barry 1989; Godwin et al. 1998)

Twelve urea and thiourea compounds, delivered from saturated solutions in propylene glycol (solubility ranged from 0.001 to 0.057 M), were evaluated as enhancers for hydrocortisone through hairless mouse skin (Godwin et al. 1998). Of the series, 1-dodecyl-3-methylthiourea (Fig. 18.3) was the most potent enhancer increasing the drug permeability coefficient ~7-fold, the amount of drug delivered transdermally in 24 h fivefold and the amount of drug within the skin twofold. Structure-activity studies indicated that the thio-substituted derivatives were more potent enhancers than the oxygen-containing molecules.

Chemical modifications of urea have clearly generated more potent enhancers than urea alone. Primarily, the addition of relatively long fatty chains that can partition into and disrupt the stratum corneum lipid packing provides moderately potent enhancers for a range of therapeutic agents. However, from the above studies, it remains unclear whether the emollient and keratolytic properties of urea are retained following chemical modification.

18.5 Urea: Safe for Skin?

In early reports, Hellgren and Larsson (1974) cautioned that whilst urea is valuable for managing hyper-keratotic skin conditions, long-term cosmetic use should be avoided since tissue soaked in saturated urea solutions for 1 week altered mechanically, and on further treatment the quaternary structure of keratin was lost. Urea is known to be a chaotropic agent and has been used at 8 M to denature/disorder proteins and other macromolecules (Taylor et al. 1995; Sogias et al. 2010). Indeed, treating the stratum corneum with 8 M urea destabilised proteins but did not alter significantly the fluidity of the stratum corneum lipids (do Couto et al. 2005).

Despite the above, urea is listed as generally regarded as safe (GRAS) by the US Food and Drug Administration (report number 103, 1978) since it is a normal body constituent, continually produced from protein metabolism. The oral LD_{50} for urea in rats is 8471 mg kg^{-1}, but it is also reported as a potential irritant to the skin, the eye and the respiratory system. In a cosmetic ingredient review, the final report of the safety assess-

ment of urea examined wide-ranging studies on the use of the material (Andersen 2005). The report noted that "urea increased the skin penetration of other compounds, including hydrocortisone" but that "no significant microscopic changes were observed in the skin of male nude mice dermally exposed to 100 % urea for 24 hours". Urea-induced irritation was also noted, though reports vary; exposure of normal skin to 60 % urea produced no significant irritation in one study, but 5 % was slightly irritating and 20 % was irritating in other reports. In treating diseased skin, a burning sensation is the most frequent adverse reaction to urea (used either alone or in combination with other agents), but few such adverse events are reported in the many clinical studies employing urea on damaged skin. The final report notes that "formulators should be aware that urea can increase the percutaneous absorption of other chemicals", but the Cosmetic Ingredient Review Expert Panel concluded that urea is safe as used in cosmetic products.

References

Alberti I, Kalia YN, Naik A, Bonny JD, Guy RH (2001) In vivo assessment of enhanced topical delivery of terbinafine to human stratum corneum. J Control Release 71:319–327

Amgaokar YM, Chikhale RV, Lade UB, Biyani DM, Umekar MJ (2011) Design, formulation and evaluation of transdermal drug delivery system of budesonide. Dig J Nanomater Biostruct 6:475–497

Ammar HO, Salama HA, Ghorab M, El-Nahhas SA, Elmotasem H (2006) A transdermal delivery system for glipizide. Curr Drug Deliv 3:333–341

Andersen FA (2005) Final report of the safety assessment of urea. Int J Toxicol 24:1–56

Aungst BJ, Roger NJ, Shefter E (1986) Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactants, sulphoxides and amides. Int J Pharm 33:225–234

Barry BW, Woodford R (1976) Proprietary hydrocortisone creams: vasoconstrictor activities and bio-availabilities of six preparations. Br J Dermatol 95:423–425

Beastall J, Guy RH, Hadgraft J, Wilding I (1986) The influence of urea on percutaneous absorption. Pharm Res 3:294–297

Bentley MVLB, Kedor ERM, Vianna RF, Collett JH (1997) The influence of lecithin and urea on the in vitro permeation of hydrocortisone acetate through skin from hairless mouse. Int J Pharm 146:255–262

Bettinger J, Maibach HI (1997) SC water-binding capacity. Cosmet Toiletries 112:49–53

Bettinger J, Gloor M, Gehring W, Wolf W (1995) Influence of emulsions with and without urea on water-binding capacity of stratum corneum. J Soc Cosmet Chem 46:247–254

Brand RM, Charron AR, Sandler VL, Jendrzejewski JL (2007) Moisturising lotions can increase transdermal absorption of the herbicide 2,4-dichlorophenoxacetic acid across hairless mouse skin. Cutan Ocul Toxicol 26:15–23

British National Formulary (2012) 63. Section 13, Pharmaceutical Press, London

Buyuktimkin S, Buyutimkin N, Rytting RH (1993) Synthesis and enhancing effect of dodecyl 2-(N, N-dimethylamino)propionate on the transepidermal delivery of indomethacin, clonidine and hydrocortisone. Pharm Res 10:1632–1637

Chi SC, Park ES, Kim H (1995) Effect of penetration enhancers on flurbiprofen permeation through rat skin. Int J Pharm 126:267–274

Cler EJ, Fourtanier A (1981) L'acide pyrrolidone carboxylique (PCA) et la peau. Intl J Cosmet Sci 3:101–113

do Couto SG, Oliverira MD, Alonso A (2005) Dynamics of proteins and lipids in the stratum corneum: effects of percutaneous permeation enhancers. Biophys Chem 116:23–31

Feldman R, Maibach HI (1974) Percutaneous penetration of hydrocortisone with urea. Arch Dermatol 109:58–59

Ferrante M, Andreeta A, Landoni MF (2010) Effect of different penetration enhancers on diclofenac permeation across horse skin. Vet J 186:312–315

Gardner M (1999) Urine therapy. Skeptical Inquirer 23(3):13–15

Gloor M, Fluhr J, Wasik B, Gehring W (2001) Clinical effect of salicylic acid and high dose urea applied in standardized NRF formulations. Pharmazie 56:810–814

Godwin DA, Player MR, Sowell JR, Michniak BB (1998) Synthesis and investigation of urea compounds as transdermal penetration enhancers. Int J Pharm 167:165–175

Grice K, Sattar H, Baker H (1973) Urea and retinoic acid in ichthyosis and their effect on transepidermal water loss and water holding capacity of stratum corneum. Acta Dermato 53:114–118

Han SK, Park YH, Kim CK (1995) Preparation of N-adamantyl n-alkylamides and evaluation of their transdermal penetration in the rabbit. Int J Pharm 126:35–40

Hellgren L, Larsson K (1974) On the effects of urea on human epidermis. Dermatologica 149:289–293

Higuchi T, Pogany SA (1987) Cyclic ureas as dermal penetration enhancers. US Patent 4,677,131

Horii Y, Tanida M, Shen J, Fujisaki Y, Fuyuki R, Hashimoto K, Niijima A, Nakashima T, Nagai K (2011) Skin application of urea-containing cream affected cutaneous arterial sympathetic nerve activity, blood flow and water evaporation. Skin Res Tech 17:75–81

Hrabalek A, Dolezal P, Krebs A, Farsa O, Sklubalova Z (1997) Urea derivatives as transdermal penetration enhancers. Chemicke Listy 91:682–683

Kim CK, Kim JJ, Chi SC, Shim CK (1993) Effect of fatty acids and urea on the penetration of ketoprofen through rat skin. Int J Pharm 99:109–118

Labouta HI, El-Khordagui LK, Schneider M (2012) Could chemical enhancement of gold nanoparticle penetration be extrapolated from established approaches for drug permeation? Skin Pharm Physiol 25:208–218

Loden M (1996) Urea-containing moisturisers influence barrier properties of normal skin. Arch Dermatol Res 288:103–107

Loden M, Bostrom P, Kneczke M (1995) Distribution and keratolytic effect of salicylic acid and urea in human skin. Skin Pharmacol 8:173–178

Loden M, Anderson AC, Lindberg M (1999) Improvement in skin barrier function in patients with atopic dermatitis after treatment with a moisturising cream (Canoderm). Br J Dermatol 140:264–268

Lu MYF, Lee D, Rao GS (1992) Percutaneous absorption enhancement of leuprolide. Pharm Res 9:1575–1579

Niazy EM (1991) Influence of oleic acid and other permeation promoters on transdermal delivery of dihydroergotamine through rabbit skin. Int J Pharm 67:97–100

Nishihata T, Rytting JH, Kamada A, Matsumoto K, Takahashi K (1990) Combined effect of alcohol and urea on the in vitro transport of indomethacin across rat dorsal skin. J Pharm Sci 79:487–489

Rawlings AV, Hardy CR (2004) Moisturization and skin barrier function. Dermatol Ther 17:43–48

Rezus YLA, Bakker HJ (2006) Effect of urea on the structural dynamics of water. Proc Natl Acad Sci U S A 103:18417–18420

Sammeta SM, Repka MA, Murthy SN (2011) Magnetophoresis in combination with chemical enhancers for transdermal drug delivery. Drug Dev Ind Pharm 37:1076–1082

Savic S, Tamburic S, Savic M, Cekic N, Milic J, Vuleta G (2004) Vehicle-controlled effect of urea on normal and SLS-irritated skin. Int J Pharm 271:269–280

Sogias IA, Khutoryanskiy VV, Williams AC (2010) Exploring the factors affecting the solubility of chitosan in water. Macromol Chem Phys 211:426–433

Soper AK, Castner EW, Luzar A (2003) Impact of urea on water structure: a clue to its properties as a denaturant? Biophys Chem 105:649–666

Sznitowska M, Janicki S, Gos T (1996) The effect of sorption promoters on percutaneous permeation of a model zwitterion – Baclofen. Int J Pharm 137:125–132

Taylor LS, York P, Williams AC, Edwards HGM, Mehta V, Jackson GS, Badcoe IG, Clarke AR (1995) Sucrose reduces the efficiency of protein denaturation by a chaotropic agent. Biochim Biophys Acta 1253:39–46

Vaddi HK, Wang LZ, Ho PC, Chan SY (2001) Effect of some enhancers on the permeation of haloperidol through rat skin in vitro. Int J Pharm 212:247–255

Valenta C, Wedenig S (1997) Effects of penetration enhancers on the in vitro percutaneous absorption of progesterone. J Pharm Pharmacol 49:955–959

Visscher MO, Tolia GT, Wickett RR, Hoath SB (2003) Effect of soaking and natural moisturising factor on stratum corneum water-handling properties. J Cosmet Sci 54:289–300

Williams AC (2003) Transdermal and topical drug delivery; from theory to clinical practice. Pharmaceutical Press, London

Williams AC, Barry BW (1989) Urea analogues in propylene glycol as penetration enhancers in human skin. Int J Pharm 56:43–50

Wohlrab W (1984) The influence of urea on the penetration kinetics of topically applied corticosteroids. Acta Dermato-Vener 64:233–238

Wong O, Huntington J, Konishi R, Rytting JH, Higuchi T (1988) Unsaturated cyclic ureas as new nontoxic biodegradable transdermal penetration enhancers. 1. Synthesis. J Pharm Sci 77:967–971

Wong O, Tsuzuki N, Nghiem B, Kuehnhoff J, Itoh T, Masaki K, Huntingdon J, Konishi R, Rytting JH, Higuchi T (1989) Unsaturated cyclic ureas as new nontoxic biodegradable transdermal penetration enhancers. II. Evaluation study. Int J Pharm 52:191–192

Woodford R, Barry BW (1984) Alphaderm cream (1% hydrocortisone and 10% urea): investigation of vasoconstrictor activity, bioavailability and application regimens in human volunteers. Curr Ther Res 35:759–767

Zbytovska J, Vavrova K, Kiselev MA, Lessieur P, Wartewig S, Neubert RHH (2009) The effects of transdermal permeation enhancers on thermotropic phase behaviour of a stratum corneum lipid model. Colloids Surf A Physicochem Eng Asp 351:30–37

Transkarbams: Transdermal Penetration-Enhancing Carbamates

19

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19.1 Introduction

Transdermal penetration enhancers are compounds that increase drug delivery through the skin by interacting with skin barrier constituents. Ideally, this interaction must be strong enough to enable sufficient drug flux but also reversible and specific enough to limit subsequent toxicity or irritation (Barry 1983, 2001; Hadgraft 1999; Marjukka Suhonen et al. 1999; Asbill et al. 2000; Kanikkannan et al. 2000; Williams and Barry 2004; Vavrova et al. 2005b; Kaushik et al. 2008; Prausnitz and Langer 2008; Ahad et al. 2009; Guy 2010; Aungst 2011). Transkarbams, such as ammonium carbamates derived from ω-amino acids (mostly 6-aminohexanoic acid or EAC, Fig. 19.1), seem to fulfill many of these requirements. In this chapter, we describe the history of transkarbams, including their discovery in 1989, their medicinal chemistry, mechanisms of action, and safety profile.

N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_19

19.2 History of Transkarbams

Transdermal penetration enhancers have been studied in our group since the early 1990s. At that time, Azone® (*N*-dodecylazepan-2-one, dodecylcaprolactam, or laurocapram; compound I, Fig. 19.2) was already well known (Rajadhyaksha 1976; Stoughton 1982), and many new enhancers were being rapidly discovered. These compounds showed vast chemical diversity, and the number of structures alone (from simple alcohols to steroids) suggested that the enhancer interaction with the skin might be rather complex. Because Azone® appeared to specifically enhance drug absorption, followed by pyrrolidone and piperidone enhancers at the turn of the 1980s and 1990s (Quan et al. 1990; Sasaki et al. 1990, 1991; Aoyagi et al. 1991; Fuhrman et al. 1997; Michniak et al. 1998), we began to work on lactams, i.e., ω-amino acid derivatives. The important molecular features of this class of enhancers may be formulated by a general structure II, i.e., a flexible lipophilic alkyl chain and a rigid, relatively polar lactam cycle (Fig. 19.2). As a matter of principle, these substances could be considered weak, nonionic surfactants.

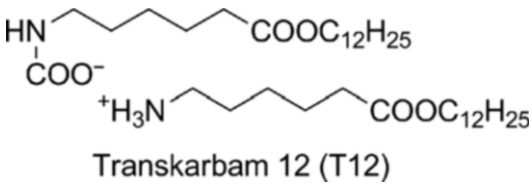


Fig. 19.1 Structure of transkarbam 12 (T12)

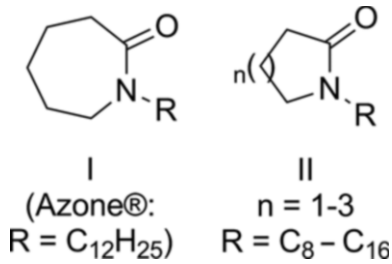
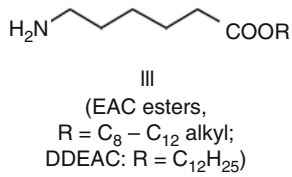


Fig. 19.2 Azone® and its derivatives (*I*) and a general structure of *N*-alkyl lactam penetration enhancers (*II*)

We decided to open the lactam cycle of Azone® and exchange the amide for an isosteric ester group to prepare 6-aminohexanoic acid (EAC) esters (compound III, Fig. 19.3). This modification was driven by the demand to reduce the toxicity of enhancers (Barry 1983, 2001) because such simple esters could be metabolized to 6-aminohexanoic acid and fatty alcohols (C8–C16), which are well-known substances, with low toxicity, that are used in medicine and cosmetics. Moreover, such enhancers could be easily prepared from cheap sources, and we expected that they would be able to be readily processed. Unless otherwise stated, the activities of the enhancers that we present in this chapter are expressed as their enhancement ratios, i.e., the ratio of the in vitro flux of a model drug, theophylline, through human or porcine skin, with or without 1 % enhancer.

19.2.1 Modifications of the Alcohol Moiety

The first results using the hexadecyl ester of EAC verified that our concept was correct because this



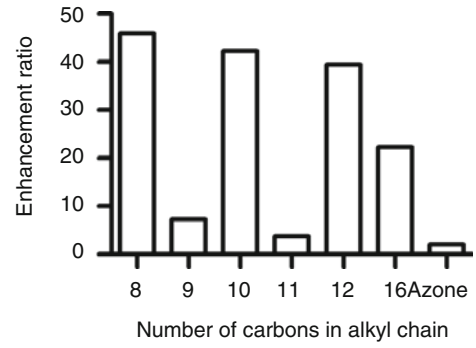


Fig. 19.3 6-Aminohexanoic acid (EAC) esters (*left panel*) and their enhancement ratios compared to Azone® (*right panel*). The enhancement ratios were determined as ratios of the flux of 2.5 % aqueous theophylline through human skin, with or without 1 % of the studied enhancers

compound was more active than Azone® (Fig. 19.3). To define some basic structure-enhancing activity relationships, we modified the length of both the alkyl chain and the linker between the nitrogen and ester. The enhancement ratios for 1 % octyl to dodecyl esters of EAC ranged between 35 and 45 when applied in water or aqueous propylene glycol, considerably exceeding that of Azone® (enhancement ratio = 5) (Dolezal et al. 1989, 1993). Interestingly, esters with an even number of carbons in the alkyl part (octyl, decyl, and dodecyl) enhanced theophylline permeation by an order of magnitude compared to nonyl and undecyl esters (Fig. 19.3). EAC esters were also effective in a lipophilic donor phase (olive oil; enhancement ratios = 17–19), while Azone® was completely inactive (Dolezal et al. 1993).

19.2.2 Modifications of the Acid Moiety

Next, we probed whether elongation of the ω-amino acid (Fig. 19.4) could increase the enhancing activity of amino esters. The enhancers were studied under the same conditions as described for the EAC esters; however, neither esters of 7-aminoheptanoic acid (7-AHE) nor 8-aminooctanoic acid (8-AOCT) reached the efficacy of EAC esters. In fact, only the octyl and nonyl esters of 7-AHE were more active than Azone® (Fig. 19.4) (Hrabalek et al. 2000).

Furthermore, to determine how terminal amino group basicity contributes to the enhancing effect, a series of *N*-acetylated EAC esters (V, Fig. 19.5) were synthesized. However, these substances lacked any activity (Hrabalek 1992).

Another disappointing result was observed with a series of 6-aminoheptanoates (VI, Fig. 19.5), i.e., compounds with methyl group next to the primary amine. Despite having the same distance between the nitrogen and ester and having a free amino group, these compounds were also inactive (Kroutil 1995).

We also prepared a series of tranexamic acid (*trans*-4-aminomethylcyclohexanecarboxylic acid) esters (VII, Fig. 19.5) as conformationally constrained analogues of EAC esters.

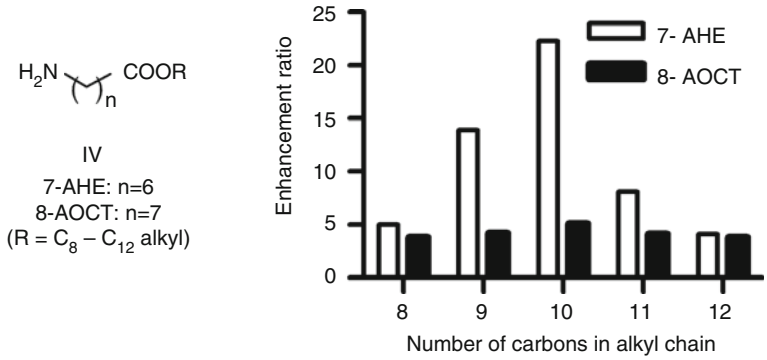


Fig. 19.4 7-Aminoheptanoic (7-AHE) and 8-aminooctanoic (8-AOCT) acid esters (*left panel*) and their enhancement ratios (*right panel*). The enhancement ratios were determined as ratios of the flux of 2.5 % aqueous theophylline through human skin, with or without 1 % of the studied enhancers

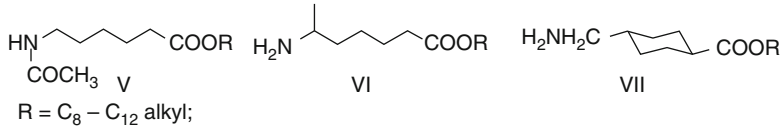


Fig. 19.5 Structural modifications of the amino group and the linking chain in EAC esters: esters of *N*-acetylhexanoic acid (*V*) and 6-aminoheptanoic acid (*VI*) were inactive; esters of tranexamic acid (*VII*) enhanced the flux of 5 % theophylline in isopropyl myristate through human skin by 4.9 times

Additionally, these compounds increased skin permeability to a much lesser extent than the EAC esters, with enhancement ratios up to 4.9 (Vavrova et al. 2005a).

The previously described results defined the basic relationships between the structure of EAC esters and their enhancing properties:

- Optimum alkyl chain length is from 8 to 12 carbons.
- Optimum distance between amino group and carbonyl group is five carbons.
- Linking chain between carbonyl and nitrogen must be unbranched and flexible.
- Amino group must be basic.
- Amino group must be terminal.

19.2.3 The Actual Structure of Transkarbams

During the aforementioned studies, the structure and purity of the synthesized enhancers were confirmed by elemental analysis, infrared spectroscopy, nuclear magnetic resonance, and mass spectrometry. The enhancers also melted in a narrow range, which is typical of pure substances. However, acidimetric titration of the dodecyl ester of EAC (DDEAC) showed a purity of only 91 %.

An explanation for this disagreement took a long time because no mistakes were found in the analytical methods and no impurities were detected. Finally, we hypothesized that the free amino group in DDEAC may capture carbon dioxide from the air to form a two-chain ammonium carbamate salt (Fig. 19.6). This reaction is analogous to the industrial production of urea, where carbon dioxide reacts with two equivalents of liquid ammonia to form ammonium carbamate, which is then dehydrated to urea. The carbon dioxide capture is reversible, as carbamate salts are unstable at higher temperatures and are sensitive to acids liberating the parent amines. This behavior is advantageous in carbon dioxide carriers, e.g., in hemoglobin (Geers and Gros 2000) or various industrial applications (Dell'Amico et al. 2003). The instability of carbamate may explain why all our analyses performed in chloroform displayed the DDEAC structure instead of the carbamate salt. Additionally, for elemental analysis, the samples were dried in vacuo at 54 °C, which effectively removed the loosely bound carbon dioxide. Therefore, this analysis also showed the structure of DDEAC.

The actual structure was then confirmed by changing the measurement conditions. First, encouraging results came from infrared spectroscopy in a solid state, which showed a band of the carbamic group at 1617 cm^{-1} and an ester doublet at 1742 a 1735 cm^{-1}, whereas in chloroform solution, the spectrum showed only a single ester band and vibration of dissolved carbon dioxide at 2337 cm^{-1} (Hrabalek et al. 2006) (Fig. 19.7). Computer modeling suggested that the ester band most likely split due to different hydrogen bonding of the two ester groups (Fig. 19.7). Furthermore, nuclear magnetic resonance (NMR) spectra, in chloroform saturated with dry pyridine, further confirmed the presence of carbamate (carbamate carbon at 161.98 ppm and two resonances at 41.02 and 40.50 ppm, corresponding to the methylene carbons next to NH_3^+ and $NHCOO^-$, respectively). Further proof came from fast atom bombardment mass spectra, which showed the carbamate anion, and elemental analysis of a sample dried at room temperature (Hrabalek et al. 2006).

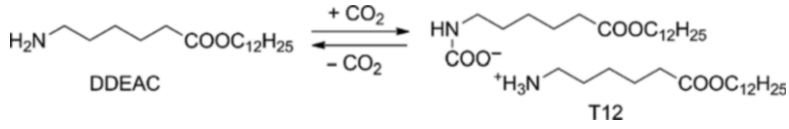


Fig. 19.6 The concept of transkarbams: amino ester (e.g., DDEAC) reacts with CO_2 to form an ammonium carbamate salt (e.g., T12). This reaction is reversible in an acidic environment or at higher temperature

Thus, we named these compounds transkarbams, i.e., TRANSdermal penetration-enhancing CARBAMates. A direct comparison of transkarbam 12 (T12) and DDEAC (the free amino ester was tested under argon to prevent its reaction with carbon dioxide) showed that T12 was responsible for the observed enhancing activity, while DDEAC was inactive (Hrabalek et al. 2006). Thus, in our initial work, we used the term "EAC esters" incorrectly, as the permeation-enhancing activities belong to transkarbams, which were recently patented as such (Hrabalek et al. 2001).

This finding also explained the fact that the basic terminal amino group is essential for the enhancing effect of this class of compounds because it must be able to react with carbon dioxide to form relatively stable ammonium carbamates. The thermotropic phase behavior of transkarbams indicates that they are stabilized by the formation of a crystalline lattice. They can even be crystallized from toluene, but the temperature cannot exceed approximately 50 °C. Above their melting point, the carbon dioxide is released (Zbytovska et al. 2004; Holas et al. 2006c). This is an important point when regarding the potential technological processing of transkarbams and the design of novel compounds bearing this moiety.

19.3 Further Structure-Activity Relationships

19.3.1 "Reverse" Transkarbams

Once we confirmed the carbamate structure, we aimed to determine the importance of the other structural features of transkarbams. First, we prepared analogous esters of amino alcohols and carboxylic acids, i.e., transkarbams with a "reversed" ester bond (general structure VIII, Fig. 19.8). These compounds showed similar enhancing potency as transkarbams, suggesting that it is not important whether these compounds are derived from amino acids or amino alcohols (Kroutil 1995).

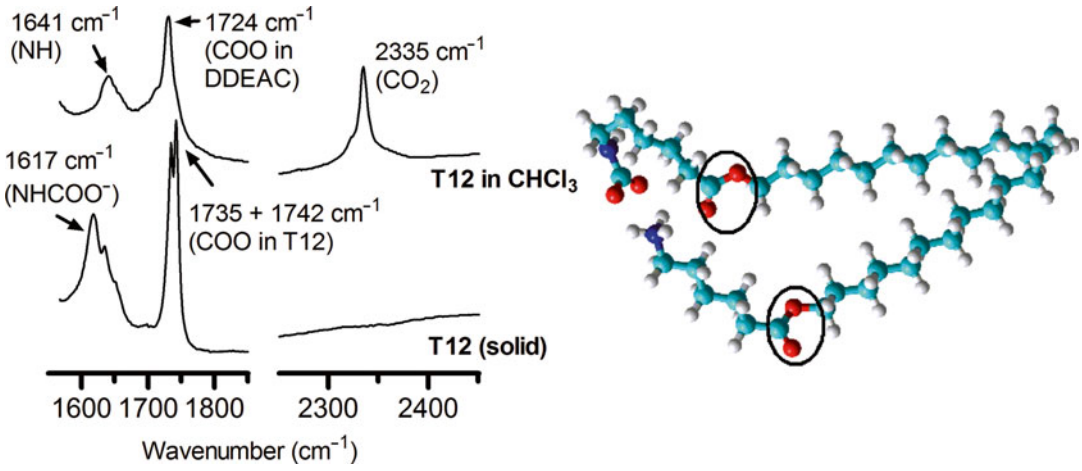


Fig. 19.7 Infrared spectra of T12 measured in a solid state (in potassium bromide) and in chloroform solution, where the carbamate salt decomposed and CO_2 was released (*left panel*). *Right panel*: computer model of T12 showing two different ester groups

$$CH_3–(CH_2)_nCOO–(CH_2)_m.NHCOO^- H_3N^+–(CH_2)_m OOC(CH_2)_nCH_3 \quad \begin{matrix} n = 6 - 10 \\ m = 5 - 6 \end{matrix}$$

VIII

Fig. 19.8 The general structure of "reverse" transkarbams, i.e., derivatives of amino alcohols

19.3.2 Alkyl Branching Close to the Ester Group

Whereas branching close to the carbamate-forming nitrogen diminished the enhancing activity of transkarbams, the effects of branching in the alkyl portion were unknown. We hypothesized that such compounds may still be active enhancers. To test this hypothesis, we synthesized a series of secondary alkyl and cycloalkyl esters. However, none of these compounds reached the same level of activity as the unbranched transkarbams. Their enhancing efficacy decreased with the degree of branching close to the ester group or cyclization (Hrabalek et al. 2005) (Fig. 19.9). One possible explanation is that the stability of the carbamic acid salt was decreased because of the lack of a crystalline lattice.

19.3.3 Chiral Enhancers

Simultaneously, we were interested in whether the action of enhancers is stereoselective because the skin barrier represents a chiral environment, and skin permeability may be different for drug enantiomers (Miyazaki et al. 1992). Thus, we prepared EAC esters with chiral octan-2-oles. However, the enhancing effects of the (*R*) and (*S*) enantiomers did not differ, suggesting that chirality was not involved in their interaction with the skin barrier (Vavrova et al. 2002). Later, we confirmed the lack of stereoselectivity in the action of various other amino acid-based enhancers (Novotny et al. 2009; Janůšová et al. 2013).

19.3.4 Enhancers with Higher Basicity

The basicity of the terminal amino group of EAC esters was found to be essential for their enhancing activity. However, the extent to which basicity or, more precisely, the nature of the amino group enhanced their activity remained unknown. Thus, we prepared esters of 6-dimethylaminohexanoic (IX) (Hrabalek et al. 2000), 6-diethylaminohexanoic (X), 6-(*N*-pyrrolidino)hexanoic (XI), 6-(*N*-piperidino) hexanoic (XII), and 6-(*N*-morpholino)hexanoic acids (XIII, Fig. 19.10) (Farsa et al. 2010). While compounds with a bulkier tertiary amino group, X–XIII, were less effective enhancers compared with the corresponding transkarbams, when applied at 2.5 %w/v in propylene glycol, dimethyl derivatives IX enhanced the permeation of theophylline through human skin by two orders of magnitude compared to control (Hrabalek et al. 2000). However, these substances apparently work via a different mechanism of action than transkarbams because they are unable to form carbamate salt.

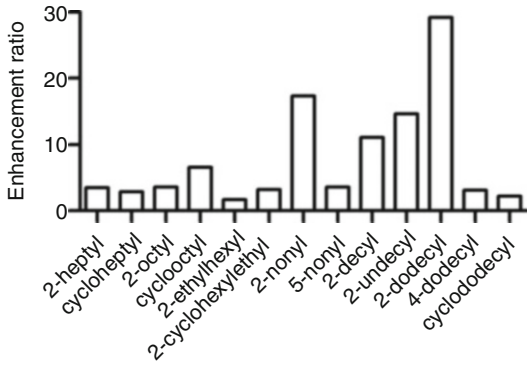


Fig. 19.9 Enhancement ratio of compounds with alkyl branching or cyclization close to the ester group. The enhancement ratios were determined as ratios of the flux of 2.5 % aqueous theophylline through human skin, with or without 1 % of the studied enhancers

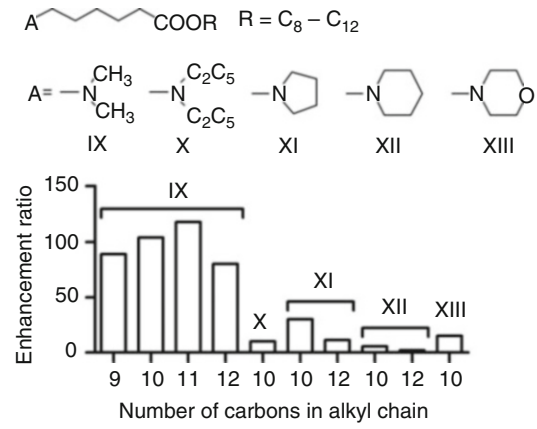


Fig. 19.10 Enhancers with tertiary amino group IX–XII and their enhancement ratios. The enhancement ratios were determined as ratios of the flux of 2.5 % theophylline in propylene glycol through human skin, with or without 5 % of the studied enhancers

At this point, our synthetic approaches split into two pathways: transkarbams and amino acid derivatives without the carbamate structure. The latter group includes potent enhancers, such as dodecyl 6-dimethylaminohexanoate (DDAK) (Hrabalek et al. 2000; Vavrova et al. 2008b, 2011) and proline derivatives (Janůšová et al. 2013). These compounds are discussed in Chap. 21 of this book.

19.3.5 Urea Derivatives

To further explore the properties of transkarbam nitrogen, we prepared derivatives of urea, both acyclic (compounds XIV and XV, Fig. 19.11) and cyclic (XII, Fig. 19.11). Urea is a part of the skin's natural moisturizing factor, and it is widely used topically for its keratoplastic and keratolytic activities. Moreover, cyclic ureas were up to three times more effective than Azone® in enhancing the permeation of indomethacin through mouse skin (Wong et al. 1989). Urea also contains carbon dioxide, i.e., it is carbamic acid amide. However, 6-ureidohexanoic acid esters, XIV, or their "reverse" analogues, i.e., ω-ureidoalkyl esters of carboxylic acids XV, showed negligible activity. Cyclic ureas, i.e., substituted imidazolin-2-ones, XVI, were more potent enhancers than the simple ureas, XIV and XV, but they did not reach the enhancing potency of transkarbams (Krebs 2001).

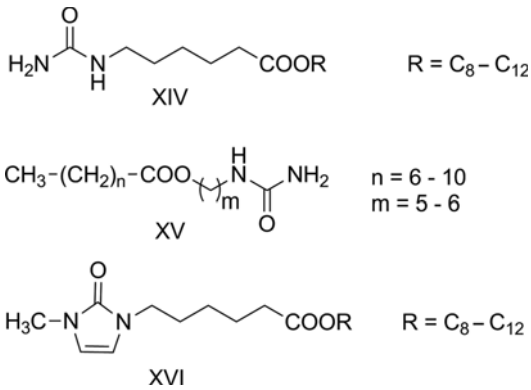


Fig. 19.11 Urea derivatives: 6-ureidohexanoic acid esters (*XIV*), ω-ureidoalkyl esters of carboxylic acids (*XV*), and esters of 6-(3-methylimidazolin-2-on-1-yl)hexanoic acid (*XVI*)

19.3.6 Transkarbam Analogues with Covalently Bound CO_2

The lower activity of urea derivatives could be explained by their higher stability compared to carbamate salts or by the fact that they are single-chain amphiphiles. To answer this question, we prepared a series of two-chain transkarbam-like derivatives with carbon dioxide covalently bound in the form of esters of carbonic acid (XVII) and carbamic acid (XVIII, Fig. 19.12) (Klimentova et al. 2006a). These structural modifications to transkarbams led to virtually inactive substances, suggesting that the carbon dioxide-containing group of transkarbams should indeed be the labile ammonium carbamate salt.

19.3.7 Transkarbams with Terminal Branching

Based on the literature describing skin permeation enhancers (Marjukka Suhonen et al. 1999) and our data obtained by step-by-step structural modifications of transkarbams, we suggested that

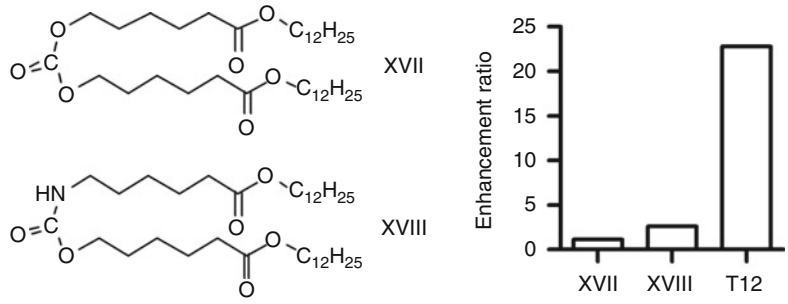


Fig. 19.12 T12 analogues with covalently bound CO_2 and their enhancement activities. The enhancement ratios were determined as ratios of the flux of 5 % theophylline in 60 % aqueous propylene glycol through pig skin, with or without 1 % of the studied enhancers

the mechanism of action of transkarbams and related substances involves their incorporation into stratum corneum (SC) lipid membranes. Such enhancer incorporation could lead to lipid fluidization, resulting in higher drug permeability. We hypothesized that more sterically demanding transkarbams may be more active enhancers because they would require more space in the skin barrier lipid lamellae.

Thus, we returned again to branched molecules. However, this time we tested esters of EAC with iso- and anteisoalcohols (XIX, Fig. 19.13) (Klimentova et al. 2008), which are compounds with terminal branching, because this type of branching is compatible with carbamate formation. The preparation of these transkarbams involved a rather complicated synthetic procedure to obtain the starting alcohols. However, none of these structural modifications resulted in more effective enhancers – the activities of terminally branched and unbranched transkarbams were roughly similar (Fig. 19.13) (Klimentova et al. 2008). We also checked the permeation-enhancing activities of the precursors, i.e., the iso- and anteisoalcohols and the corresponding acids, but none were better enhancers than their unbranched counterparts (Klimentova et al. 2006b).

19.3.8 Transkarbams: Isosteres of the Ester Group

We were also interested in determining the role of the ester bond in transkarbam activity. Previous results suggested that this group may be "reversed" without losing enhancing activity; however, this group is also sensitive to steric hindrance, e.g., branching, which decreases the enhancing potency. On the other hand, most of our results pointed to the central role of the carbamate salt. Thus, we were interested in what would happen if we substituted the ester with other groups, namely, amide, ketone, hydrocarbon chain, carbonate, carbamate, and urea (Fig. 19.14).

First, we prepared amide, ketone, and hydrocarbon analogues of transkarbams. To our surprise, these compounds were an order of magnitude less active than T12 (Fig. 19.14) (Holas et al. 2006a). We also tried to substitute the carboxylic ester with esters of carbonic acid (carbonate) and carbamic acid (carbamate). Although carbonates were relatively good enhancers, neither of these compounds reached the level of activity observed for T12 (Fig. 19.14) (Holas et al. 2006b). In addition, urea derivatives were even less active. Thus, similar to the carbamate salt, the ester bond is an essential part of transkarbams.

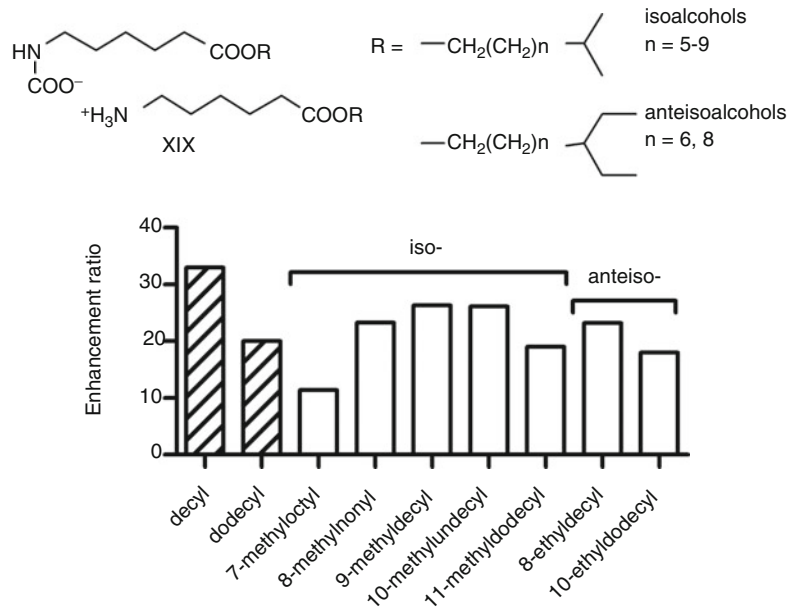
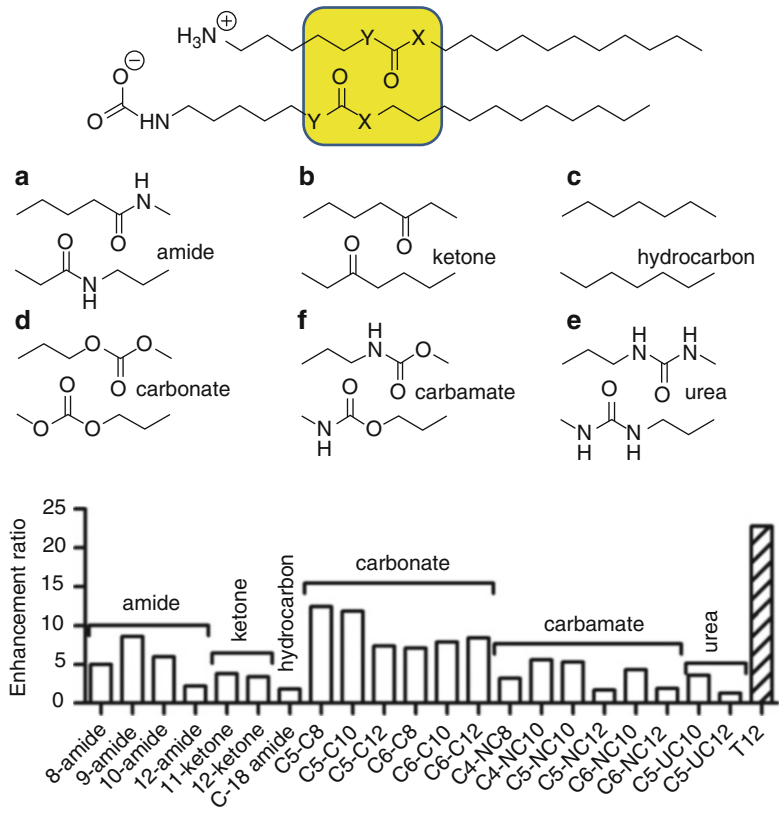


Fig. 19.13 Transkarbams with terminal branching and their enhancement ratios. The enhancement ratios were determined as ratios of the flux of 5 % theophylline in 60 % aqueous propylene glycol through pig skin, with or without 1 % of the studied enhancers



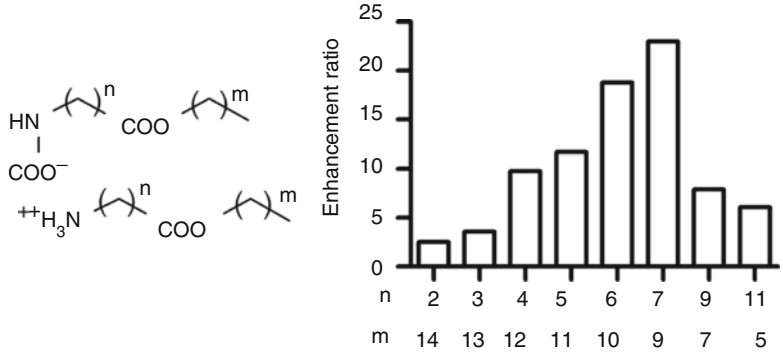


Fig. 19.14 Isosteres of ester group in transkarbams and their enhancement ratios. The enhancement ratios were determined as ratios of the flux of 5 % theophylline in 60 % aqueous propylene glycol through pig skin, with or without 1 % of the studied enhancers

Fig. 19.15 Isomers of T12 with different ester group positions and their enhancing activities. The enhancement ratios were determined as ratios of the flux of 5 % theophylline in 60 % aqueous propylene glycol through pig skin, with or without 1 % of the studied enhancers

19.3.9 Ester Position in Transkarbams

We also varied the ester position in the T12 molecule, i.e., the overall length of the molecule was kept constant, and the distance between the nitrogen and ester carbonyl was altered from 2C to 11C (Fig. 19.15). The enhancing activities of these transkarbams suggested that the optimum position of the ester bond is 4C–7C from the nitrogen (Novotny et al. 2010).

Next, we sought to understand why the ester bond in transkarbams is so important. The infrared

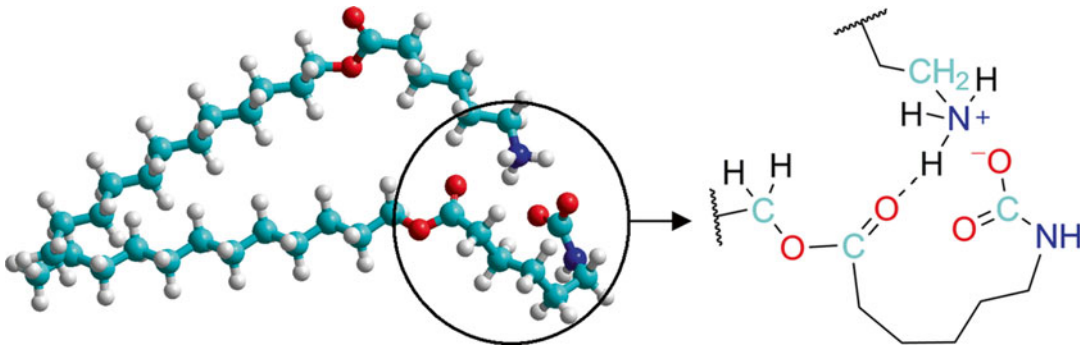


Fig. 19.16 A possible explanation of the importance of the ester in transkarbams. The ester carbonyl oxygen in the anionic chain forms an intramolecular hydrogen bond with the ammonium group, leading to a "cycle" that either stabilizes the carbamate polar head at room temperature or contributes to its action in the stratum corneum

spectra of these enhancers indicated that the ester carbonyl vibration split into two bands (Zbytovska et al. 2004), while the isosteric carbamic acid esters only showed one ester band. These data suggest different hydrogen bonding of the two ester carbonyls in transkarbams. A computer model of T12 revealed that the ester group in the anionic part of T12 loops back to the carbamate polar head and forms a "cycle" via a hydrogen bond to the ammonium salt (Fig. 19.16). This "cycle" seems to stabilize the carbamate structure at room temperature or somehow contribute to its action in SC, and it explains the importance of the ester and its position in transkarbams.

19.4 Mechanism of Action of Transkarbams

19.4.1 Similarity to Ceramides

In general, enhancers can improve skin permeability by various mechanisms (Smith and Maibach 1995; Marjukka Suhonen et al. 1999; Barry 2001; Williams and Barry 2004). The most potent enhancers are believed to interact with SC intercellular lipids (mainly ceramides) because these lipid lamellae provide the major permeation pathway for drugs (Albery and Hadgraft 1979; Potts and Guy 1992). Ideally, such interactions involve changes in lipid chain order or packing and are reversible.

When considering the mechanism(s) of action of transkarbams, we first noted that, in contrast to other known enhancers, transkarbams are two-chain, lipid-like compounds. The T12 molecule is almost as long as the sphingosine chain in ceramides (Fig. 19.17), and several of their other physicochemical parameters are roughly similar (Table 19.1). Thus, T12 most likely has a good affinity for the SC lipid membranes. This characteristic may be important for the incorporation of T12 into SC lipid membranes, i.e., the polar head of T12 may be located in the hydrophilic part of the membrane, while the lipophilic chains protrude into the membrane's hydrophobic core.

On the other hand, the aforementioned ester group isosteres of transkarbams have the same length and similar physicochemical parameters, yet their enhancing activity is much lower. Therefore, there must be an additional mechanism explaining the exceptional activity of transkarbams.

19.4.2 Decomposition of Carbamate in SC

Because the ammonium carbamate polar head is essential for the activity of transkarbams, we aimed to determine how this group improves drug flux through the skin. In general, carbamic acid salts are sensitive to pH; in acidic conditions, they release carbamic acid, which immediately decomposes to carbon dioxide (Fig. 19.18). Because SC lipids contain a significant proportion of free fatty acids, we hypothesized that they may be acidic enough to

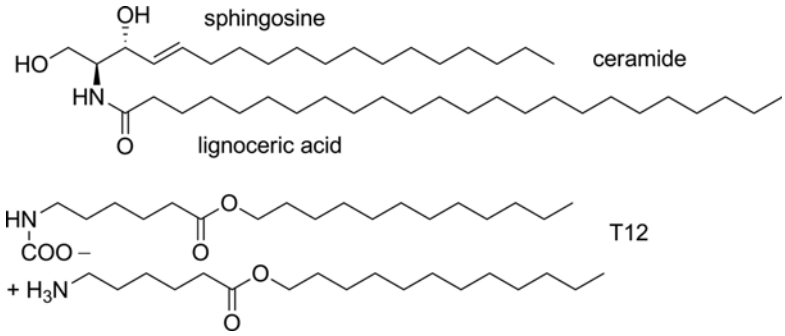


Fig. 19.17 Comparison of ceramide NS (*N*-lignoceroylsphingosine) and T12

Table 19.1 Selected physicochemical properties of ceramide NS (*N*-lignoceroylsphingosine), T12, and Azone®

Compound	logP	Mol. refractivity [Å^3]	Polarizability [Å^3]	Length [Å]
Ceramide	13.54	207.71	83.03	21.15 + 29.19
T12	9.12	185.64	73.28	19.8
Azone®	5.22	87.44	34.47	15.1

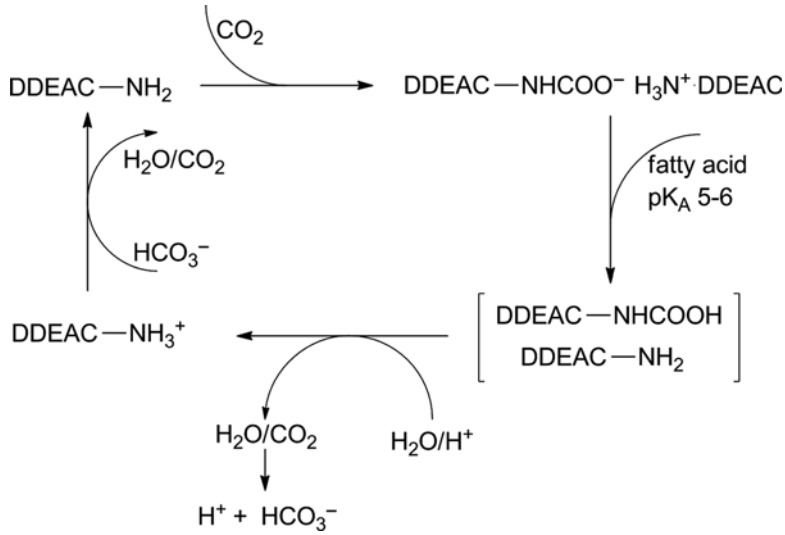


Fig. 19.18 The equilibrium between T12 and DDEAC in relation to pH. The source of protons for the carbamate decomposition may be the fatty acids in the stratum corneum lipid barrier

mediate the decomposition of transkarbams and release carbon dioxide. These changes might result in higher tension between SC lipids, their fluidization, and increased skin permeability.

The proposed decomposition of T12 was first confirmed by gravimetric analysis in the presence of palmitic acid, showing a weight loss corresponding to carbon dioxide. To verify that the released substance was carbon dioxide, we monitored the reaction by infrared spectroscopy. Within minutes of the mixing of T12 with palmitic acid, the typical carbamate carbonyl band at 1617 cm^{-1} disappeared, and an ester doublet at 1742 cm^{-1} and 1735 cm^{-1} merged into a single peak. Simultaneously, a carbon dioxide vibration appeared and increased (Fig. 19.19). Similar decomposition of T12 was also observed in isolated SC lipids (Novotny et al. 2011).

Thus, transkarbams are decomposed in the SC intercellular lipid lamellae into carbon dioxide and two protonated DDEAC molecules. The released carbon dioxide may form transient "bubbles" (similar to those induced by electroporation (Prausnitz and Langer 2008)) that disorder the SC

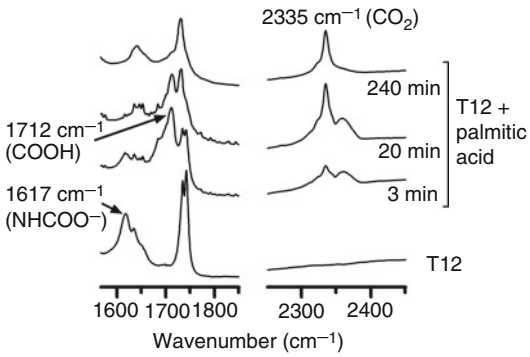


Fig. 19.19 Decomposition of T12 into protonated DDEAC and CO_2 in the presence of palmitic acid. Similar decomposition occurs in the stratum corneum lipids

lipid membranes. Alternatively, the lipid barrier permeability may be increased by the structural changes connected with the decomposition of transkarbams, e.g., by the repulsive forces between the released, positively charged DDEAC.

19.4.3 Dual Mechanism of Action

In 2008, we studied the effects of various enhancers on the transdermal and dermal delivery of adefovir (Vavrova et al. 2008a). T12 was a better enhancer than Azone®, dodecyl dimethylaminopropionate (DDAIP) (Buyuktimkin et al. 1993), dodecanol, or isopropyl myristate. T12 increased adefovir flux by up to 49-fold. To our surprise, the highest T12 activity for adefovir was found at pH 4, and it completely disappeared at pH 7 and above. At such acidic pH, only protonated DDEAC, and not T12, could have been present in the donor sample. Thus, our hypothesis that T12, i.e., the ammonium carbonate, was the only active enhancer species had to be revisited.

First, we compared the enhancing activity of T12 and its decomposition product, protonated DDEAC, and found that the latter species might have been responsible for a portion of the T12 activity (Novotny et al. 2010). Later, we confirmed that the action of transkarbams is indeed dual: carbamate salt (or its decomposition reaction) starts to increase skin permeability, and upon its decomposition in the SC, the released protonated EAC ester continues acting as a permeation enhancer. The advantage of this dual effect is that the "daughter" enhancer, despite being less active, broadens the scope of action of the parent carbamate because it also enhances permeation of hydrophilic drugs (Novotny et al. 2011).

19.5 Enhancing Activities, Biodegradability, Toxicity, and Skin Irritation

In addition to theophylline, which we used to screen for new enhancers, T12 also enhanced the transdermal permeation of clotrimazole by 7.7 times, flobufen (4-(2′,4′-difluorobiphenyl-4-yl)-2-methyl-4-oxobutyric acid) by 5 times, griseofulvin by 24 times (Hrabalek et al. 2006), adefovir by 49 times (Vavrova et al. 2008a), hydrocortisone by 141 times (Novotny et al. 2010), butorphanol by 2.5 times (Svozil et al. 2007), 5-flurouracil by 88 times, and acyclovir by 7.8 times (Hrabalek et al. 2001).

Moreover, T12 is rapidly (half-life of ~30 min) hydrolyzed by porcine esterase, proving the design principle of this class of enhancers (Hrabalek et al. 2006). Because esterases are present in the human epidermis (Montagna 1955), the hydrolysis is likely to occur in vivo as well, preventing the action of T12 on living cells. In addition, sensitive analytical methods are available for the determination of T12 (Hrabalek et al. 2006; Pasakova et al. 2006a, b; Folbrova 2007).

Initial toxicity studies suggested that these compounds may be relatively safe. The acute toxicity of T12 (at that time termed DDEAC) after intraperitoneal administration to mice was lower than that of Azone®: median lethal doses (LD_{50}) were 352 mg/kg and 232 mg/kg, respectively (Dolezal et al. 1993).

Further studies confirmed the favorable safety profile of T12. No signs of toxicity were observed in rats in the acute oral toxicity test (according to OECD guideline No. 423) at doses of 50, 300, and 2000 mg/kg over the 24 h after drug administration or during the 14-day observation period. A 28-day repeated daily dose dermal toxicity

study in rats, with a 14-day treatment-free period (according to OECD guideline No. 410), revealed no significant clinical symptoms, including site of administration, in a group receiving 0.1 % T12. A slight skin erythema was observed with 2 % T12, and a skin erythema and crusts were observed at a 10 % concentration (which is 10 times higher concentration than needed for penetration enhancement). After the administration of T12 was stopped, the skin recovered within 5–14 days. Significant changes in total leucocyte count were observed, which correlated with the skin reaction. No changes in body weight, food consumption, organ weights, red blood cell parameters, or serum chemistry parameters and no hepato- or nephrotoxicity were found at any of the concentrations used.

The index of dermal irritation (according to OECD guideline No. 404) was 0.25 (category nonirritating) and 1.67 (category slightly irritating) for 0.5 % and 5 % suspension of T12 in propylene glycol, respectively. T12 did not act as a contact allergen in the closed patch sensitization test (Buehler's method, according to OECD guideline No. 406). Moreover, T12 had no mutagenic potential in bacteria (Ames reverse mutation test, according to OECD guideline No. 471), and T12 had no cytotoxic effect on the bone marrow of rats (micronucleus test, according to OECD guideline No. 474).

Conclusion

In this chapter, we summarized the current knowledge on the group of transdermal penetration-enhancing carbamates, or transkarbams. The history of transkarbams almost reached a happy ending in 1999, when our patent on the use of amino acid derivatives as transdermal penetration enhancers was purchased by a Czech pharmaceutical company, Herbacos-Bofarma, s.r.o., which is a subsidiary of Bochemie Group. US and European patents followed on the actual carbamate structures of these enhancers. As a part of a joint project of the Czech Ministry of Industry and Trade, pharmacological and toxicological studies continued together with the technological development of eight formulations. However, in 2009, Recordati SpA acquired Herbacos-Bofarma, and the research on transkarbams was stopped.

However, we should admit that in 1989, we were lucky when choosing the pilot structure of amino acid esters. If we had decided to synthesize linear amide as the first, most logical modification of Azone®, the transkarbam group would have not been developed at all because we would have produced ineffective enhancers, and the whole group of open Azone® analogues would have appeared to be unpromising.

Finally, although we have not succeeded in introducing transkarbams into clinical practice, we believe that this work presents valuable new knowledge for the field of transdermal penetration enhancers and the behavior of ammonium carbamate salts in general. The detailed structure-activity relationships led to a discovery of another class of promising enhancers, 6-dimethylaminohexanoates, and these structural requirements can be used for a more rational design of compounds that could broaden the range of transdermally applicable drugs.

Acknowledgments We thank the Czech Science Foundation for its financial support (project 207/11/0365).

References

Ahad A, Aqil M, Kohli K, Chaudhary H, Sultana Y, Mujeeb M, Talegaonkar S (2009) Chemical penetration enhancers: a patent review. Expert Opin Ther Pat 19(7):969–988

Albery WJ, Hadgraft J (1979) Percutaneous absorption: in vivo experiments. J Pharm Pharmacol 31(3):140–147

Aoyagi T, Yamamura M, Suzuki N, Matsui K, Nagase Y (1991) Preparation of alkyl-substituted pyrrolidone derivatives and their evaluation as transdermal penetration enhancers. Drug Des Discov 8(1):37–46

Asbill CS, El-Kattan AF, Michniak B (2000) Enhancement of transdermal drug delivery: chemical and physical approaches. Crit Rev Ther Drug Carrier Syst 17(6): 621–658

Aungst BJ (2011) Absorption enhancers: applications and advances. AAPS J 14(1):10–18

Barry BW (1983) Dermatological formulations: percutaneous absorption. Marcel Dekker, New York

Barry BW (2001) Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci 14(2):101–114

Buyuktimkin S, Buyuktimkin N, Rytting JH (1993) Synthesis and enhancing effect of dodecyl 2-(N, N-dimethylamino)propionate on the transepidermal delivery of indomethacin, clonidine, and hydrocortisone. Pharm Res 10(11):1632–1637

Dell'Amico DB, Calderazzo F, Labella L, Marchetti F, Pampaloni G (2003) Converting carbon dioxide into carbamato derivatives. Chem Rev 103(10):3857–3897

Dolezal P, Hrabalek A, Mericka P, Klimesova V, Klimes J, Sochor J, Semecky V, Budnikova R (1989) Urychlovač transdermální penetrace. CZ Pat 276300

Dolezal P, Hrabalek A, Semecky V (1993) epsilon-Aminocaproic acid esters as transdermal penetration enhancing agents. Pharm Res 10(7):1015–1019

Farsa O, Dolezal P, Hrabalek A (2010) Esters and amides of hexanoic acid substituted with tertiary amino group in terminal position and their activity as transdermal permeation enhancers. J Serb Chem Soc 75(5):595–603

Folbrova J (2007) Hodnocení transkarbamu 12 a jeho nečistot pomocí HPLC s MS detekcí. PharmD. thesis, Charles University in Prague, Hradec Kralove

Fuhrman LC, Michniak BB, Behl CR, Malick AW (1997) Effect of novel penetration enhancers on the transdermal delivery of hydrocortisone: an in vitro species comparison. J Control Release 45(2):199–206

Geers C, Gros G (2000) Carbon dioxide transport and carbonic anhydrase in blood and muscle. Phys Rev 80(2):681–715

Guy RH (2010) Transdermal drug delivery. Handb Exp Pharmacol 197:399–410

Hadgraft J (1999) Passive enhancement strategies in topical and transdermal drug delivery. Int J Pharm 184(1):1–6

Holas T, Vavrova K, Klimentova J, Hrabalek A (2006a) Synthesis and transdermal permeation-enhancing activity of ketone, amide, and alkane analogs of Transkarbam 12. Bioorg Med Chem 14(9):2896–2903

Holas T, Vavrova K, Sima M, Klimentova J, Hrabalek A (2006b) Synthesis and transdermal permeation-enhancing activity of carbonate and carbamate analogs of Transkarbam 12. Bioorg Med Chem 14(23): 7671–7680

Holas T, Zbytovska J, Vavrova K, Berka P, Madlova M, Klimentova J, Hrabalek A (2006c) Thermotropic phase behavior of long-chain alkylammonium-alkylcarbamates. Thermochim Acta 441(2):116–123

Hrabalek A (1992) Potenciální akceleranty transdermální penetrace na bázi isosterních obměn substituovaných cyklických amidů (Potential transdermal penetration enhancers based on isosteric modifications of substituted cyclic amides) [Ph.D. thesis]. Charles University in Prague, Hradec Kralove

Hrabalek A, Dolezal P, Farsa O, Sklubalova Z, Kunes J (2000) Esters of 6-dimethylaminohexanoic acid as skin penetration enhancers. Pharmazie 55(10):759–761

Hrabalek A, Dolezal P, Farsa O, Krebs A, Kroutil A, Roman M, Sklubalova Z (2001) Omega-amino acid derivatives, processes of their preparation and their use. US 6187938

Hrabalek A, Vavrova K, Dolezal P, Machacek M (2005) Esters of 6-aminohexanoic acid as skin permeation enhancers: the effect of branching in the alkanol moiety. J Pharm Sci 94(7):1494–1499

Hrabalek A, Dolezal P, Vavrova K, Zbytovska J, Holas T, Klimentova J, Novotny J (2006) Synthesis and enhancing effect of transkarbam 12 on the transdermal delivery of theophylline, clotrimazole, flobufen, and griseofulvin. Pharm Res 23(5):912–919

Janůšová B, Školová B, Tükörová K, Wojnarová L, Šimůnek T, Mladěnka P, Filipský T, Říha M, Roh J, Palát K, Hrabálek A, Vávrová K (2013) Amino acid derivatives as transdermal permeation enhancers. J Control Release 165(2):91–100

Kanikkannan N, Kandimalla K, Lamba SS, Singh M (2000) Structure-activity relationship of chemical penetration enhancers in transdermal drug delivery. Curr Med Chem 7(6):593–608

Kaushik D, Batheja P, Kilfoyle B, Rai V, Michniak-Kohn B (2008) Percutaneous permeation modifiers: enhancement versus retardation. Expert Opin Drug Deliv 5(5):517–529

Klimentova J, Hrabalek A, Vavrova K, Holas T, Kroutil A (2006a) Synthesis and transdermal penetration-enhancing activity of carbonic and carbamic acid esters–comparison with transkarbam 12. Bioorg Med Chem Lett 16(7):1981–1984

Klimentova J, Kosak P, Vavrova K, Holas T, Hrabalek A (2006b) Influence of terminal branching on the transdermal permeation-enhancing activity in fatty alcohols and acids. Bioorg Med Chem 14(23):7681–7687

Klimentova J, Kosak P, Vavrova K, Holas T, Novotny J, Hrabalek A (2008) Transkarbams with terminal branching as transdermal permeation enhancers. Bioorg Med Chem Lett 18(5):1712–1715

Krebs A (2001) Akceleranty transdermální penetrace na bázi derivátů močoviny (Transdermal penetration enhancers based on urea derivatives) [Ph.D. thesis]. Charles University in Prague, Hradec Kralove

Kroutil A (1995) Estery bazických alkoholů jako potenciální akceleranty transdermální penetrace (Esters of basic alcohols as potential transdermal penetration enhancers). Master thesis, Charles University in Prague, Hradec Kralove

Marjukka Suhonen T, Bouwstra JA, Urtti A (1999) Chemical enhancement of percutaneous absorption in relation to stratum corneum structural alterations. J Control Release 59(2):149–161

Michniak BB, Player MR, Godwin DA, Lockhart CC, Sowell JW (1998) In vitro evaluation of azone analogs as dermal penetration enhancers – V. Miscellaneous compounds. Int J Pharm 161(2):169–178

Miyazaki K, Kaiho F, Inagaki A, Dohi M, Hazemoto N, Haga M, Hara H, Kato Y (1992) Enantiomeric difference in percutaneous penetration of propranolol through rat excised skin. Chem Pharm Bull 40(4): 1075–1076

Montagna W (1955) Histology and cytochemistry of human skin. 9. The distribution of non-specific esterases. J Biophys Biochem Cytol 1(1):13–16

Novotny J, Kovarikova P, Novotny M, Janusova B, Hrabalek A, Vavrova K (2009) Dimethylamino acid

esters as biodegradable and reversible transdermal permeation enhancers: effects of linking chain length, chirality and polyfluorination. Pharm Res 26(4):811–821

Novotny M, Hrabalek A, Janusova B, Novotny J, Vavrova K (2010) Transkarbams as transdermal permeation enhancers: effects of ester position and ammonium carbamate formation. Bioorg Med Chem Lett 20(9):2726–2728

Novotny M, Klimentova J, Janusova B, Palat K, Hrabalek A, Vavrova K (2011) Ammonium carbamates as highly active transdermal permeation enhancers with a dual mechanism of action. J Control Release 150(2): 164–170

Pasakova I, Klimes J, Sochor J, Hrabalek A (2006a) Optimization of HPLC chromatographic conditions for determination of Transkarbam 12 and its degradation products. J Pharm Biomed Anal 42(1):136–142

Pasakova I, Sochor J, Klimes J, Hrabalek A (2006b) Preliminary pharmaceutico-analytical evaluation of transkarbam 12 using liquid chromatography. J Sep Sci 29(11):1595–1599

Potts RO, Guy RH (1992) Predicting skin permeability. Pharm Res 9(5):663–669

Prausnitz MR, Langer R (2008) Transdermal drug delivery. Nat Biotechnol 26(11):1261–1268

Quan DY, Higuchi RI, Takayama K, Higashiyama K, Nagai T (1990) Enhancing effect of piperidone derivatives on the percutaneous absorption of indomethacin. Drug Des Deliv 6(1):61–71

Rajadhyaksha VJ (1976) Inventor Vehicle composition containing 1-substituted azacycloheptan-2-ones. US Patent 3,989,816

Sasaki H, Kojima M, Nakamura J, Shibasaki J (1990) Acute toxicity and skin irritation of pyrrolidone derivatives as transdermal penetration enhancer. Chem Pharm Bull (Tokyo) 38(8):2308–2310

Sasaki H, Kojima M, Mori Y, Nakamura J, Shibasaki J (1991) Enhancing effect of pyrrolidone derivatives on transdermal penetration of 5-fluorouracil, triamcinolone acetonide, indomethacin, and flurbiprofen. J Pharm Sci 80(6):533–538

Smith EW, Maibach HI (1995) Percutaneous penetration enhancers. CRC Press, Boca Raton

Stoughton RB (1982) Enhanced percutaneous penetration with 1-dodecylazacycloheptan-2-one. Arch Dermatol 118(7):474–477

Svozil M, Dolezal P, Hrabalek A, Mericka P (2007) In vitro studies on transdermal permeation of butorphanol. Drug Dev Ind Pharm 33(5):559–567

Vavrova K, Hrabalek A, Dolezal P (2002) Enhancement effects of (R) and (S) enantiomers and the racemate of a model enhancer on permeation of theophylline through human skin. Arch Dermatol Res 294(8):383–385

Vavrova K, Hrabalek A, Dolezal P, Holas T, Klimentova J (2005a) Biodegradable derivatives of tranexamic acid as transdermal permeation enhancers. J Control Release 104(1):41–49

Vavrova K, Zbytovska J, Hrabalek A (2005b) Amphiphilic transdermal permeation enhancers: structure-activity relationships. Curr Med Chem 12(19):2273–2291

Vavrova K, Lorencova K, Klimentova J, Novotny J, Holy AN, Hrabalek A (2008a) Transdermal and dermal delivery of adefovir: effects of pH and permeation enhancers. Eur J Pharm Biopharm 69(2):597–604

Vavrova K, Lorencova K, Novotny J, Holy A, Hrabalek A (2008b) Permeation enhancer dodecyl 6-(dimethylamino)hexanoate increases transdermal and topical delivery of adefovir: influence of pH, ion-pairing and skin species. Eur J Pharm Biopharm 70(3):901–907

Vavrova K, Kovarikova P, Skolova B, Libalova M, Roh J, Cap R, Holy A, Hrabalek A (2011) Enhanced topical and transdermal delivery of antineoplastic and antiviral acyclic nucleoside phosphonate cPr-PMEDAP. Pharm Res 28(12):3105–3115

Williams AC, Barry BW (2004) Penetration enhancers. Adv Drug Deliv Rev 56(5):603–618

Wong O, Tsuzuki N, Nghiem B, Kuehnhoff J, Itoh T, Masaki K, Huntington J, Konishi R, Rytting JH, Higuchi T (1989) Unsaturated cyclic ureas as new nontoxic biodegradable transdermal penetration enhancers. II. Evaluation study. Int J Pharm 52(3):191–202

Zbytovska J, Raudenkolb S, Wartewig S, Hubner W, Rettig W, Pissis P, Hrabalek A, Dolezal P, Neubert RH (2004) Phase behaviour of transkarbam 12. Chem Phys Lipids 129(1):97–109

Amino Acid-Based Transdermal Penetration Enhancers

20

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20.1 Introduction

Transdermal drug delivery offers an attractive alternative to conventional routes of drug administration. Transdermal drug delivery can provide sustained and controlled drug delivery over long time periods, avoids metabolism by the liver, and increases patient compliance (Prausnitz et al. 2004; Brown et al. 2006; Prausnitz and Langer 2008; Guy 2010). However, development of transdermal patches for drugs with a molecular mass larger than 500 Da is hindered primarily by low skin permeability. Human skin evolved to impede the penetration of exogenous molecules and the loss of water from the body; the major barrier is its uppermost layer, the stratum corneum (Forslind et al. 1997; Wertz 2000; Bouwstra et al. 2001; Madison 2003; Bouwstra and Ponec 2006; Elias 2008; Proksch et al. 2008; Menon and Kligman 2009).

In recent decades, many techniques have emerged to decrease skin barrier resistance to enable successful transdermal drug delivery (Hadgraft 1999; Asbill et al. 2000; Barry 2001; Williams and Barry 2004; Benson 2005; Tiwary et al. 2007). One of the approaches to increase drug flux through the skin into systemic circulation is the use of transdermal permeation enhancers, also known as penetration enhancers, percutaneous absorption promoters, or accelerants. These enhancers comprise a large,

N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_20

chemically heterogeneous group of compounds, including small molecules with solvent properties such as water, alcohols (e.g., ethanol), glycols (e.g., propylene glycol), sulphoxides (e.g., dimethylsulphoxide), esters (e.g., ethyl acetate), and amides (e.g., urea, dimethylacetamide), as well as larger molecules usually of amphiphilic structure, such as fatty acids (e.g., oleic acid), fatty alcohols (e.g., dodecanol), long-chain esters (e.g., dodecyl esters of amino acids), acyclic and cyclic amides (e.g., azepanones and pyrrolidones), surfactants (e.g., *N*-lauroylsarcosine), terpenes (e.g., farnesol), acetals (e.g., alkyldioxolanes), and others. The first compound specifically designed as a permeation enhancer was Azone® (1-dodecylazepan-2-one or laurocapram), patented in 1976 (Rajadhyaksha 1976; Stoughton 1982); it was followed in the 1980s and 1990s by many surfactant-like compounds with a C10–C12 chain. Detailed classifications of enhancers and their structure-activity relationships are discussed elsewhere (Smith and Maibach 1995; Kanikkannan et al. 2000; Williams and Barry 2004; Vavrova et al. 2005b).

The mechanisms by which these compounds promote drug flux are numerous. They can interact with the drug in the formulation, changing its thermodynamic activity, or interact with the skin barrier, i.e., the stratum corneum. The direct interactions with the stratum corneum involve altering the solvent properties of this skin layer, thereby changing drug partitioning and drug interaction with stratum corneum proteins (intracellular keratin or corneodesmosomes) or with the stratum corneum intercellular lipid lamellae (Smith and Maibach 1995; Marjukka Suhonen et al. 1999; Barry 2001; Williams and Barry 2004). The most potent enhancers are believed to act by influencing the properties of the intercellular stratum corneum lipids because they provide the major permeation pathway for most drugs (Albery and Hadgraft 1979; Potts and Guy 1992). This process may include lipid extraction or reversible changes of the lipid chain order (Karande et al. 2005).

Most of the potent enhancers, however, affect viable epidermal cells and provoke significant skin irritation. One of the rare exceptions to this rule is an alanine derivative dodecyl 2-(dimethylamino)propanoate (DDAIP, NexACT®, NexMed, USA, a subsidiary of Apricus Biosciences, Inc.) (Buyuktimkin et al. 1993) – most likely because of its biodegradability by epidermal esterases. To identify additional enhancers or combinations of enhancers that possess high potency and low irritation risk, Mitragotri's group developed a high-throughput screening method based on the effect of enhancers on the skin's electrical properties (Karande and Mitragotri 2002; Karande et al. 2004, 2005). They demonstrated that there exist classes of enhancers for which potency and irritation are not closely related (Karande et al. 2005). One of the compounds that combined apparent efficacy without noticeable irritation potential was *N*-lauroylsarcosine (Karande et al. 2006, 2007), i.e., another amino acid derivative.

Amino acid derivatives seem to be a promising class of permeation enhancers, especially those with a hydrophobic "tail" attached to an amino acid "head" via a biodegradable linkage, e.g., an ester bond (Janůšová et al. 2013). Such an amphiphilic enhancer could incorporate into the stratum corneum lipid barrier and disrupt the tight arrangement of the membrane lipids. Then, after reaching the enzymatically active nucleated epidermis, its labile bond could be hydrolyzed, thus releasing known nontoxic compounds with much lower irritation potential (Fig. 20.1).

In this chapter, we present an overview of acyclic amino acid-based permeation enhancers. The cyclic derivatives, i.e., lactams, including pyrrolidones and azones, were reviewed in detail in other chapters of this volume.

20.2 α-Amino Acid Derivatives

20.2.1 Glycine Derivatives

Glycine is the smallest of the 20 amino acids commonly found in proteins. The first permeation enhancers based on *N*-substituted glycine esters were prepared in 1989 (Wong et al. 1989, 1990). The most active enhancers in this series, dodecyl *N,N*-dimethylaminoacetate (DDAA, **1**, Fig. 20.2) and its decyl analog, were 2 and 3.8

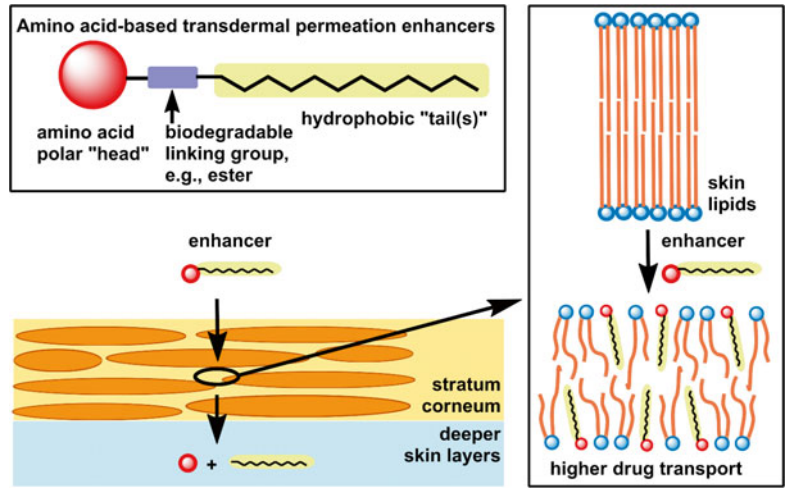


Fig. 20.1 The concept of biodegradable amino acid transdermal permeation enhancers

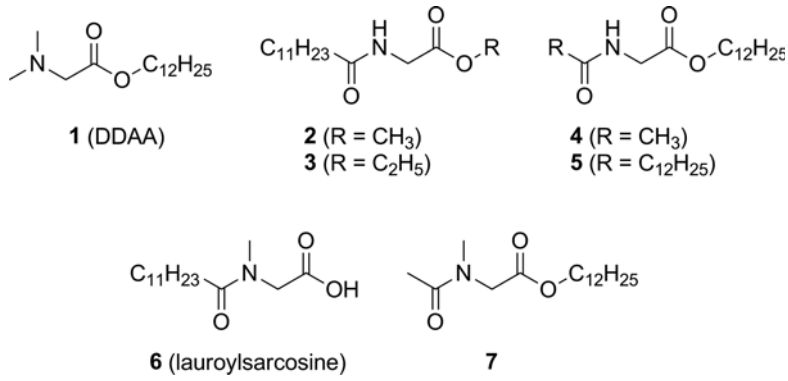


Fig. 20.2 Glycine (**1**–**5**) and sarcosine (**6**, **7**) derivatives

times more active, respectively, in enhancing the transport of indomethacin from petrolatum through snake skin than the standard enhancer Azone®. It was also 2.5 times more active than its putative decomposition product, dodecanol. In contrast, enhancers with bulkier substituents on nitrogen, i.e., diethylamino or 4-methylpiperazinyl groups, did not reach Azone® potency.

The enhancing activity of DDAA was also proved for the basic drug clonidine in aqueous vehicles with pH 3–7 (enhancement ratios, i.e., ratio of drug flux with and without enhancer, up to 11) (Fleeker et al. 1989). Hirvonen et al. confirmed DDAA activity on human epidermis and for a broader scope of drugs, including indomethacin, 5-fluorouracil, and propranolol hydrochloride (Hirvonen et al. 1991). Furthermore, DDAA increased the permeation of 5-fluorouracil through snake skin by 24 times. This effect was comparable to the activity of Azone® (enhancement ratio = 23) and 4.8 and 12 times higher than the effects of dodecanol and oleic acid, respectively (enhancement ratios = 5 and 2) (Turunen et al. 1993).

The mechanisms of the permeation-enhancing activity of DDAA involved both the stratum corneum proteins and lipids (Wong et al. 1989). Its enhancement and irritation in rabbit pinna skin were reversible within 4 days (Hirvonen et al. 1993). This reversibility was confirmed by differential scanning calorimetric experiments showing recovery of the stratum corneum lipid endotherms within the same time (Hirvonen et al. 1994). Furthermore, all mice that had been injected subcutaneously with DDAA at a dose of 1 g/kg daily for 7 days survived to 14 days, proving the low toxicity of this enhancer (Wong et al. 1990).

Another potent glycine permeation enhancer, ethyl *N*-dodecanoylglycine (compound **3**, Fig. 20.2), increased permeation of indomethacin through rat skin by 5.2 times (at 1 % in 50 % ethanol), making it approximately two times more active than similar enhancers based on alanine, valine, leucine, and isoleucine (Suzuki et al. 1999). This enhancer (**3**) also increased permeation of sodium salicylate, ketoprofen, prednisolone, and pindolol by 4.3, 1.5, 5.0, and 19.1 times, respectively. Interestingly, substitution of the 12C acyl in compound **3** by an alkyl of the same length markedly decreased its activity.

On the other hand, methyl *N*-dodecanoylglycine **2** (Fig. 20.2) was the least active in the series of *N*-dodecanoylamino acid methyl esters, increasing the permeability coefficient of hydrocortisone through hairless mouse skin only 2.6 times. In comparison, the enhancement ratios of alanine (**9**) and proline-based esters (**20**) were 11.6 and 13.9, respectively (see below) (Fincher et al. 1996).

In 2003, we prepared a series of double-chain enhancers based on glycine, serine, and dipeptides combining these two amino acids (Vavrova et al. 2003a, b). These enhancers were designed as ceramide analogs to test the hypothesis that a degree of similarity should exist between an enhancer molecule and ceramides for its incorporation into the stratum corneum lipid membranes. The most active enhancer of this series was a dodecyl ester of *N*-dodecanoylglycine **5** (Fig. 20.2) that, when dispersed in water at 1 % concentration, enhanced theophylline permeation through human and porcine skin by 12.5 and 4.5 times, respectively. Symmetric shortening of both hydrocarbon chains decreased its activity (Vavrova et al. 2003b), whereas shortening only the acyl to 2 carbons (compound **4**) resulted in a higher permeation-enhancing potency (Janůšová et al. 2013). Of note, *N*-oleoyl derivatives of both glycine and serine dodecyl esters decreased the flux of theophylline by ~50 % in pig skin. The compounds having a dipeptide polar head did not show any significant enhancement, suggesting that this polar head is most likely too large to be incorporated into the stratum corneum intercellular lipids (Vavrova et al. 2003b).

20.2.2 Sarcosine Derivatives

Sarcosine, i.e., *N*-methylglycine, is an intermediate in glycine metabolism found in muscles and other bodily tissues. In fact, an *N,N*-dimethylglycine-based enhancer DDAA (**1**) can also be regarded as a derivative of *N*-methylsarcosine. The most widely known enhancer derived from this natural amino acid is *N*-lauroylsarcosine (compound **6**, Fig. 20.2) (Tsubota et al. 1987), which is also used as an anionic surfactant. Combinations of this enhancer with vitamin E and squalene were shown to alleviate *N*-lauroylsarcosine's skin irritation potential and to increase isosorbide dinitrate absorption in rats (Aioi et al. 1993). More than 10 years later, this sarcosine derivative **6** was identified as a very promising enhancer with high efficacy without noticeable irritation potential by high-throughput screening, particularly in combination with another 12C amphiphile, sorbitan monolaurate (Karande et al. 2004, 2006, 2007).

The mechanisms by which this sarcosine derivative **6** in aqueous ethanol acts were studied by differential scanning calorimetry and infrared spectroscopy (Kim et al. 2008). The permeability of fluorescein, a model permeant, across human epidermis increased up to 47-fold and correlated well with changes in the stratum corneum lipid organization. The skin electrical resistance measurements showed a rapid onset of the enhancer action; the most effective combination of 2 % enhancer **6** and 50 % ethanol reached its maximum potency in just 4 h. The fluidization of the stratum corneum lipids was suggested to be the major factor contributing to this enhancer's efficacy as deduced from the shifts in the infrared methylene vibrations, although the authors also found some lipid extraction and changes in protein conformation.

N-Lauroylsarcosine **6** was further modified to mask the free carboxyl and thus change its anionic properties to nonionic. The *N*-lauroylsarcosine dodecyl ester was inactive, but shortening of the acyl chain to 2 carbons in *N*-acetylsarcosine dodecyl ester (compound **7**, Fig. 20.2) resulted in a potent enhancer that increased the transdermal flux of theophylline by 6.2 times in water and 15.5 times in 60 % propylene glycol (Janůšová et al. 2013).

20.2.3 Alanine Derivatives

One of the most interesting permeation enhancers from the group of α-amino acid derivatives is a dodecyl ester of *N,N*-dimethylalanine DDAIP (compound **8**, Fig. 20.3), prepared in 1993 (Buyuktimkin et al. 1993). It is one of the rare examples of enhancers used in clinical practice and is also known as NexACT® drug delivery technology, proprietary to NexMed, USA, a subsidiary of Apricus Biosciences, Inc. It differs from DDAA only in the presence of methyl group at C2. DDAIP enhanced the permeation of indomethacin, clonidine, and hydrocortisone across shed snake skin. Its biodegradability into dimethylalanine and dodecanol, with a half-life of 18.5 min, was demonstrated in vitro in the presence of porcine esterase. When compared with reference enhancers Azone® and dodecanol, DDAIP was a more active enhancer by 4.7 and 7.5 times for indomethacin, 1.7 and 3.1 times more active for clonidine, and 2.4 and 2.8 times more active for hydrocortisone, respectively. These data confirmed that DDAIP was the active compound rather than its decomposition compound dodecanol. In the case of indomethacin, the enhancement was partially explained by the interaction between the acidic indomethacin carboxyl and basic tertiary amino group of DDAIP (Buyuktimkin et al. 1996). Interestingly, a DDAIP isomer with a "reversed" ester bond 1-(*N,N*-dimethylamino)propan-2-ol dodecanoate (DAIPD) is also a potent and biodegradable enhancer (Buyuktimkin et al. 1995b).

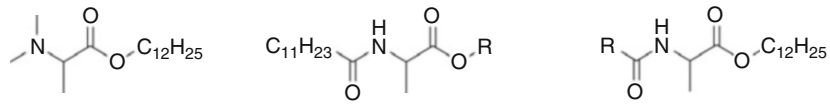
A direct comparison of DDAIP and its parent compound, DDAA, using 5-fluorouracil as a model hydrophilic drug with poor skin permeability, revealed that DDAIP is an almost three times more potent enhancer than DDAA – enhancement ratios after pretreatment of snake skin with 5 μl of DDAIP, DDAA, Azone®, dodecanol, and oleic acid were 69, 24, 23, 5, and 2, respectively, compared to untreated skin (Turunen et al. 1993).

The importance of DDAIP chirality was also studied to test the hypothesis that its interaction with the skin constituents is stereoselective (Novotny et al. 2009). However, no differences in activity were found between (*R*), (*S*), and racemic DDAIP. Another interesting observation from that study was that a substitution of a hydrocarbon tail in DDAIP with a fluorocarbon resulted in a loss of activity.

Importantly, DDAIP and its hydrochloride salt have low toxicity, are rapidly metabolized by esterases, and are well tolerated by the skin (Pfister et al. 2006). The mechanisms by which DDAIP promotes drug permeation include disordering the lipid organization (Turunen et al. 1994; Suhonen et al. 1997; Wolka et al. 2004), keratin interaction (Buyuktimkin et al. 1995a), and drug complexation (Buyuktimkin et al. 1996).

Other examples of alanine-derived permeation enhancers include *N*-dodecanoylalanine methyl and ethyl esters (compounds **9** and **10**, Fig. 20.3). Methyl ester **9** increased the absorption of hydrocortisone through hairless mouse skin by nearly 12 times (Fincher et al. 1996), while ethyl ester **10** enhanced permeation of indomethacin through rat skin 2.6 times (Suzuki et al. 1999).

Another enhancer with similar or slightly higher activity than Azone® was obtained by "switching" the chain position of compound **10**, leading to an *N*-acetylalanine dodecyl ester **11** (Fig. 20.3). This compound, at 1 %w/v in 60 % propylene glycol, enhanced theophylline flux through porcine skin 12.6 times (Janůšová et al. 2013). In contrast, increasing the ester alkyl chain to 12C in the double-chain lipid-like compound **12** led to a loss of its activity.



8 (DDAIP)

9 (R = CH_3)
10 (R = C_2H_5)

11 (R = CH_3)
12 (R = $C_{12}H_{25}$)

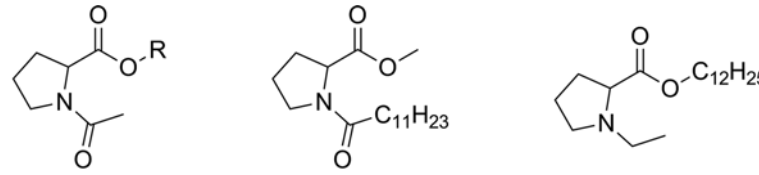
Fig. 20.3 Alanine derivatives

20.2.4 Proline Derivatives

Proline is the only α-amino acid with the amino group in a cycle. This position leads to a lower flexibility of such an enhancer's polar head and, in the case of nitrogen substitution, lower hydrogen-bonding ability. Pentyl *N*-acetylprolinate (compound **13**, Fig. 20.4) proved to be an effective enhancer for benzoic acid through human skin and is nontoxic in low doses; however, it possesses a CNS-depressant effect in mice at 50 mg/kg dose intraperitoneally (Harris et al. 1995). One year later, an *N*-dodecanoylproline methyl ester (**20**) was prepared and studied together with a series of other amino acid derivatives (Fincher et al. 1996). It was more than three times more active than pentyl *N*-acetylprolinate **13** in enhancing the delivery of hydrocortisone through hairless mouse skin, reaching an enhancement ratio of 13.9.

In 1999, a series of *N*-acetylproline esters with a chain length from 5C to 18C (compounds **13**–**19**, Fig. 20.4) were studied using hairless mouse skin. The most active enhancers were the undecyl, dodecyl, and oleyl esters. Dodecyl ester **17** was the best enhancer for hydrocortisone, increasing its permeability coefficient by 34 times, which was 1.6 and 2.4 times higher than the activity of Azone® and pentyl *N*-acetylprolinate **13**, respectively. In the case of the second model drug, benazepril, the undecyl (**16**) and oleyl (**19**) esters were the most effective, with enhancement ratios of 40. However, this increase was less than the increase demonstrated by Azone®, which enhanced benazepril permeation by 68 times. This example well illustrates the fact that the enhancing potencies are drug specific. This finding complicates the development of new enhancers (apart from other factors) because initial screening using a model drug or skin electrical properties would identify active enhancers from inactive compounds, but the exact permeation-enhancing potency may be completely different for a particular drug.

Recently, we have prepared a series of amino acid (proline, sarcosine, alanine, β-alanine, and glycine) permeation enhancers (Janůšová et al. 2013). The double-chain substances displayed no enhancing effect, whereas single-chain substances significantly increased skin permeability. From this series, dodecyl *N*-acetylprolinate **17**, also termed L-Pro2, was the most active enhancer for the model drug theophylline, reaching an enhancement ratio of 40 at 1 % concentration. Under the same conditions, Azone® was inactive, and DDAIP, dodecyl 6-dimethylaminohexanoate (DDAK, see later), and transkarbam 12 (see later) reached ER values of 7.8, 23, and 19, respectively. Surprisingly, substitution of acetyl by ethyl group in compound **21** led to a marked decrease or loss of activity. No stereoselectivity was observed; both L- and D-enantiomers enhanced the drug flux with the same potency. Enhancer **17** acted synergistically with propylene glycol, and infrared studies revealed that compound **17** forms a separate liquid-ordered phase in the stratum corneum lipids and has no significant effect on proteins. The enhancing activity of compound **17** was at least partially reversible, as measured by skin electrical impedance. Toxicity in HaCaT keratinocyte and 3 T3 fibroblast cell lines was comparable to standard enhancers. Furthermore, this enhancer was rapidly decomposed in plasma. In vivo transdermal absorption studies in rats confirmed the enhancing activity of compound **17** and suggested its negligible skin toxicity and minimal effect on transepidermal water loss.



13-19 (R = C_5 – C_{18}) **20** **21**

Fig. 20.4 Proline derivatives

20.2.5 Serine Derivatives

L-Serine is a key amino acid in the biosynthesis of ceramides, forming the "polar head" of these skin barrier sphingolipids. Enhancer **22** (Fig. 20.5), based on this amino acid, was first prepared in 1996 as a part of larger series of *N*-dodecanoyl amino acid methyl esters (Fincher et al. 1996). It increased the flux of hydrocortisone through hairless mouse skin by 6.3 times, while only the alanine derivative **9** and proline-based analog **20** were better enhancers under those conditions.

Our group studied the action of double-chain ceramide-like L-serine derivatives in human skin. The chain length was found to be crucial; compound **23** with two 12C chains enhanced skin permeability for theophylline by 2.7 times (Vavrova et al. 2003a, b), whereas its homolog **24**, termed 14S24, with chain lengths 14C and 24C, i.e., the same as in skin ceramides (Fig. 20.5), behaved as a skin barrier repair agent (Vavrova et al. 2004, 2007).

20.2.6 Other α-Amino Acid Permeation Enhancers

Other α-amino acids incorporated into permeation enhancer molecules were valine (**25**), leucine (**27**), phenylalanine (**30**), tyrosine (**31**), tryptophane (**32**), and methionine (compound **33**, Fig. 20.6); these amino acids enhanced permeation of hydrocortisone through hairless mouse skin by 3.5, 5.5, 5.3, 4.8, and 4.1 times, respectively (Fincher et al. 1996). However, this increase was less than with the abovementioned alanine **9** and proline **20** derivatives (enhancement ratios of 11.6 and 13.9, respectively).

Valine (**26**), leucine (**28**), and isoleucine (**29**) derivatives were also studied for their enhancing ability for indomethacin through rat skin, reaching enhancement ratios of 2.2, 1.9, and 2.4, respectively. This increase was comparable to the activity of an analogous alanine derivative **10** but two times less than that of a glycine derivative **3** under the same conditions (Suzuki et al. 1999).

Lysine was also esterified with unbranched alcohols (**34**, Fig. 20.6) to yield substances enhancing permeation through both the skin and gastric epithelium (Fix and Pogany 1985). Notably, these compounds can also be classified as ε-amino acid derivatives and are structurally very similar to transkarbams and DDAK (see below).

20.3 β-Amino Acid Derivatives

In an attempt to increase the permeation-enhancing activity of DDAIP, its "straight-chain" isomer dodecyl 3-dimethylaminopropionate (compound **35**, Fig. 20.7), i.e., a β-alanine derivative, was prepared (Novotny et al. 2009). Indeed, this change led to 2.6-fold increase in activity for theophylline through porcine skin in vitro (the enhancement ratios of 1 % DDAIP and **35** were 5.9 and 15, respectively).

On the other hand, when the enhancing potency of 1 % dodecyl esters of *N*-acetylalanine **11** and β-alanine **36** in 60 % propylene glycol was compared, no significant difference was found. They improved the flux of theophylline through pig skin by 12.6 and 11.0 times (Janůšová et al. 2013).

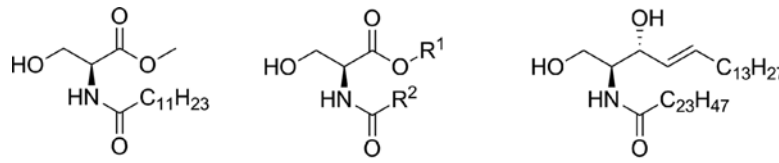


Fig. 20.5 Serine derivatives and ceramide NS (non-hydroxyacylsphingosine)

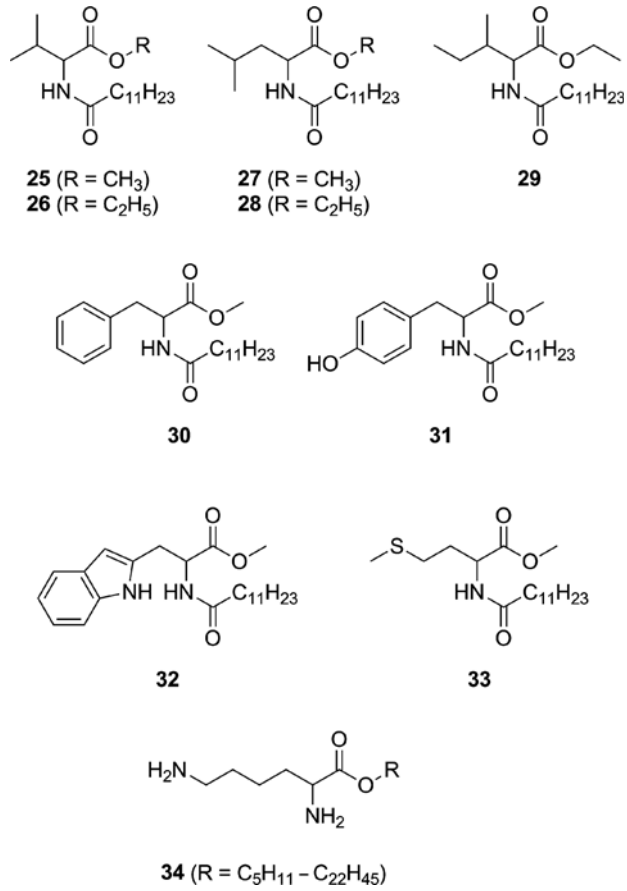


Fig. 20.6 Other α-amino acid-based permeation enhancers

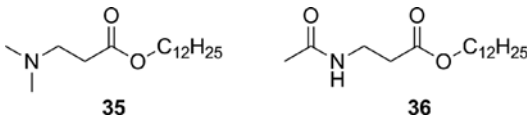


Fig. 20.7 β-Alanine derivatives

20.4 ω-Amino Acid Derivatives

Another important group of amino acid-based permeation enhancers is derived from ω-amino acids, i.e., those with a terminal amino group. 6-Aminohexanoates were originally designed in our group in 1989 as open Azone® analogs with the amide bond replaced by an ester bond (Fig. 20.8) (Dolezal et al. 1989). The hypothesis behind such a structure was to replace the 7-membered ring of Azone® by a more flexible chain and to substitute the amide bond with a more readily biodegradable linker. These compounds showed exceptional activity a full order of magnitude higher than Azone® (Dolezal et al. 1993; Hrabalek et al. 1994, 2001). Later, it was found that these compounds trap atmospheric carbon dioxide and that the active enhancer is actually a two-chain ammonium carbamate (Hrabalek et al. 2006; Novotny et al. 2011). The carbamate enhancers (termed transkarbams), their properties (Dolezal et al. 1993; Hrabalek et al. 2006; Vavrova et al. 2008a; Novotny et al. 2011), their toxicity (Hrabalek et al. 2006), and their structure-activity relationships (Hrabalek et al. 1994, 2005; Vavrova et al. 2002, 2005a; Holas et al. 2006a, b; Klimentova et al. 2006, 2008; Novotny et al. 2010) are discussed in Chap. 21 of this volume.

The structure of the most promising compound of the transkarbam series, transkarbam 12, has been a subject of hundreds of synthetic

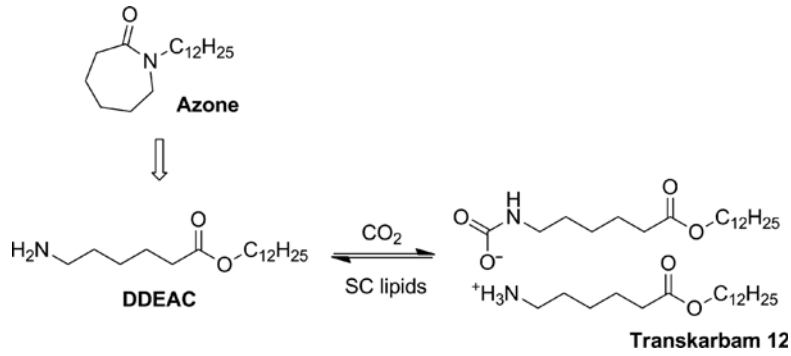


Fig. 20.8 Design of 6-aminohexanoic acid derivatives and discovery of transkarbams

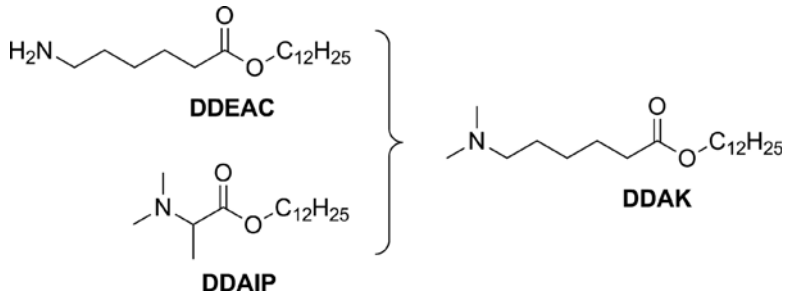


Fig. 20.9 Design of DDAK by a combination of DDEAC and DDAIP structural features

modifications (Vavrova et al. 2002, 2005a; Hrabalek et al. 2005; Holas et al. 2006a, b; Klimentova et al. 2006, 2008; Novotny et al. 2010, 2011). Unfortunately, most of them were unsuccessful until the discovery of 6-dimethylaminohexanoic acid dodecyl ester (DDAK) (Hrabalek et al. 2000; Novotny et al. 2009; Farsa et al. 2010). This compound can be viewed as a "hybrid" of a dimethylalanine enhancer DDAIP and dodecyl 6-aminohexanoate, i.e., a precursor and also a degradation product of transkarbam 12 (Fig. 20.9).

DDAK was found to be a more potent enhancer than DDAIP for theophylline (enhancement ratios of DDAK and DDAIP were 17.3 and 5.9, respectively), hydrocortisone (43.2 and 11.5), and adefovir (13.6 and 2.8), while DDAIP was a better enhancer for indomethacin (8.7 and 22.8) (Novotny et al. 2009). DDAK was rapidly metabolized by porcine esterase, with a half-life of 17 min. Electrical impedance of a DDAK-treated skin barrier rapidly recovered, demonstrating at least partial reversibility of its action in the skin barrier.

The length of the linking chain between the ester carbonyl and the tertiary amino group was found to be relatively unimportant, with the highest enhancing potencies found for 4C–6C, i.e., derivatives of 4-aminobutanoic acid, 5-aminopentanoic acid, and DDAK, but with only slightly decreased values for shorter 3-aminopropanoate and longer 8-aminooctanoate (Novotny et al. 2009).

DDAK was also studied as a permeation enhancer for transdermal delivery of highly hydrophilic drugs from the group of acyclic nucleoside phosphonates, including the broad-spectrum antiviral adefovir (Vavrova et al. 2008b) and potent antineoplastic N^6-cPr-PMEDAP (N^6-cyclopropyl-2,6-diamino-9-[2-(phosphonomethoxy)ethyl]purine) (Vavrova et al. 2011). In the case of adefovir, DDAK was more active than DDAIP. It increased adefovir flux through human skin in vitro by 179 times, with a maximum at pH ~6, and also increased its concentration in viable epidermis and dermis by 33–61 times. With the diaminopurine derivative N^6-cPr-PMEDAP, the highest permeation rates were also found at pH 6, reaching enhancement ratios of up to 61.

The concentrations of DDAK inducing a 50 % decrease in viability (IC_{50}) of HaCaT keratinocytes

and 3 T3 fibroblasts after 48 h of treatment were 76 and 175 μM, respectively (the experiment was run for 48 h, because at 24 h, the enhancers were not sufficiently toxic to reach IC_{50}) (Janůšová et al. 2013). Furthermore, oral toxicity tests in mice and rats showed that all animals survived a DDAK dose of 2 g/kg without any signs of toxicity (Novotny et al. 2009).

Conclusion

Amino acid-based amphiphilic compounds with an ester linker seem to be a promising class of permeation enhancers. The alanine derivative DDAIP is one of the few enhancers in use clinically because of its high potency and advantageous safety profile. Recently, two experimental enhancers – a proline-derived L-Pro2 and 6-dimethylaminohexanoate DDAK – showed even higher permeation-enhancing activities, and their preliminary toxicological evaluations suggest that they merit further study. We consider these compounds to be the leading structures for further synthetic modifications and follow-up toxicological studies.

Acknowledgments We thank the Czech Science Foundation for its financial support (project 207/11/0365).

References

Aioi A, Kuriyama K, Shimizu T, Yoshioka M, Uenoyama S (1993) Effects of vitamin-E and squalene on skin irritation of a transdermal absorption enhancer, lauroylsarcosine. Int J Pharm 93(1–3):1–6

Albery WJ, Hadgraft J (1979) Percutaneous absorption: in vivo experiments. J Pharm Pharmacol 31(3): 140–147

Asbill CS, El-Kattan AF, Michniak B (2000) Enhancement of transdermal drug delivery: chemical and physical approaches. Crit Rev Ther Drug Carrier Syst 17(6): 621–658

Barry BW (2001) Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci 14(2):101–114

Benson HA (2005) Transdermal drug delivery: penetration enhancement techniques. Curr Drug Deliv 2(1):23–33

Bouwstra JA, Ponec M (2006) The skin barrier in healthy and diseased state. Biochim Biophys Acta 1758(12): 2080–2095

Bouwstra J, Pilgram G, Gooris G, Koerten H, Ponec M (2001) New aspects of the skin barrier organization. Skin Pharmacol Appl Skin Physiol 14(Suppl 1): 52–62

Brown MB, Martin GP, Jones SA, Akomeah FK (2006) Dermal and transdermal drug delivery systems: current and future prospects. Drug Deliv 13(3):175–187

Buyuktimkin S, Buyuktimkin N, Rytting JH (1993) Synthesis and enhancing effect of dodecyl 2-(N, N-dimethylamino)propionate on the transepidermal delivery of indomethacin, clonidine, and hydrocortisone. Pharm Res 10(11):1632–1637

Buyuktimkin N, Buyuktimkin S, Rytting JH (1995a) Alkyl *N,N*-disubstituted-amino acetates. In: Smith EW, Maibach HI (eds) Percutaneous penetration enhancers. CRC Press, New York, pp 91–102

Buyuktimkin S, Buyuktimkin N, Rytting JH (1995b) Preparation and enhancing effect of 1-(N, N-dimethylamino)-2-propanol dodecanoate (DAIPD) on the transepidermal penetration of clonidine, hydrocortisone, and indomethacin through shed snake skin. Int J Pharm 118(1):113–119

Buyuktimkin S, Buyuktimkin N, Rytting JH (1996) Interaction of indomethacin with a new penetration enhancer, dodecyl 2-(N, N-dimethylamino)propionate (DDAIP): its effect on transdermal delivery. Int J Pharm 127(2):245–253

Dolezal P, Hrabalek A, Mericka P, Klimesova V, Klimes J, Sochor J et al (1989) Urychlovač transdermální penetrace. CZ Pat 276300

Dolezal P, Hrabalek A, Semecky V (1993) epsilon-Aminocaproic acid esters as transdermal penetration enhancing agents. Pharm Res 10(7):1015–1019

Elias PM (2008) Skin barrier function. Curr Allergy Asthma Rep 8(4):299–305

Farsa O, Dolezal P, Hrabalek A (2010) Esters and amides of hexanoic acid substituted with tertiary amino group in terminal position and their activity as transdermal permeation enhancers. J Serb Chem Soc 75(5):595–603

Fincher TK, Yoo SD, Player MR, Sowell JW Sr, Michniak BB (1996) In vitro evaluation of a series of N-dodecanoyl-L-amino acid methyl esters as dermal penetration enhancers. J Pharm Sci 85(9):920–923

Fix JA, Pogany SA (1985) Lysine esters used as absorption enhancing agents. EP-0162747

Fleeker C, Wong O, Rytting JH (1989) Facilitated transport of basic and acidic drugs in solutions through snakeskin by a new enhancer–dodecyl N,N-dimethylamino acetate. Pharm Res 6(6):443–448

Forslind B, Engstrom S, Engblom J, Norlen L (1997) A novel approach to the understanding of human skin barrier function. J Dermatol Sci 14(2):115–125

Guy RH (2010) Transdermal drug delivery. Handb Exp Pharmacol 197:399–410

Hadgraft J (1999) Passive enhancement strategies in topical and transdermal drug delivery. Int J Pharm 184(1):1–6

Harris WT, Tenjarla SN, Holbrook JM, Smith J, Mead C, Entrekin J (1995) n-pentyl N-acetylprolinate. A new skin penetration enhancer. J Pharm Sci 84(5):640–642

Hirvonen J, Rytting JH, Paronen P, Urtti A (1991) Dodecyl N,N-dimethylamino acetate and azone enhance drug penetration across human, snake, and rabbit skin. Pharm Res 8(7):933–937

Hirvonen J, Sutinen R, Paronen P, Urtti A (1993) Transdermal penetration enhancers in rabbit pinna skin: duration of action, skin irritation, and in vivo/in vitro comparison. Int J Pharm 99(2–3):253–261

Hirvonen J, Rajala R, Vilhervaara P, Laine E, Paronen P, Urtti A (1994) Mechanism and reversibility of penetration enhancer action in the skin: a DSC study. Eur J Pharm Biopharm 40(2):81–85

Holas T, Vavrova K, Klimentova J, Hrabalek A (2006a) Synthesis and transdermal permeation-enhancing activity of ketone, amide, and alkane analogs of Transkarbam 12. Bioorg Med Chem 14(9):2896–2903

Holas T, Vavrova K, Sima M, Klimentova J, Hrabalek A (2006b) Synthesis and transdermal permeation-enhancing activity of carbonate and carbamate analogs of Transkarbam 12. Bioorg Med Chem 14(23): 7671–7680

Hrabalek A, Dolezal P, Roman M, Machacek M, Sklubalova Z (1994) Esters of omega-amino acids as flexible penetration enhancers. Pharmazie 49(5):325–328

Hrabalek A, Dolezal P, Farsa O, Sklubalova Z, Kunes J (2000) Esters of 6-dimethylaminohexanoic acid as skin penetration enhancers. Pharmazie 55(10):759–761

Hrabalek A, Dolezal P, Farsa O, Krebs A, Kroutil A, Roman M et al (2001) Omega-amino acid derivatives, processes of their preparation and their use. US 6187938

Hrabalek A, Vavrova K, Dolezal P, Machacek M (2005) Esters of 6-aminohexanoic acid as skin permeation enhancers: the effect of branching in the alkanol moiety. J Pharm Sci 94(7):1494–1499

Hrabalek A, Dolezal P, Vavrova K, Zbytovska J, Holas T, Klimentova J et al (2006) Synthesis and enhancing effect of transkarbam 12 on the transdermal delivery of theophylline, clotrimazole, flobufen, and griseofulvin. Pharm Res 23(5):912–919

Janůšová B, Školová B, Tükörová K, Wojnarová L, Šimůnek T, Mladěnka P et al (2013) Amino acid derivatives as transdermal permeation enhancers. J Control Release 165(2):91–100

Kanikkannan N, Kandimalla K, Lamba SS, Singh M (2000) Structure-activity relationship of chemical penetration enhancers in transdermal drug delivery. Curr Med Chem 7(6):593–608

Karande P, Mitragotri S (2002) High throughput screening of transdermal formulations. Pharm Res 19(5):655–660

Karande P, Jain A, Mitragotri S (2004) Discovery of transdermal penetration enhancers by high-throughput screening. Nat Biotechnol 22(2):192–197

Karande P, Jain A, Ergun K, Kispersky V, Mitragotri S (2005) Design principles of chemical penetration enhancers for transdermal drug delivery. Proc Natl Acad Sci U S A 102(13):4688–4693

Karande P, Jain A, Mitragotri S (2006) Insights into synergistic interactions in binary mixtures of chemical permeation enhancers for transdermal drug delivery. J Control Release 115(1):85–93

Karande P, Jain A, Arora A, Ho MJ, Mitragotri S (2007) Synergistic effects of chemical enhancers on skin permeability: a case study of sodium lauroylsarcosinate and sorbitan monolaurate. Eur J Pharm Sci 31(1):1–7

Kim YC, Park JH, Ludovice PJ, Prausnitz MR (2008) Synergistic enhancement of skin permeability by N-lauroylsarcosine and ethanol. Int J Pharm 352(1–2): 129–138

Klimentova J, Hrabalek A, Vavrova K, Holas T, Kroutil A (2006) Synthesis and transdermal penetration-enhancing activity of carbonic and carbamic acid esters–comparison with Transkarbam 12. Bioorg Med Chem Lett 16(7):1981–1984

Klimentova J, Kosak P, Vavrova K, Holas T, Novotny J, Hrabalek A (2008) Transkarbams with terminal branching as transdermal permeation enhancers. Bioorg Med Chem Lett 18(5):1712–1715

Madison KC (2003) Barrier function of the skin: "la raison d'etre" of the epidermis. J Invest Dermatol 121(2): 231–241

Marjukka Suhonen T, Bouwstra JA, Urtti A (1999) Chemical enhancement of percutaneous absorption in relation to stratum corneum structural alterations. J Control Release 59(2):149–161

Menon GK, Kligman AM (2009) Barrier functions of human skin: a holistic view. Skin Pharmacol Physiol 22(4):178–189

Novotny J, Kovarikova P, Novotny M, Janusova B, Hrabalek A, Vavrova K (2009) Dimethylamino acid esters as biodegradable and reversible transdermal permeation enhancers: effects of linking chain length, chirality and polyfluorination. Pharm Res 26(4): 811–821

Novotny M, Hrabalek A, Janusova B, Novotny J, Vavrova K (2010) Transkarbams as transdermal permeation enhancers: effects of ester position and ammonium carbamate formation. Bioorg Med Chem Lett 20(9):2726–2728

Novotny M, Klimentova J, Janusova B, Palat K, Hrabalek A, Vavrova K (2011) Ammonium carbamates as highly active transdermal permeation enhancers with a dual mechanism of action. J Control Release 150(2):164–170

Pfister W, Li M, Frank D (2006) Development of the novel permeation enhancers dodecyl-2-*N,N-dimethylaminopropionate* (DDAIP) and HCl salt: physiochemical properties, preclinical safety and in vitro permeation enhancement. AAPS J 8(S2)

Potts RO, Guy RH (1992) Predicting skin permeability. Pharm Res 9(5):663–669

Prausnitz MR, Langer R (2008) Transdermal drug delivery. Nat Biotechnol 26(11):1261–1268

Prausnitz MR, Mitragotri S, Langer R (2004) Current status and future potential of transdermal drug delivery. Nat Rev Drug Discov 3(2):115–124

Proksch E, Brandner JM, Jensen JM (2008) The skin: an indispensable barrier. Exp Dermatol 17(12):1063–1072

Rajadhyaksha VJ (1976) Inventor Vehicle composition containing 1-substituted azacycloheptan-2-ones. US patent 3,989,816

Smith EW, Maibach HI (1995) Percutaneous penetration enhancers. CRC Press, Boca Raton

Stoughton RB (1982) Enhanced percutaneous penetration with 1-dodecylazacycloheptan-2-one. Arch Dermatol 118(7):474–477

Suhonen TM, Pirskanen L, Raisanen M, Kosonen K, Rytting JH, Paronen P et al (1997) Transepidermal delivery of beta-blocking agents: evaluation of enhancer effects using stratum corneum lipid liposomes. J Control Release 43(2–3):251–259

Suzuki E, Okabe H, Saito T (1999) Percutaneous absorption promoter, a tape plaster and a method of promoting percutaneous absorption. JP 4034274 (1992), USRE36138E

Tiwary AK, Sapra B, Jain S (2007) Innovations in transdermal drug delivery: formulations and techniques. Recent Pat Drug Deliv Formul 1(1):23–36

Tsubota S, Nakagawa T, Kobayashi K, Azuma M (1987) Transdermal and transmucosal pharmaceutics. Jap Pat 62–96430

Turunen TM, Buyuktimkin S, Buyuktimkin N, Urtti A, Paronen P, Rytting JH (1993) Enhanced delivery of 5-fluorouracil through shed snake skin by 2 new transdermal penetration enhancers. Int J Pharm 92(1–3):89–95

Turunen TM, Urtti A, Paronen P, Audus KL, Rytting JH (1994) Effect of some penetration enhancers on epithelial membrane lipid domains: evidence from fluorescence spectroscopy studies. Pharm Res 11(2):288–294

Vavrova K, Hrabalek A, Dolezal P (2002) Enhancement effects of (R) and (S) enantiomers and the racemate of a model enhancer on permeation of theophylline through human skin. Arch Dermatol Res 294(8):383–385

Vavrova K, Hrabalek A, Dolezal P, Holas T, Zbytovska J (2003a) L-Serine and glycine based ceramide analogues as transdermal permeation enhancers: polar head size and hydrogen bonding. Bioorg Med Chem Lett 13(14):2351–2353

Vavrova K, Hrabalek A, Dolezal P, Samalova L, Palat K, Zbytovska J et al (2003b) Synthetic ceramide analogues as skin permeation enhancers: structure-activity relationships. Bioorg Med Chem 11(24): 5381–5390

Vavrova K, Zbytovska J, Palat K, Holas T, Klimentova J, Hrabalek A et al (2004) Ceramide analogue 14S24 ((S)-2-tetracosanoylamino-3-hydroxypropionic acid tetradecyl ester) is effective in skin barrier repair in vitro. Eur J Pharm Sci 21(5):581–587

Vavrova K, Hrabalek A, Dolezal P, Holas T, Klimentova J (2005a) Biodegradable derivatives of tranexamic acid as transdermal permeation enhancers. J Control Release 104(1):41–49

Vavrova K, Zbytovska J, Hrabalek A (2005b) Amphiphilic transdermal permeation enhancers: structure-activity relationships. Curr Med Chem 12(19):2273–2291

Vavrova K, Hrabalek A, Mac-Mary S, Humbert P, Muret P (2007) Ceramide analogue 14S24 selectively recovers perturbed human skin barrier. Br J Dermatol 157(4):704–712

Vavrova K, Lorencova K, Klimentova J, Novotny J, Holy AN, Hrabalek A (2008a) Transdermal and dermal delivery of adefovir: effects of pH and permeation enhancers. Eur J Pharm Biopharm 69(2):597–604

Vavrova K, Lorencova K, Novotny J, Holy A, Hrabalek A (2008b) Permeation enhancer dodecyl 6-(dimethylamino)hexanoate increases transdermal and topical delivery of adefovir: influence of pH, ion-pairing and skin species. Eur J Pharm Biopharm 70(3):901–907

Vavrova K, Kovarikova P, Skolova B, Libalova M, Roh J, Cap R et al (2011) Enhanced topical and transdermal delivery of antineoplastic and antiviral acyclic nucleoside phosphonate cPr-PMEDAP. Pharm Res 28(12): 3105–3115

Wertz PW (2000) Lipids and barrier function of the skin. Acta Derm Venereol Suppl (Stockh) 208:7–11

Williams AC, Barry BW (2004) Penetration enhancers. Adv Drug Deliv Rev 56(5):603–618

Wolka AM, Rytting JH, Reed BL, Finnin BC (2004) The interaction of the penetration enhancer DDAIP with a phospholipid model membrane. Int J Pharm 271(1–2): 5–10

Wong O, Huntington J, Nishihata T, Rytting JH (1989) New alkyl N, N-dialkyl-substituted amino acetates as transdermal penetration enhancers. Pharm Res 6(4): 286–295

Wong O, Nishiahta T, Rytting JH (1990) Biodegradable absorption enhancers. US 4980378

Peptides as Skin Penetration Enhancers for Low Molecular Weight Drugs and Macromolecules

21

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Contents

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21.1 Introduction

The skin is the largest and most easily accessible organ of the human body for drug delivery (Zaffaroni 1991), and as a route of drug administration, it offers many advantages such as ease of application, patient compliance, absence of first-pass metabolism, and reduced side effects (Scheindlin 2004). For the aforementioned reasons, transdermal drug delivery (TDD) has received a lot of attention and emerged as one of the most successful non-oral controlled delivery option. Despite its success in terms of market demand and wide acceptance by patients, till today only a handful of drugs are marketed as TDD systems. The fundamental reason limiting the transdermal access to many drugs is the impermeable nature of the outermost layer of the skin, stratum corneum (SC), which prevents the permeation of most compounds. Nevertheless, many other drugs and/or drug candidates lie outside the gamut of physicochemical requirements for sufficient skin permeation which will benefit if the SC properties are altered and/or manipulated

N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_21

transiently. A number of physical and chemical skin penetration enhancement approaches have been tested in the past to promote the drug delivery through the skin (Kanikkannan et al. 2000; Barry 2004; Rizwan et al. 2009). For example, chemical enhancers such as alcohols, terpenes, fatty acids, surfactants, pH variations, and physical approaches such as microneedles, tape stripping, ultrasonic waves, and iontophoresis have been used to enhance transdermal drug delivery (Kanikkannan et al. 2000; McAllister et al. 2000; Mitragotri et al. 2000; Barry 2004; Narishetty and Panchagnula 2004, 2005; Nanda et al. 2006; Rizwan et al. 2009). Ideally, skin permeation enhancers are expected to be pharmacologically inert, nontoxic, nonirritating, cost-effective, and must not permanently compromise the barrier function of the skin. However, most of the reported approaches have one or more drawbacks, to list few: irritation potential of chemical enhancers at high concentration, invasiveness of microneedles or tape stripping, destabilization of protein or peptide drugs by organic solvent-based enhancers such as alcohol or DMSO, and high cost and complex production process of the equipment for physical enhancement techniques such as ultrasonic waves and iontophoresis. Recently, peptides are gaining popularity as non-invasive and safe skin penetration enhancers for both small molecular drugs and macromolecules such as proteins or short-interfering ribonucleic acids (siRNAs) (Rothbard et al. 2000; Lopes et al. 2005; Chen et al. 2006; Kim et al. 2007; Desai et al. 2010; Hsu and Mitragotri 2011; Uchida et al. 2011; Cohen-Avrahami et al. 2012; Kumar et al. 2012; Shah et al. 2012). This chapter is focused on the discovery of various classes of peptides as skin permeability enhancers and their mechanism of permeation enhancement.

21.2 Peptides as Skin Penetration Enhancers

Over the last decade, several peptides have been identified and tested for their abilities to enhance the penetration of small/large molecules into and across the skin (Rothbard et al. 2000; Chen et al. 2006; Cohen-Avrahami et al. 2010, 2012; Kumar et al. 2012; Shah et al. 2012). These peptides are called skin penetration enhancement peptides (SPEPs). SPEPs offer number of advantages for TDD; they are safe, cost-effective, noninvasive in application, and thus better compliance from patients. SPEPs can be broadly classified into three major categories based on their origin of discovery:

1. Cell-penetrating peptides (CPPs)
2. Antimicrobial peptides (AMPs)
3. Phage peptides (peptides identified from phage display library technology)

21.3 Cell-Penetrating Peptides (CPPs)

21.3.1 Overview

Cell-penetrating peptides (CPPs), also known as protein transduction domains (PTDs), are short peptides (up to 30 amino acids in length) with an ability to translocate through the plasma membrane of a cell and enter the cytoplasm (Patel et al. 2007; Madani et al. 2011; Baoum et al. 2012). Usually, CPPs are cationic or amphipathic at physiological pH (Lindberg et al. 2011; Madani et al. 2011; Nakase et al. 2012). Although numerous CPPs have been reported, some of the most commonly used CPPs include transactivator of transcription (TAT) peptide, YARA, polyarginine, penetratin, transportan, and Pep-1 (Chauhan et al. 2007; Jones and Sayers 2012). The major advantage of CPPs is that they penetrate the cell membrane at low micromolar concentrations *in vivo* and *in vitro* without causing significant membrane damage or toxicity to the cell (Chauhan et al. 2007; Madani et al. 2011; Jones and Sayers 2012; Nakase et al. 2012). In addition to entering into the cells by themselves, CPPs can also carry associated cargo molecules into the cells with high efficiency (Jones and Sayers 2012). CPPs have been used to deliver several cargo molecules such as proteins, oligonucleotides, solid lipid nanoparticles, and liposomes into the cells (as reviewed in (Chauhan et al. 2007; Patel et al. 2007; Lindberg et al. 2011; Bechara and Sagan 2013). Considering

the similarity in the nature and barrier properties of the cell membrane and the skin's outermost layer, in recent years, CPPs have been tested to enhance the topical/transdermal delivery of the above cargo molecules (Rothbard et al. 2000; Lim et al. 2003; Hou et al. 2007; Shah et al. 2012).

The studies on CPPs as skin penetration enhancers can be broadly classified into two major categories based on their interaction with the cargo molecules: (1) cargos delivered through covalent conjugation with the CPPs and (2) cargos delivered by incubating the skin along with CPPs without any chemical attachment between CPPs and cargos.

21.3.2 Conjugated CPPs

In initial studies, CPPs were tested for their ability to deliver macromolecules such as proteins or peptides into and/or across the skin (Jin et al. 2001; Lim et al. 2003). Topical delivery of peptides and proteins has become an attractive field for both pharmaceutical and cosmetic applications (Ma et al. 2002; Partidos et al. 2002; Gorouhi and Maibach 2009; Sundaram et al. 2009). In addition, delivery of several peptide antigens through the dermis was investigated for the development of needle-free topical vaccines (Schutze-Redelmeier et al. 2004; Itoh and Celis 2005; Frankenburg et al. 2007). However, the delivery of macromolecules either into or across the skin is very challenging due to barrier functions of the skin (Langer 2004). CPPs have been used to deliver various kinds of conjugated proteins or peptides, as small as 3 amino acids long to as large as 527 amino acids long, across the skin (Jin et al. 2001; Lim et al. 2003). CPPs have been linked to therapeutic peptides/proteins either by chemical conjugation or synthesized together as a single peptide chain (for small peptides) or genetically fused and expressed together as a single protein (for large peptides/proteins) (Jin et al. 2001; Zhao et al. 2012). These fusion proteins/peptides have been successfully tested for their delivery into or across the skin after topical application (Jin et al. 2001; Zhao et al. 2012).

The first study to test the ability of a known CPP to cross the SC of the skin was reported by Rothbard et al., in the year 2000 (Rothbard et al. 2000). They have shown that an established CPP, polyarginine (R7), could penetrate through the SC and enter into the epidermis of the mouse skin within 2 h of application (Rothbard et al. 2000). The amount of peptide penetrated and the depth of skin penetration were dependent on the concentration of the peptide and the time of incubation (Rothbard et al. 2000). More importantly, R7 peptide delivered a macromolecule, cyclosporin A (CsA) (Mol. Wt. 1202.61 Da), across the cutaneous barrier of mouse and human skin, when conjugated together (R7-CsA) (Rothbard et al. 2000). Cyclosporine was delivered into the skin to inhibit inflammation in the mouse models of contact dermatitis. After topical application, enough amount of R7–CsA has reached dermal T lymphocytes to inhibit cutaneous inflammation (Rothbard et al. 2000). More importantly, the amount of R7-CsA conjugate delivered into the skin and the free drug released from the conjugate was sufficient enough to elicit therapeutic response in the mouse model of contact dermatitis (Rothbard et al. 2000).

One of the most well-studied and efficient CPP is the amino acid residue 47–57 (YGRKKRRQRRR) of human immunodeficiency virus-1 transactivator of transcription protein (HIV-1 TAT), which is popularly known as the TAT peptide. A conjugate of TAT peptide with a tripeptide glycine-lysine-histidine (GKH) was successfully used to deliver GKH into the deeper layers of the skin for cosmetic purposes (lipolysis). GKH is derived from parathyroid hormone and is involved in lipolysis (Lim et al. 2003). Conjugation with the TAT peptide enhanced the penetration of GKH into excised hairless mice skin by more than 36 times (Lim et al. 2003). In addition to small peptides, CPPs have also delivered large therapeutic proteins, such as catalase with 527 amino acids (a.a.), into the skin after topical application (Jin et al. 2001). In this study, CPPs were associated with large proteins through genetic fusion. The gene fragments coding for several CPPs (TAT; polyarginine, R9; and polylysine; K9) were fused with

the genes of large therapeutic proteins (catalase, 527 a.a.; and CuZn SOD1, 154 a.a.), and the fused gene is expressed to produce a single fusion protein. In this fusion protein, the sequence of CPPs are present at the N-terminal part of the therapeutic protein (Jin et al. 2001). With the presence of CPP sequences at the N-terminal, these fusion proteins have gained the ability to penetrate deep into the viable epidermis and dermis layers of the mouse skin (Jin et al. 2001). A fusion protein with R9 (RRRRRRRRR) at the N-terminus was more efficient in skin penetration than fusion proteins with Tat (RKKRRQRRR) peptide (Jin et al. 2001). Similarly, TAT peptide enhanced the penetration of another protein, epidermal growth factor (53 amino acids), into the epidermis and hair follicles of the porcine skin when associated together as fusion protein (Zhao et al. 2012).

By molecular modification and optimization of TAT peptide, more efficient cell-transducing peptide, YARA (YARAAARQARA) peptide, was discovered (Ho et al. 2001). Lopes et al. have compared the skin penetration ability of the YARA peptide with the TAT peptide and a non-transducing peptide (YKAc) (Lopes et al. 2005). Interestingly, after 4 h of application, the amount of YARA peptide entered into the epidermis and dermis layers of the skin was not significantly different than that of the TAT peptide (Lopes et al. 2005). However, both peptides entered the skin in higher amounts than the nontransducing YKAc peptide (Lopes et al. 2005). In addition to entering into the skin, YARA and TAT peptides, when conjugated, carried a 13-amino-acid-long hydrophilic peptide P20 (derived from heat shock protein 20) into viable layers of the porcine ear skin. However, the transdermal delivery was negligible after 8 h of incubation (Lopes et al. 2005). Although YARA peptide is more efficient than TAT peptide in cell transduction; the efficiency of these two peptides to carry P20 into the skin was comparable (Lopes et al. 2005).

It is very well established that the skin is an attractive target for topical immunization because of the presence of antigen-processing immune cells (Langerhans and dendritic cells) in the epidermis and dermis layers of the skin (Celluzzi and Falo 1997; Banchereau and Steinman 1998; Campton et al. 2000; Seo et al. 2000). Antigens or pathogens entering topically into the viable layers of the skin are engulfed by antigen-presenting cells (APCs) such as Langerhans and dendritic cells, residing locally at the epidermis and dermis. Once receiving the antigen, APCs migrate to the lymph node, where they interact with the T cells and B cells for the generation of immune response against the antigen. However, most of the antigens used for immunization are either small peptides or proteins, which cannot penetrate into the viable layers of the skin after topical application. Schutze-Redelmeier et al. have reported that antennapedia transduction sequence peptide (ANTP) could deliver an eight-amino-acid-long antigenic peptide originated from ovalbumin protein ($OVA_{257–264}$) into the mouse skin after topical application (Schutze-Redelmeier et al. 2004). After topical application, significantly higher amount of ANTP-conjugated $OVA_{257–264}$ peptide was found in the epidermis and dermis layers of the skin as compared to unconjugated $OVA_{257–264}$ peptide (Schutze-Redelmeier et al. 2004). In addition, topical application of ANTP-conjugated $OVA_{257–264}$ peptide also resulted in the generation of significantly stronger immune response as compared to free $OVA_{257–264}$ peptide (Schutze-Redelmeier et al. 2004). The immune response was strong enough, to generate therapeutically effective anti-tumor immunity in mice with the help of proper vaccine adjuvants (Schutze-Redelmeier et al. 2004). This study showed the feasibility of topical delivery of vaccine antigens with conjugated CPPs as penetration enhancers.

Although conjugation of macromolecules with CPPs enhances their penetration into the skin, degradation and rapid clearance of drug molecules from the skin has always been a concern. One way to overcome this challenge is by encapsulating the drug molecules inside a particulate carrier, which not only protects the drug from degradation but also releases the drug in a controlled manner for prolonged period of time (Teeranachaideekul et al. 2008; Desai et al. 2010; Pople and Singh 2010). Although particulate delivery systems offer the above advantages,

irrespective of the material used, rigid particles above 10 nm in diameter do not penetrate the intact skin (Souto and Muller 2008). To address this problem, Singh et al. have conjugated CPPs to nanoparticles and tested their ability to permeate through SC (Patlolla et al. 2010; Shah et al. 2012). Transducing TAT peptide or a non-transducing YKA peptide (YKALRISRKLAK) was conjugated onto the surface of a fluorescent dye (DID oil, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine perchlorate) and encapsulated nano-lipid crystal nanoparticles (FNLCN). Subsequently, their ability to penetrate into viable layers of the skin was tested (Patlolla et al. 2010). After 24 h of incubation, nonconjugated and YKA peptide-conjugated nanoparticles were localized in the appendages of the skin. However, TAT-conjugated nanoparticles penetrated into the deeper layers of the skin such as the epidermis (Patlolla et al. 2010). When a drug molecule, celecoxib (CXb), was encapsulated inside these nanoparticles, not only the drug was delivered into the skin, but also the release of Cxb from the nanoparticles was sustained for prolonged period of time (Patlolla et al. 2010). The presence of more arginines and thus more positive charge on TAT peptide (six arginines) compared to YKA peptide (two arginines) might have played a role in the superior ability of TAT peptide in mediating skin penetration of nanoparticles.

In a follow up study, Singh et al. have compared the ability of CPPs with different number of arginines present in their sequence (polyarginines: R8 (8 arginines), R11 (11 arginines), and R15 (15 arginines) and TAT peptide (6 arginines)) in enhancing the penetration of nanostructured lipid carriers (NLCs) into the skin (Shah et al. 2012). NLCs conjugated with a CPP containing 11 arginine groups (R11) have shown higher amounts of penetration into the skin and also into deeper layers of the skin compared to NLCs conjugated with other peptides tested (Shah et al. 2012). The results have suggested that the ability to enhance the skin penetration was increased when arginine number in the CPP was increased from 6 and 8 to 11 (Shah et al. 2012). However, further increase in arginine number from 11 to 15 had resulted in reduction in the ability to enhance the skin penetration (Shah et al. 2012). It could be due to the saturation effect or steric hindrance caused by long chain length of the arginine peptides (Shah et al. 2012). This study indicates that there may be an optimum positive charge, which is required to enhance the interaction of the CPPs with the skin (Shah et al. 2012). Similarly, a polyarginine with nine arginines (R9) has been shown to be more efficient in enhancing the penetration of sodium diclofenac-containing reversed hexagonal liquid crystals ($H_{II}LC$) when conjugated on the surface in comparison to RALA peptide containing five arginines (RALARALARALRALAR) on the surface (Cohen-Avrahami et al. 2010, 2012). The ability to enhance the skin penetration of $H_{II}LC$ by R9 was lost, when R9 was present inside the $H_{II}LC$ instead of conjugated onto the surface. Therefore, it is important for CPPs to be on the surface for proper interaction with the skin.

Overall, these studies suggest that CPP conjugation to the cargo molecules (macromolecules and nanoparticles) could enhance their skin penetration.

21.3.3 Nonconjugated CPPs

The previous section has emphasized the role of CPPs in delivering covalently attached therapeutic moieties or nanoparticles into the skin. However, covalent attachment of CPPs to cargo molecules may pose several challenges, such as high cost and extra time for synthesis and purification, loss of activity after conjugation, and challenges associated with purification and stability. CPPs such as Pep-1, MPG, and Pep-2 are reported to deliver cargos, such as peptides, proteins, and antibodies into different cells both *in vitro* and *in vivo*, without conjugation to cargos (Morris et al. 1997, 2001, 2007; Simeoni et al. 2003, 2005; Crombez et al. 2007). Similar to these intracellular delivery studies, Wang et al. have shown that arginine-rich peptides (R9, TAT) could promote the entry of the green fluorescent protein (GFP) into the deeper layers of the skin when incubated together on the mouse skin (*in vivo*). After 20 min of incubating skin with CPPs

and GFP, strong green fluorescence was observed among the epidermis, dermis, panniculus adiposus, and even hypodermis (Wang et al. 2006). Interestingly, when incubated together (co-incubated), R9 peptide resulted in penetration of GFP into the skin within 1 min of incubation and reached maximum after 20 min (Wang et al. 2006). Prolonged incubation with the R9 peptide resulted in further enhancement in skin penetration of GFP. Similarly, TAT peptide has also been shown to enhance the penetration of GFP across the rat skin without conjugation (Lohcharoenkal et al. 2011).

In another study, Lopes et al. have tested the abilities of YARA and WLR (WLRRIKAWLRRIKAWLRRIKA) peptides as skin penetration enhancers without conjugating them to the cargo molecule (Lopes et al. 2008). The skin penetration of P20 peptide alone was increased by two- to threefold when incubated together with YARA and WLR peptides, respectively (Lopes et al. 2008). The WLR peptide was more efficient (2.8-fold) in enhancing the penetration of P20 into the epidermis and dermis layers of the skin as compared to YARA peptide (2.3-fold) (Lopes et al. 2008). This result could be attributed to the higher positive charge of the WLR peptide (six arginines) as compared to YARA peptide (three arginines) (Lopes et al. 2008). Interestingly, the ability of YARA peptide to enhance the skin penetration of P20 peptide was significantly higher when these two peptides were covalently conjugated (5–7 times) in comparison to when they were incubated together as independent peptides (co-incubation) (2.3 times). However, co-incubation offers several advantages as mentioned earlier in this section.

The skin penetration of sodium diclofenac (Na-DFC) embedded in reversed hexagonal mesophases was enhanced when co-incubated with CPPs such as RALA peptide (RALARALARALRALAR) and penetratin (RQIKIWFQNRRMKWKK) (Cohen-Avrahami et al. 2010, 2012). Both the peptides enhanced the skin permeability coefficient of Na-DFC without changing its lag time (Cohen-Avrahami et al. 2010, 2012). Hexagonal mesophase serves as an excellent gel matrix for drug solubilization (Cohen-Avrahami et al. 2010, 2012). ATR-FTIR analysis suggests that penetratin accelerated the structural transition of skin lipids from hexagonal to liquid and thus enhanced the diffusion rate of Na-DFC through the SC (Cohen-Avrahami et al. 2012).

In addition to small molecular drugs, proteins, and peptides, CPPs have also been shown to enhance the delivery of siRNAs into the skin after topical application. Topical delivery of siRNAs has great therapeutic significance for treating various skin diseases, transcutaneous vaccination, and improving skin properties (Partidos et al. 2003; Lopes et al. 2005). However, transdermal delivery of siRNA is also limited due to its low permeability through various skin barriers such as the SC (multiple lipid bilayers) and viable epidermal layers (paracellular connection by tight junctions) (Guan et al. 2008; Uchida et al. 2011). Uchida et al. in 2011 have successfully delivered siRNAs deeper into the skin layers (viable epidermis) and hair follicles by incubating together with TAT peptide and tight junction-modulating peptide (AT1002, amino acid sequence: FCIGRL), to alter SC and tight junction barriers, respectively. In contrast, siRNAs were localized only to hair follicles when given with TAT peptide alone (Uchida et al. 2011).

In summary, CPPs have applications in noninvasive topical skin delivery of drugs and nanoparticles. The CPP-mediated skin delivery of cargo molecules (drugs, proteins, peptides, siRNAs, and nanoparticles) depends on the nature of CPP, conjugation method (covalent or noncovalent type of attachment), and characteristics of the cargo protein.

21.4 Antimicrobial Peptides (AMPs)

21.4.1 Overview

Antimicrobial peptides (AMPs), which are involved in host innate immunity, share many structural similarities with CPPs (Splith and Neundorf 2011). AMPs are short peptides, usually less than 60 amino acids, and consists of

blocks of cationic (+4 to +6 charge) and hydrophobic amino acids that are spatially separated. This provides amphipathic properties to the AMPs (Hancock 1997; Powers and Hancock 2003). Cationic charge and amphipathic arrangement are the basis of their interaction with anionic lipid bilayers of the microbial cellular membranes (Splith and Neundorf 2011). Considering the presence of anionic lipid bilayers in SC of the human skin, AMPs have been hypothesized to interact with the skin lipids and thereby alter the skin permeability to drugs.

21.4.2 Magainin

Magainin is the most studied AMP for altering the permeability of the skin to various drugs (Kim et al. 2007, 2008a, b, 2010). Magainin is a 23-amino-acid-long AMP (GIGKFLHSAKKFGKAFVGEIMNS) isolated from the skin of the African frog (*Xenopus laevis*) (Zasloff 1987). Magainin is known to form pores in the bacterial cell membranes and hence also called as pore-forming peptide (Matsuzaki et al. 1994; Matsuzaki 1998). It has a net +4 charge and binds to negatively charged phospholipid membranes with the aid of electrostatic interactions and permeabilizes the lipid bilayers (Matsuzaki et al. 1997; Kim et al. 2008a). Considering the ability of magainin to interact with the lipid membranes, its potential utility as a skin penetration enhancer was evaluated by Prausnitz et al. group (Kim et al. 2007, 2010).

21.4.3 Magainin as Skin Penetration Enhancer

Magainin increases the membrane permeability in bacteria by creating pores in the lipid membrane, and this often leads to cell lysis (Matsuzaki et al. 1994; Matsuzaki 1998). Kim et al. have shown that magainin could also disrupt the monolayer of synthetic vesicles made up of lipids with similar biophysical characteristics to the lipids present in the human SC (Kaushik et al. 2001). However, treating the skin with magainin alone could not enhance the penetration of a small molecule, fluorescein (323 Da), into the skin (Kim et al. 2007). Interestingly, the addition of magainin to other skin penetration enhancers (N-lauroyl sarcosine (NLS) in Ethanolic solution) significantly improved their ability to enhance the penetration of fluorescein into the skin (Kim et al. 2007). This may be due to the fact that SC consists of multiple lipid bilayers in comparison to monolayered lipid vesicles tested in the previous study (Kim et al. 2007). To disrupt multiple lipid bilayers, magainin may require the help of other chemical skin penetration enhancers (Kim et al. 2007). This theory was further supported by the observation that magainin alone (rhodamine labeled magainin) showed negligible penetration into the SC; however, when incubated together with NLS and ethanolic solution, it could penetrate into deeper layers of SC (10–15 μm) (Kim et al. 2007). Magainin was proposed to form angstrom (A^0)-scale pores in the lipid layers of the skin (Kim et al. 2008a). Angstrom-scale pores are known to be large enough only to transport small molecules. In consistent with this hypothesis, magainin enhanced the transport of only small molecules such as fluorescein (323 Da), which has radius of approximately $5A^0$ (Prausnitz and Noonan 1998), but not the penetration of larger molecules such as calcein (623 DA, radius = $6A^0$) or FITC-dextran (3000 Da, radius = $16A^0$) (Oliver et al. 1992; Edwards et al. 1995).

In further studies, interaction of magainin with either skin lipids or drug molecules was optimized to increase its skin penetration enhancement ability. Increasing the concentration of magainin (up to 1 mM) or its time of incubation with the skin (up to 12 h) enhanced the penetration of fluorescein molecules into the skin (Kim et al. 2008b). In addition to interaction of magainin with negatively charged skin lipids, its interaction with the drug molecule also seems to be important factor for its ability to increase skin penetration of drugs. Magainin acquires positive, neutral, and negative charges at pH 7.4, 10, and 11, respectively (Kim et al. 2008a). At pH 7.4, positively charged magainin electrostatically interacts with negatively charged fluorescein molecules and skin lipids. However, at pH 11, these interactions are

disturbed (Kim et al. 2008a). The observation that magainin enhanced the penetration of fluorescein at pH 7.4 but failed at pH 11.0 suggests that the electrostatic interaction of magainin with drug molecule and/or skin lipids may be vital for its skin penetration enhancement abilities (Kim et al. 2008a). The importance of magainin interaction with the drug molecule for its skin penetration ability is confirmed by the observation that magainin could not enhance the penetration of positively charged molecule (granisetron) into the skin, even at pH 7.4.

The role of the electrostatic interactions of magainin in skin penetration enhancement was further confirmed by the following two studies. Neutralizing the charge of magainin by incubating it with increasing salt concentration or replacement of positively (+4) charged magainin with negatively charged anti-magainin (−4) peptide decreased the magainin's ability to enhance the skin penetration of negatively charged fluorescein molecule (Kim et al. 2008a, 2010). Although several modifications of the magainin such as increase in positive charge or hydrophobicity, or change of certain amino acids, etc., have been shown to improve its antimicrobial activity, these modifications could not further enhance its ability to increase skin permeability (Kim et al. 2010). These studies clearly suggest that in addition to the net charge on the magainin, the appropriate amino acid composition is also critical for its ability to change skin permeability of certain drugs (Kim et al. 2010).

In summary, magainin peptide could enhance the skin penetration of small molecules when used in combination with other chemical skin penetration enhancers. The amino acid composition of the magainin and its interaction with the charged drug molecules are crucial for its effect on skin penetration.

21.5 Phage Peptides

21.5.1 Overview

Several peptides such as CPPs and AMPs were shown to act as skin penetration enhancers (Lopes et al. 2005; Wang et al. 2006; Kim et al. 2007, 2008b). But majority of these peptides were large in size (12–23 amino acids) and hence costly and difficult to synthesize. Moreover, some of them, such as magainin, may need the help of other chemical skin penetration enhancers (Kim et al. 2007) to enhance the permeability of the skin. Therefore, it was of great significance to find smaller peptides (five to six amino acids), which could enhance the skin penetration. Phage display technology enabled us to identify such smaller peptides (Chen et al. 2006; Hsu and Mitragotri 2011; Kumar et al. 2012).

21.5.2 Phage Display Technology

Bacteriophages are bacterial viruses, which usually cannot penetrate through biological barriers, such as the skin (Haq et al. 2012). Phage display technology is based on screening a library of bacteriophages (billions of them), where each bacteriophage expresses unique random peptide on its surface, to identify small peptide sequences that could carry bacteriophages across the biological barriers such as the skin (Haq et al. 2012). This technique has also been used successfully to identify peptides that could cross other biological barriers such as the blood-brain barrier and intestine (Duerr et al. 2004; Chen et al. 2006; Wan et al. 2009).

21.5.3 SPEPS Identified by Phage Display Peptide Library Screening

To identify skin penetration-enhancing peptides, the screening of phage display peptide libraries was performed both *in vivo* and *in vitro* on mouse and pig skins (Chen et al. 2006; Hsu and Mitragotri 2011; Kumar et al. 2012). In these screenings, phage particles were applied onto the skin and were allowed to penetrate into and across the skin. Phages that have penetrated were collected, amplified, and again applied onto the skin (Chen et al. 2006; Hsu and Mitragotri 2011; Kumar et al. 2012). After few rounds of screening,

the sequence of the peptide present on the surface of the bacteriophages that were consistently penetrating into or through the skin was identified by genomic DNA sequencing of the penetrated phages. The identified peptides were synthesized and further investigated as skin penetration enhancers.

By screening cyclic peptide (C7C) displayed phage library by using mouse skin (*in vivo*), Chen et al. have identified the TD-1 peptide (ACSSSPSKHCG), which not only enhanced the penetration of phages across mouse skin when expressed on a phage surface coat protein but also significantly increased the permeation of insulin through the skin when incubated together with insulin (Chen et al. 2006). Most importantly, insulin delivered with the help of TD-1 peptide resulted in significant reduction of serum glucose levels in rats (Chen et al. 2006). The effect of TD-1 peptide on the delivery of insulin was dependent on the amount of the peptide applied (Chen et al. 2006). In other studies, TD-1 peptide was also shown to deliver other macromolecules such as human growth hormone (hGH) and siRNAs into the skin (Zhang et al. 2010; Lin et al. 2012). When TD-1 peptide was incubated together with siRNAs, it enhanced the penetration of topically applied siRNAs into the epidermis and subcutaneous tissues of the skin and resulted in efficient downregulation of the target protein expression (GAPDH). In contrast, scrambled peptide failed to enhance the penetration of siRNAs (Lin et al. 2012). Although TD-1 peptide is not expected to deliver 100 % of the applied siRNAs, the functional efficiency of siRNAs delivered topically using TD-1 peptide was comparable to the efficiency of siRNAs delivered by intradermal injection (Lin et al. 2012) in inhibiting the target protein expression. This may be due to the large surface area of the skin that was exposed to siRNAs after topical application as compared to restricted area exposed by intradermal injection (Lin et al. 2012). Skin penetration enhancement mediated by TD-1 peptide is highly sequence specific. Even a single amino acid substitution in the TD-1 peptide resulted in significant loss in its ability to enhance the skin penetration (Chen et al. 2006).

TD-1 peptide was discovered by using mouse system. It enhanced the skin penetration of insulin mainly through hair follicles (Chen et al. 2006). Although the discovery of TD-1 peptide as a skin penetration enhancer was an exciting advancement, its use in humans is limited because of the fact that the hair follicle density in mice or rats is several times higher than in humans and also mouse skin is more permeable than human skin (Priborsky and Muhlbachova 1990; Ghosh et al. 2000; Prausnitz 2006). Therefore, mouse/rat skin may not be a clinically relevant model for human TDD application.

To address this issue, in later studies, screening of PDLs was performed using porcine skin. This is because porcine skin is more close to human skin in terms of both lipid composition and hair follicular density (Dick and Scott 1992; Hammond et al. 2000). By screening PDL using porcine skin, Hsu et al. have identified SPACE (skin permeating and cell entering) peptide (ACTGSTQHQCG), which could cross human, porcine, as well as mouse skin (Hsu and Mitragotri 2011). Importantly, SPACE peptide delivered macromolecular cargos across porcine SC (Hsu and Mitragotri 2011). For example, when conjugated to SPACE peptide, streptavidin (159 amino acid protein) penetrated into the epidermis and dermis of porcine skin (Hsu and Mitragotri 2011). SPACE peptide-mediated skin penetration enhancement of cargo molecules was size dependent. Increase in cargo size affected skin penetration enhancement effect of SPACE peptide (Hsu and Mitragotri 2011). Interestingly, SPACE peptide could also cross the cellular membranes, similar to CPPs. SPACE peptide could penetrate into different cell lines such as keratinocytes, fibroblasts, endothelial cells (HUVECs), and breast cancer cells (MDA-MB-231) (Hsu and Mitragotri 2011). Considering the ability of SPACE peptide to cross the skin and cellular barriers, it has been used for topical delivery of siRNAs. SPACE peptide conjugated to siRNAs enhanced the delivery of siRNAs into the skin (Hsu and Mitragotri 2011). Importantly, these conjugated siRNAs enter the cell and are efficient in downregulating the target protein expression (GFP in endothelial

cells and GAPDH and IL-10 in mice skin) (Hsu and Mitragotri 2011). The efficiency of SPACE peptide-mediated siRNA delivery was dependent on the amount of the peptide applied and the time of incubation on the mice skin. Higher dose of the peptide and longer application time resulted in higher knockdown of the target genes (Hsu and Mitragotri 2011).

SPACE peptide was found primarily effective in enhancing skin penetration of only conjugated molecules (Hsu and Mitragotri 2011). Considering the limitation associated with the conjugation technique, Kumar et al. have identified a novel peptide (T2 peptide: LVGVFH) that enhanced the penetration of small molecules across the porcine skin without the need of conjugation (Kumar et al. 2012). T2 peptide is a linear peptide unlike TD-1 or SPACE peptides. T2 peptide enhanced the penetration of bacteriophages across porcine and mouse skin (Kumar et al. 2012). Pretreatment of the skin with synthetic T2 peptide at pH 4.5 resulted in significant enhancement in the permeability of the skin to several small molecules with different lipophilicities, such as fluorescein isothiocyanate (hydrophobic), rhodamine-123 hydrochloride (hydrophilic), and 5-fluorouracil (5-FU) (hydrophilic) across the skin (Kumar et al. 2012). The major challenge in the skin penetration of hydrophilic drugs such as 5-FU is their partitioning into the lipid layers of SC (Kumar et al. 2012). T2 peptide enhanced the partitioning of both hydrophilic (5-FU and rhodamine 123 hydrochloride) and hydrophobic (fluorescein isothiocyanate) molecules into the lipid layers of SC by altering the SC lipid structures (Kumar et al. 2012). Pretreating the skin with T2 peptide increased the steady-state flux (J_{ss}), permeability coefficient (K_p), and cumulative amount (Q_{24}) of 5-FU crossing the skin by three- to fourfold in comparison to untreated skin (Kumar et al. 2012).

In summary, PDL technology has been successfully employed to discover small peptides which enhanced the delivery of small and macromolecules into and across the skin without the need of any other penetration enhancers.

21.6 Mechanism of Peptides as Skin Penetration Enhancers

Introduced just a decade ago, SPEPs are still evolving as a new class of skin penetration enhancers. Although many studies have been reported on SPEPs, the common mechanism by which these peptides enhance the skin penetration is yet to be elucidated. More than one mechanism may be involved for the enhancement of skin penetration by SPEPs (Chen et al. 2006; Kim et al. 2007; Hsu and Mitragotri 2011; Kumar et al. 2012). In general, drugs may penetrate through the skin via intracellular pathway, intercellular lipid pathway, intercellular pathway through appendages, and/or through hair follicles (Prausnitz et al. 2004). SPEPs have been shown to act on one or more of these pathways to enhance skin penetration.

TD-1 peptide, identified via PDL screening using mouse skin, penetrates and delivers associated cargos (insulin) into the hair follicles of the mouse/rat skin (Chen et al. 2006), and insulin diffuses from there into deeper layers of the skin (Chen et al. 2006). Similarly, polyarginine (a CPP) delivered the conjugated drug-loaded nanoparticles into the hair follicles, and the drug released from these nanoparticles diffuses into the different layers of the skin or across the skin (Shah et al. 2012). Although skin penetration via hair follicles delivers the cargo molecules into the skin, it may not be relevant to human application because of the variability in the hair follicle density in different species or between individuals. However, this may be advantageous in delivering therapeutic molecules specifically targeted to hair follicles.

Permeation of molecules through intercellular lipids (lipid bilayer) represents the classical mechanism for transdermal delivery (Hsu and Mitragotri 2011). Some SPEPs such as penetratin, magainin, and T2-peptide have been reported to alter the lipid bilayer structure of the skin (Kim et al. 2007; Cohen-Avrahami et al.

2012; Kumar et al. 2012), and this was proposed as a possible mechanism for their ability to enhance the skin penetration. Alteration in lipid acyl chain, after treatment with these peptides, was indicated either by shift in lipid peaks of SC to higher wave numbers (C-H stretching absorbance) or by increase in the height and area of these lipid peaks (Kim et al. 2007; Cohen-Avrahami et al. 2012; Kumar et al. 2012), observed via Fourier transform infrared (FTIR) spectroscopy. This alteration suggests a change in the molecular conformation of the skin lipids, which may cause fluidization of the lipid bilayers and thus results in the enhancement of skin permeability (Cornwell et al. 1994; Kim et al. 2007; Cohen-Avrahami et al. 2012; Kumar et al. 2012). Instead of interacting with SC lipids, some SPEPs are shown to interact with the proteins present in the SC. SPACE peptide interacts with keratin, a corneocyte protein, to enhance the skin permeability (Hsu and Mitragotri 2011). Similarly, magainin was shown to alter the secondary structures of the proteins present in SC from α-helix to β-sheet structure (Kim et al. 2007). Magainin alters the structural arrangement of both lipids and proteins present in the SC to enhance skin penetration (Kim et al. 2007).

Most of the SPEPs discovered so far are positively charged at pHs that they were successfully used (such as magainin, TAT, Pep-1, YARA, etc.) This suggests that the cationic nature of the SPEPs (Table 21.1) may play a very important role in their interaction with the anionic lipids of the SC or in their skin penetration enhancement ability (Kim et al. 2007, 2008b, 2010; Kumar et al. 2012). For example, a peptide with 11 arginines enhances the skin penetration more efficiently than peptides with six or eight arginines. However, additional increase in arginines to 15 did not result in further increase in skin penetration enhancement (Jin et al. 2001; Lopes et al. 2008). Similarly, increase in charge of the magainin peptide from +4 to +5 did not result in further skin penetration enhancement (Kim et al. 2010). The above studies suggest that an optimum positive charge may be necessary for these peptides to function as skin penetration enhancers.

In addition to the charge on the peptide, many other physicochemical properties of the SPEPs may also play a critical role in their function. For example, even a single amino acid substitution in TD-1 peptide eliminates its skin penetration enhancement ability (Chen et al. 2006). In case of T-2 peptide, it only enhances the skin permeability at pH 4.5, but not at pH 7.4. It was proposed that the histidine present at the C-terminal part of the T-2 peptide acquires a positive charge at pH 4.5, and this charge may be necessary for its interaction with the skin lipids. When histidine in T2 peptide was replaced with alanine (H6A peptide), the peptide lost its ability to enhance the skin penetration. This suggests that the histidine present at the C-terminal part of the T-2 peptide is critical for its function. Interestingly, SPEPs identified by multiple random PDL screenings also have histidines at the C-terminal part of the peptide. This suggests that the amino acid histidine may be involved in the interaction with the anionic lipids of the SC. The total or local charge on the peptide, the secondary structure of the peptide, the rigidity of the peptide, and the availability of particular amino acids for interaction with the skin vary when the SPEPs are conjugated or nonconjugated to the cargo molecules. The above properties of the SPEPs may also alter depending on the type of cargo molecule and the linker used during conjugation and transport. Therefore, more studies are warranted to further understand on how the physicochemical properties of the peptides influence their interaction with the skin.

Overall, the interaction of SPEPs with the skin and their function as skin penetration enhancers depend on multiple factors such as the physicochemical properties of the peptide, type of the skin (mice, porcine, or human), buffer conditions, time of the treatment (pretreatment vs. co-treatment), type of cargo molecules, and the type of interaction with the cargo molecules (conjugated vs. nonconjugated).

Table 21.1 List and sequence of various skin penetration-enhancing peptides studied in the literature

S.No.	Name	Sequence	Category	Application	Conjugation	Species	Reference
1	Arginine-rich intracellular delivery (AID) peptides (R9)	RRRRRRRRR	CPP	*In vitro*	Nonconjugated	Mouse	Wang et al. (2006)
2	Antennapedia transduction sequence (ANTP)	RQIKIWFQNRRMKWKK	CPP	*In vivo*	Conjugated	Mouse	Schutze-Redelmeier et al. (2004)
3	Arginine oligomers (R7)	RRRRRRR	CPP	*In vivo*	Conjugated	Mouse and human	Rothbard et al. (2000)
4	Polyarginines	R8: RRRRRRRR R11: RRRRRRRRRRR R15: RRRRRRRRRRRRRRR	CPP	*In vitro*	Conjugated	Rats	Shah et al. (2012)
5	Penetratin	RQIKIWFQNRRMKWKK	CPP	*In vitro*	Nonconjugated	Porcine	Cohen-Avrahami et al. (2012)
6	RALA	RALARALARALRALAR	CPP	*In vitro*	Nonconjugated	Porcine	Cohen-Avrahami et al. (2010)
7	Protein transduction domain (PTD)	YARA: YARAAARQARA TAT: YGRKKRRQRRR	CPP	*In vitro*	Conjugated	Porcine	Lopes et al. (2005)
8	Protein transduction domain (PTD)	YARA: YARAAARQARA WLR: WLRRIKAWLRRIKAWLRRIKA	CPP	*In vitro*	Nonconjugated	Porcine	Lopes et al. (2008)
9	Tight junction modulator	AT1002: FCIGRL		*In vivo*	Nonconjugated	Mice	Uchida et al. (2011)
10	YKA and TAT peptides	YKA: YKALRISRKLAK TAT: YGRKKRRQRRR	CPP	*In vitro*	Conjugated	Rats	Patlolla et al. (2010)
11	Magainin	GIGKFLHSAKKFGKAFVGEIMNS	AMP	*In vitro*	Nonconjugated	Porcine	Kim et al. (2007)
12	TD-1	ACSSSPSKHCG	Phage peptides	*In vivo*	Nonconjugated	Mouse/rat	Chen et al. (2006)
13	TD-1	ACSSSPSKHCG	Phage peptides	*In vivo*	Nonconjugated	Porcine	Zhang et al. (2010)
14	TD-1	ACSSSPSKHCG	Phage peptides	*In vivo*	Nonconjugated	Rat	Lin et al. (2012)

15	SPACE	ACTGSTQHQCG	Phage peptides	*In vitro*	Conjugated	Porcine/mouse/ human	Hsu and Mitragotri (2011)
16	T2	LVGVFH	Phage peptides	*In vitro*	Nonconjugated	Porcine	Kumar et al. (2012)

Abbreviations: *CPP* cell-penetrating peptide, *SC* stratum corneum, *TAT* transactivator of transcription, *R9* nine arginine amino acid residues (RRRRRRRRR), *R7* seven arginine amino acid residues (RRRRRRR), *K9* nine lysine amino acid residues (KKKKKKKKK), *siRNA* short-interfering ribonucleic acids, *OVA* ovalbumin, *CsA* cyclosporin A, *GKH* glycine-lysine-histidine, *CuZn SOD1* copper-zinc superoxide dismutase-1, *ANTP* antennapedia transduction sequence, *APCs* antigen-presenting cells, *FNLCN* fluorescent dye encapsulated nano-lipid crystal nanoparticles, *Cxb* celecoxib, *NLCs* nanostructured lipid carriers, *($H_{II}LC$)* hexagonal lyotropic liquid crystals, *Na-DFC* sodium diclofenac, *ATR-FTIR* attenuated total reflectance Fourier transform infrared spectroscopy, *IL-10* interleukin 10, *GFP* green fluorescent protein, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase

Amino acid abbreviations: *A* alanine, *P* proline, *Q* glutamine, *C* cysteine, *R* arginine, *D* aspartate, *S* serine, *E* glutamate, *T* threonine, *F* phenylalanine, *G* glycine, *V* valine, *H* histidine, *W* tryptophan, *I* isoleucine, *Y* tyrosine, *K* lysine, *L* leucine, *M* methionine

Conclusion

Transdermal drug delivery offers several advantages and has great clinical implications both in pharmaceuticals and cosmetics. Delivering active drugs through the skin is a challenging aspect. Further, drug delivery through innovative methods has been a great strategy to enhance the efficiency of both existing and new drugs. Recently, peptides have been explored to enhance the delivery of active molecules into and/or across the skin without causing any significant skin damage. Especially, these peptides (SPEPs) have been used to deliver larger cargos such as proteins, siRNAs, or nanoparticles into the skin. Although this will open a new avenue for successful percutaneous/transdermal delivery of small and macromolecules, the field is still at very early stage. More studies are needed to understand the interaction of these peptides with the skin and its short-term and long-term implications. And, there is also a need to develop more of small SPEPs (five to ten amino acids), which offer several advantages compared to large peptides, such as more cost-effective due to smaller in size, easy to synthesize, and less likely to develop an immune response against the peptide. With more efficient SPEPs at disposal and with the understanding of the molecular mechanism for their penetration enhancement, SPEPs offer an exciting future for dermal/transdermal delivery of drug molecules and dermal delivery of cosmetics.

References

Banchereau J, Steinman RM (1998) Dendritic cells and the control of immunity. Nature 392(6673):245–252

Baoum A, Ovcharenko D et al (2012) Calcium condensed cell penetrating peptide complexes offer highly efficient, low toxicity gene silencing. Int J Pharm 427(1): 134–142

Barry BW (2004) Breaching the skin's barrier to drugs. Nat Biotechnol 22(2):165–167

Bechara C, Sagan S (2013) Cell-penetrating peptides: 20 years later, where do we stand? FEBS Lett 587(12): 1693–1702

Campton K, Ding W et al (2000) Tumor antigen presentation by dermal antigen-presenting cells. J Invest Dermatol 115(1):57–61

Celluzzi CM, Falo LD Jr (1997) Epidermal dendritic cells induce potent antigen-specific CTL-mediated immunity. J Invest Dermatol 108(5):716–720

Chauhan A, Tikoo A et al (2007) The taming of the cell penetrating domain of the HIV Tat: myths and realities. J Control Release 117(2):148–162

Chen Y, Shen Y et al (2006) Transdermal protein delivery by a coadministered peptide identified via phage display. Nat Biotechnol 24(4):455–460

Cohen-Avrahami M, Aserin A et al (2010) H-II mesophase and peptide cell-penetrating enhancers for improved transdermal delivery of sodium diclofenac. Colloids Surf B Biointerfaces 77(2):131–138

Cohen-Avrahami M, Libster D et al (2012) Penetratin-induced transdermal delivery from H-II mesophases of sodium diclofenac. J Control Release 159(3):419–428

Cornwell PA, Barry BW et al (1994) Wide-angle X-ray diffraction of human stratum corneum: effects of hydration and terpene enhancer treatment. J Pharm Pharmacol 46(12):938–950

Crombez L, Charnet A et al (2007) A non-covalent peptide-based strategy for siRNA delivery. Biochem Soc Trans 35(Pt 1):44–46

Desai P, Patlolla RR et al (2010) Interaction of nanoparticles and cell-penetrating peptides with skin for transdermal drug delivery. Mol Membr Biol 27(7):247–259

Dick IP, Scott RC (1992) Pig ear skin as an in-vitro model for human skin permeability. J Pharm Pharmacol 44(8):640–645

Duerr DM, White SJ et al (2004) Identification of peptide sequences that induce the transport of phage across the gastrointestinal mucosal barrier. J Virol Methods 116(2):177

Edwards DA, Prausnitz MR et al (1995) Analysis of enhanced transdermal transport by skin electroporation. J Control Release 34(3):211–221

Frankenburg S, Grinberg I et al (2007) Immunological activation following transcutaneous delivery of HR-gp100 protein. Vaccine 25(23):4564–4570

Ghosh B, Reddy LH et al (2000) Comparison of skin permeability of drugs in mice and human cadaver skin. Indian J Exp Biol 38(1):42–45

Gorouhi F, Maibach HI (2009) Role of topical peptides in preventing or treating aged skin. Int J Cosmet Sci 31(5):327–345

Guan CP, Zhou MN et al (2008) The skin: an indispensable barrier. Exp Dermatol 17(12):1059–1062

Hammond SA, Tsonis C et al (2000) Transcutaneous immunization of domestic animals: opportunities and challenges. Adv Drug Deliv Rev 43(1):45–55

Hancock RE (1997) Peptide antibiotics. Lancet 349(9049): 418–422

Haq IU, Chaudhry WN et al (2012) Bacteriophages and their implications on future biotechnology: a review. Virol J 9:9

Ho A, Schwarze SR et al (2001) Synthetic protein transduction domains: enhanced transduction potential *in vitro* and *in vivo*. Cancer Res 61(2):474–477

Hou YW, Chan MH et al (2007) Transdermal delivery of proteins mediated by non-covalently associated arginine-rich intracellular delivery peptides. Exp Dermatol 16(12):999–1006

Hsu T, Mitragotri S (2011) Delivery of siRNA and other macromolecules into skin and cells using a peptide enhancer. Proc Natl Acad Sci U S A 108(38):15816–15821

Itoh T, Celis E (2005) Transcutaneous immunization with cytotoxic T-cell peptide epitopes provides effective antitumor immunity in mice. J Immunother 28(5):430–437

Jin LH, Bahn JH et al (2001) Transduction of human catalase mediated by an HIV-1 TAT protein basic domain and arginine-rich peptides into mammalian cells. Free Radic Biol Med 31(11):1509–1519

Jones AT, Sayers EJ (2012) Cell entry of cell penetrating peptides: tales of tails wagging dogs. J Control Release 161(2):582–591

Kanikkannan N, Kandimalla K et al (2000) Structure-activity relationship of chemical penetration enhancers in transdermal drug delivery. Curr Med Chem 7(6):593–608

Kaushik S, Krishnan A et al (2001) Magainin-mediated disruption of stratum corneum lipid vesicles. Pharm Res 18(6):894–896

Kim YC, Ludovice PJ et al (2007) Transdermal delivery enhanced by magainin pore-forming peptide. J Control Release 122(3):375–383

Kim YC, Late S et al (2008a) Biochemical enhancement of transdermal delivery with magainin peptide: modification of electrostatic interactions by changing pH. Int J Pharm 362(1–2):20–28

Kim YC, Ludovice PJ et al (2008b) Optimization of transdermal delivery using magainin pore-forming peptide. J Phys Chem Solid 69(5–6):1560–1563

Kim YC, Ludovice PJ et al (2010) Transdermal delivery enhanced by antimicrobial peptides. J Biomed Nanotechnol 6(5):612–620

Kumar S, Sahdev P et al (2012) Identification of a novel skin penetration enhancement Peptide by phage display Peptide library screening. Mol Pharm 9(5):1320–1330

Langer R (2004) Transdermal drug delivery: past progress, current status, and future prospects. Adv Drug Deliv Rev 56(5):557–558

Lim JM, Chang MY et al (2003) Penetration enhancement in mouse skin and lipolysis in adipocytes by TAT-GKH, a new cosmetic ingredient. J Cosmet Sci 54(5): 483–491

Lin CM, Huang K et al (2012) A simple, noninvasive and efficient method for transdermal delivery of siRNA. Arch Dermatol Res 304(2):139–144

Lindberg S, Copolovici DM et al (2011) Therapeutic delivery opportunities, obstacles and applications for cell-penetrating peptides. Ther Deliv 2(1):71–82

Lohcharoenkal W, Manosaroi A et al (2011) Potent enhancement of GFP uptake into HT-29 cells and rat skin permeation by coincubation with tat peptide. J Pharm Sci 100(11):4766–4773

Lopes LB, Brophy CM et al (2005) Comparative study of the skin penetration of protein transduction domains and a conjugated peptide. Pharm Res 22(5):750–757

Lopes LB, Furnish E et al (2008) Enhanced skin penetration of P20 phosphopeptide using protein transduction domains. Eur J Pharm Biopharm 68(2):441–445

Ma Y, Zhao H et al (2002) Topical treatment with growth factors for tympanic membrane perforations: progress towards clinical application. Acta Otolaryngol 122(6): 586–599

Madani F, Lindberg S et al (2011) Mechanisms of cellular uptake of cell-penetrating peptides. J Biophys 2011 :414729

Matsuzaki K (1998) Magainins as paradigm for the mode of action of pore forming polypeptides. Biochimica Et Biophysica Acta (Reviews on Biomembranes) 1376(3):391–400

Matsuzaki K, Murase O et al (1994) Orientational and aggregational states of magainin 2 in phospholipid bilayers. Biochemistry 33(11):3342–3349

Matsuzaki K, Nakamura A et al (1997) Modulation of magainin 2-lipid bilayer interactions by peptide charge. Biochemistry 36(8):2104–2111

McAllister DV, Allen MG et al (2000) Microfabricated microneedles for gene and drug delivery. Annu Rev Biomed Eng 2:289–313

Mitragotri S, Farrell J et al (2000) Determination of threshold energy dose for ultrasound-induced transdermal drug transport. J Control Release 63(1–2): 41–52

Morris MC, Vidal P et al (1997) A new peptide vector for efficient delivery of oligonucleotides into mammalian cells. Nucleic Acids Res 25(14):2730–2736

Morris MC, Depollier J et al (2001) A peptide carrier for the delivery of biologically active proteins into mammalian cells. Nat Biotechnol 19(12):1173–1176

Morris MC, Gros E et al (2007) A non-covalent peptide-based carrier for *in vivo* delivery of DNA mimics. Nucleic Acids Res 35(7), e49

Nakase I, Akita H et al (2012) Efficient intracellular delivery of nucleic acid pharmaceuticals using cell-penetrating peptides. Acc Chem Res 45(7): 1132–1139

Nanda A, Nanda S et al (2006) Current developments using emerging transdermal technologies in physical enhancement methods. Curr Drug Deliv 3(3):233–242

Narishetty STK, Panchagnula R (2004) Transdermal delivery of zidovudine: effect of terpenes and their mechanism of action. J Control Release 95(3):367–379

Narishetty STK, Panchagnula R (2005) Effect of L-menthol and 1,8-cineole on phase behavior and molecular organization of SC lipids and skin permeation of zidovudine. J Control Release 102(1):59–70

Oliver JD 3rd, Anderson S et al (1992) Determination of glomerular size-selectivity in the normal rat with Ficoll. J Am Soc Nephrol 3(2):214–228

Partidos CD, Beignon AS et al (2002) Applying peptide antigens onto bare skin: induction of humoral and cellular immune responses and potential for vaccination. J Control Release 85(1–3):27–34

Partidos CD, Beignon AS et al (2003) Immunity under the skin: potential application for topical delivery of vaccines. Vaccine 21(7–8):776–780

Patel LN, Zaro JL et al (2007) Cell penetrating peptides: intracellular pathways and pharmaceutical perspectives. Pharm Res 24(11):1977–1992

Patlolla RR, Desai PR et al (2010) Translocation of cell penetrating peptide engrafted nanoparticles across skin layers. Biomaterials 31(21):5598–5607

Pople PV, Singh KK (2010) Targeting tacrolimus to deeper layers of skin with improved safety for treatment of atopic dermatitis. Int J Pharm 398(1–2): 165–178

Powers JPS, Hancock REW (2003) The relationship between peptide structure and antibacterial activity. Peptides 24(11):1681–1691

Prausnitz MR (2006) A peptide chaperone for transdermal drug delivery. Nat Biotechnol 24(4):416–417

Prausnitz MR, Noonan JS (1998) Permeability of cornea, sclera, and conjunctiva: a literature analysis for drug delivery to the eye. J Pharm Sci 87(12): 1479–1488

Prausnitz MR, Mitragotri S et al (2004) Current status and future potential of transdermal drug delivery. Nat Rev Drug Discov 3(2):115–124

Priborsky J, Muhlbachova E (1990) Evaluation of in-vitro percutaneous absorption across human skin and in animal models. J Pharm Pharmacol 42(7):468–472

Rizwan M, Aqil M et al (2009) Enhanced transdermal drug delivery techniques: an extensive review of patents. Recent Pat Drug Deliv Formul 3(2):105–124

Rothbard JB, Garlington S et al (2000) Conjugation of arginine oligomers to cyclosporin A facilitates topical delivery and inhibition of inflammation. Nat Med 6(11):1253–1257

Scheindlin S (2004) Transdermal drug delivery: PAST, PRESENT, FUTURE. Mol Interv 4(6):308–312

Schutze-Redelmeier MP, Kong S et al (2004) Antennapedia transduction sequence promotes anti tumour immunity to epicutaneously administered CTL epitopes. Vaccine 22(15–16):1985–1991

Seo N, Tokura Y et al (2000) Percutaneous peptide immunization via corneum barrier-disrupted murine skin for experimental tumor immunoprophylaxis. Proc Natl Acad Sci U S A 97(1):371–376

Shah PP, Desai PR et al (2012) Enhanced skin permeation using polyarginine modified nanostructured lipid carriers. J Control Release 161(3):735–745

Simeoni F, Morris MC et al (2003) Insight into the mechanism of the peptide-based gene delivery system MPG: implications for delivery of siRNA into mammalian cells. Nucleic Acids Res 31(11):2717–2724

Simeoni F, Morris MC et al (2005) Peptide-based strategy for siRNA delivery into mammalian cells. Methods Mol Biol 309:251–260

Souto EB, Muller RH (2008) Cosmetic features and applications of lipid nanoparticles (SLN, NLC). Int J Cosmet Sci 30(3):157–165

Splith K, Neundorf I (2011) Antimicrobial peptides with cell-penetrating peptide properties and vice versa. Eur Biophys J 40(4):387–397

Sundaram H, Mehta RC et al (2009) Topically applied physiologically balanced growth factors: a new paradigm of skin rejuvenation. J Drugs Dermatol 8(5 Suppl Skin Rejuenation):4–13

Teeranachaideekul V, Boonme P et al (2008) Influence of oil content on physicochemical properties and skin distribution of Nile red-loaded NLC. J Control Release 128(2):134–141

Uchida T, Kanazawa T et al (2011) Development of an efficient transdermal delivery system of small interfering RNA using functional peptides, Tat and AT-1002. Chem Pharm Bull 59(2):196–201

Wan XM, Chen YP et al (2009) Identification of nose-to-brain homing peptide through phage display. Peptides 30(2):343–350

Wang YH, Chen CP et al (2006) Arginine-rich intracellular delivery peptides noncovalently transport protein into living cells. Biochem Biophys Res Commun 346(3):758–767

Zaffaroni A (1991) Overview and evolution of therapeutic systems. Ann N Y Acad Sci 618:405–421

Zasloff M (1987) Magainins, a class of antimicrobial peptides from Xenopus skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. Proc Natl Acad Sci U S A 84(15):5449–5453

Zhang T, Qu H et al (2010) Transmembrane delivery and biological effect of human growth hormone via a phage displayed peptide *in vivo* and *in vitro*. J Pharm Sci 99(12):4880–4891

Zhao B, Guo Y et al (2012) Genetically engineered epidermal growth factor conjugate crosses cell membrane. Appl Biochem Biotechnol 166(6):1463–1471

Peptide-Mediated Transdermal Drug Delivery

22

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22.1 Transdermal Drug Delivery

Transdermal drug delivery (TDD) offers an advantageous mode of drug administration by eliminating first-pass hepatic metabolism and providing sustained drug release for a prolonged period of time. It is painless as compared to needles and therefore offers superior patient compatibility. However, the skin is the first line of defense of an organism and the last barrier separating the organism from its hostile environment of viruses, pathogens, and toxics. Evolved to impede the flux of exogenous molecules into the body, the skin naturally offers a very low permeability to the movement of foreign molecules across it. A unique hierarchical structure of lipid rich matrix with embedded keratinocytes in the upper strata (15 μm) of the skin, stratum corneum (SC), is responsible for this barrier (Bouwstra 1997). In addition to its role as a barrier, both physical and biological, the skin performs a complimentary role, that of a transport regulator. The skin routinely regulates the flux of water molecules into and out of the body. It also permits the influx of a variety of small molecules that are fairly lipophilic with high partition coefficient (log P >1.5) and have molecular weight (MW) less than 500 Da (Bos and Meinardi 2000). As a result, there has been a natural bias of transdermal delivery systems to cash in on therapeutics that meet these requirements. Drug molecules

N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_22

currently administered via the transdermal route are typically characterized by high log *P* (>1.5) and low MW (<500 Da) taking advantage of natural selectivity of skin membrane. The biggest challenge in transdermal drug delivery today is to open the skin safely and reversibly to hydrophilic drugs of high MW.

Several technological advances have been made in the past couple of decades to overcome this challenge. These advances can be broadly divided into two categories: (1) physical approaches including but not limited to iontophoresis, sonophoresis, microneedles, and microporation that use some form of physical energy to modulate the SC ultrastructure and (2) chemical approaches that employ chemical moieties to modulate skin transport barrier (Prausnitz et al. 2004). Each of these methods has its individual benefits and limitations. Chemical formulation-based approaches, in particular, have a number of unique advantages such as design simplicity, flexibility, and ease of application over a large area. A transdermal delivery device based on chemical enhancers is essentially a combination of active drug and drug-compatible, skin-modulating excipients in a Band-Aid-like adhesive reservoir. Such a device has logistic and utilitarian benefits as compared to sophisticated devices based on ultrasound, iontophoresis, or other physical methods.

22.2 Chemical Enhancers Based Transdermal Drug Delivery

Chemical enhancers, also referred to as chemical penetration enhancers or chemical permeation enhancers (CPEs), are moieties that can alter the SC barrier to promote the flux of therapeutic molecules across the skin. About 350 different chemicals have been introduced as CPEs in the last four decades after the first documented report on the systemic absorption of dimethyl sulfoxide (DMSO) placed on human skin in 1965 (Kligman 1965; Stoughton 1965; Perlman and Wolfe 1966; Karande et al. 2005). These CPEs include surfactants (charged and uncharged), fatty acids (and their salts), fatty alcohols, fatty amines, and organic solvents. Only a handful of these, however, are actually used in practice (Berti and Lipsky 1995). This discrepancy results from the fact that among all the enhancers that have been tested, only a few induce a significant (therapeutic) enhancement of drug transport (Walters 1989; Finnin and Morgan 1999). Furthermore, skin irritation and safety issues limit the applications of several enhancers (Green 2000; Draize et al. 1944; Lee et al. 2000; Lashmar et al. 1989). Attempts have been made to synthesize novel CPEs, such as Azone® (Stoughton 1982; Takanashi et al. 1999; Akimoto and Nagase 2003). However, achieving sufficient potency without irritancy has proved somewhat challenging (Fang et al. 2003).

Identification of chemicals to increase skin permeability has been an area of high activity in the last three decades (Asbill et al. 2000; Bauerova et al. 2001; Kanikkannan and Singh 2002). After an initial rise in the number of CPEs in the 1980s, the active pool of CPEs has reached a plateau in the last decade. In an era where new chemical entities are being discovered at an exponential rate (as indicated by the entries in the Chemical Abstract Services), the plateau in the number of CPE molecules is rather surprising (only one in 100,000 known molecules represents a CPE) (Karande et al. 2005). This anomaly partly originates from slow rates of chemical enhancer syntheses and low throughput of screening techniques. In parallel, a lack of fundamental understanding of mechanistic principles that define CPE performance has promoted the use of somewhat empirical machineries to identify new CPEs (Karande et al. 2005). Consequently, most studies aimed at identifying new CPEs have been focused on exploring very narrow search spaces bound by a handful of chemical functionalities identified in the past. Not surprisingly, CPEs have not reached their expected potential in transdermal or topical systems so far.

22.3 Peptides in Transdermal Delivery

Peptides have a long history of use in the treatment of a wide variety of skin disorders. Several peptides have known therapeutic uses including bacitracin, palmitoyl-glycyl-histidyl-lysine tripeptide

(Lopes et al. 2005; Nasrollahi et al. 2012). In addition, peptides, consisting of T-helper (Th), B-cell, and cytotoxic T-lymphocyte (CTL) epitopes have also been used as subunit vaccines across the skin. Skin bypasses the problem of short half-lives associated with peptides by being able to continuously deliver them, thus being able to achieve a more sustained response (Partidos et al. 2003; Lee et al. 2008; Oyston et al. 2009; Desai et al. 2010; Namjoshi and Benson 2010). Additionally, cyclic peptides are now being used for treating various skin disorders, including penicillin, vancomycin, cyclosporin, echinocandins, and bleomycin (Namjoshi and Benson 2010). In general, cyclic peptides possess increased chemical and enzymatic stability, better pharmacodynamic properties, and receptor selectivity as compared to linear peptides. Several inflammatory skin disorders such as eczema, dermatitis, psoriasis, and urticaria are currently being treated with cyclic peptides (Namjoshi and Benson 2010). Peptides have also been demonstrated to be efficacious in treating certain forms of cancer, especially melanoma. Allman et al. have demonstrated the use of a cyclic oligopeptide cyclic Arg-Gly-Asp-DPhe-Val in inhibiting the growth of melanoma xenografts in nude mice. The peptide showed a strong anti-angiogenesis effect by binding with alpha vitronectin beta 3 integrin, highly expressed by melanoma cells (Allman et al. 2000). Finally, peptides can also function as therapeutics against microbial growth in the skin. These are known as antimicrobial peptides (AMPs) and have been known to kill bacteria, fungi, and certain viruses. They are rapidly released at high local concentrations in response to an injury or an infection and thus act as cutaneous antimicrobial shield in addition to enzymes and reactive oxygen species already present in the skin (Zasloff 2002; Bardan et al. 2004; Schauber and Gallo 2007).

22.4 Peptides as Transdermal Drug Delivery Agents

Peptides represent an attractive class of molecules as potential skin permeation enhancers or carriers for transdermal drug delivery. Several properties that make peptides excellent drugs are also potentially the ones that make them excellent candidates as drug delivery agents. Peptides are amenable to modular design and synthesis from their constituent amino acids and are less likely to cause adverse reactions such as dermal toxicity or systemic immunogenicity. Peptides offer several advantages over conventional approaches due to their ease of use, diversity and ability to specifically target various cellular subtypes within the skin, and ease of conjugation with drugs (Namjoshi and Benson 2010). The key premise is to increase the permeability of the skin and disrupt its barrier properties with peptides, so that drugs or other cargoes can then be delivered across it. There are currently three pathways of drug transport across the skin, namely, intracellular pathway, intercellular lipid pathway, and the transappendageal pathway which is through hair follicles, sebaceous, and sweat glands (Desai et al. 2010; Kumar et al. 2012). Intercellular lipid pathway has been most commonly used pathway for the delivery of cargoes. There have been numerous methods used to identify peptides as transdermal delivery agents, most commonly being the phage display method where large peptide libraries (10^8–10^9) are displayed on virus particles for screening (Chen et al. 2006; Hsu and Mitragotri 2011; Kumar et al. 2012). Cargoes have been delivered across the skin either in free form or in a conjugated form. In some cases, pretreatment with the delivery peptides seems to be better in enhancing skin permeation of cargoes as compared to co-application (Kumar et al. 2012). There mainly exist two categories of peptides as transdermal delivery agents.

22.4.1 Cell-Penetrating Peptides (CPPs)

These are also referred to as protein transduction domains (PTDs) or as membrane transduction peptides (MTPs). CPPs are typically around 30 amino acids in length, water soluble, positively charged, and/or amphipathic in nature. They have the ability of penetrating across the cell membrane

without the use of any receptors or causing any measurable membrane damage (Desai et al. 2010). CPPs have been grouped into three general categories based on their origin:

(a) Protein-derived CPPs, referring to those originating from naturally occurring protein.
(b) Chimeric CPPs, consisting of different protein domains.
(c) Model CPPs, referring to those developed based on structure-function relationships. These do not bear any homology to natural sequences.

CPPs have been shown to deliver cargoes across membranes either through conjugation with the cargo or by facilitating the co-delivery of the cargo. Although the exact mechanism of internalization into the cells is still unknown, cellular endocytosis has been proposed to be the main pathway involved in their activity. It is found to strongly depend upon the properties of both the CPP and the cargo employed. The best known CPPs are TAT (Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg), penetratin (Arg-Gln-Ile-Lys-Ile-Trp-Phe-Gln-Asn-Arg-Arg-Met-Lys-Trp-Lys-Lys), herpes simplex virus protein virus tegument protein 22, and arginine oligomers (Lopes et al. 2005; Veldhoen et al. 2008; Cohen-Avrahami et al. 2012; Nasrollahi et al. 2012). The noninvasive mode of delivery of cargoes by CPPs has led to various applications for basic research and for the development of new therapeutics. CPPs have been used to label cells or subcellular structures either through the delivery of magnetic nanoparticles or through fluorescent tags conjugated to CPPs. Additionally, several therapeutic drugs have been delivered into various cancerous cells, across the blood-brain barrier, and across human and mice skin (Nasrollahi et al. 2012). They have been increasingly used to deliver small molecules, small interfering ribonucleic acid (siRNA) nucleotides, drug-encapsulated nanoparticles, proteins, and peptides (Delehanty et al. 2010; Hsu and Mitragotri 2011; Kumar et al. 2012). Although trans-activating transcriptional activator (Tat) and penetratin were the first to be discovered, several other CPPs including transportan, multiple antigenic peptides (Lys-Leu-Ala-Leu-Lys-Leu-Ala-Leu-Lys-Ala-Leu-Lys-Ala-Ala-Leu-Lys-Leu-Ala), KALA (Trp-Glu-Ala-Lys-Leu-Ala-Lys-Ala-Leu-Ala-Lys-Ala-Leu-Ala-Lys-His-Leu-Ala-Lys-Ala-Leu-Ala-Lys-Ala-Leu-Lys-Ala-Cys-Glu-Ala), P1 (Met-Gly-Leu-Gly-Leu-His-Leu-Leu-Val-Leu-Ala-Ala-Ala-Leu-Gln-Gly-Ala-Trp-Ser-Gln-Pro-Lys-Lys-Lys-Arg-Lys-Val), MPG (Gly-Ala-Leu-Phe-Leu-Gly-Phe-Leu-Gly-Ala-Ala-Gly-Ser-Thr-Met-Gly-Ala-Trp-Ser-Gln-Pro-Lys-Lys-Lys-Arg-Lys-Val), Pep1 (Lys-Glu-Thr-Trp-Trp-Glu-Thr-Trp-Trp-Thr-Glu-Trp-Ser-Gln-Pro-Lys-Lys-Lys-Arg-Lys-Val), Arg9 (Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg), human hormone calcitonin, and hCT (Leu-Gly-Thr-Tyr-Thr-Gln-Asp-Phe-Asn-Lys-Phe-His-Thr-Phe-Pro-Gln-Thr-Ala-Ile-Gly-Val-Gly-Ala-Pro) have since been reported (Cohen-Avrahami et al. 2012; Nasrollahi et al. 2012).

22.4.2 Membrane-Penetrating Peptides (MPPs)

MPPs generally act via membrane depolarization, creation of physical holes, and disrupting the organization of the membrane lipids. In nature, they are actively involved in initiating both the innate and the adaptive immunities and therefore are sometimes referred to as "host defense peptides (HDPs)." They have been found both in animal and plant kingdoms and enable their survival against a wide variety of bacteria, fungi, viruses, and protozoa (Zasloff 2002; Bardan et al. 2004; Oyston et al. 2009; Delehanty et al. 2010). Antimicrobial peptides (AMPs) are the most well-known peptides in this class. In general, MPPs vary in length from 12 to 100 amino acids, are positively charged and known to interact with cell or bacterial membranes with hydrophobic amino acids, and are broadly categorized into cathelicidins and defensins. In the skin, they play a key role in wound healing and regeneration. Magainin, a 23-amino acid peptide, is currently the only known MMP known to enhance the skin permeability in combination with a known surfactant N-lauroyl sarcosine

(NLS). These compounds in combination were able to disrupt the organization of the extracellular lipids in the SC layer and thereby increasing the skin permeability for the delivery of fluorescein (Kim et al. 2007).

22.5 Peptide-Mediated Transdermal Delivery

There is now a significant body of literature that points to the potential of using peptides as delivery agents or vectors for transdermal drug delivery. Not intended to be comprehensive but rather supporting this observation, we review key representative studies that demonstrate the applications and merits of peptides for transdermal drug delivery of a wide range of therapeutic and diagnostic payloads.

22.5.1 Delivery of Protein and Peptide Therapeutics

Several large MW and hydrophilic peptides have been recognized as therapeutically active with the ability to treat a variety of skin diseases. However, their transdermal delivery across the skin at therapeutic doses has proven to be challenging due to poor skin permeability. P20 (Trp-Leu-Arg-Arg-Ala-Ser-Ala-Pro-Leu-Pro-Gly-Leu-Lys) is an example of one such peptide. Recently, however, Lopes et al. have shown that CPPs such as YARA (Tyr-Ala-Arg-Ala-Ala-Ala-Arg-Gln-Ala-Arg-Ala) and TAT (Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg) are able to penetrate the porcine ear skin in vitro and carry P20 conjugated to them. Penetration was observed well into the epidermal layers (Lopes et al. 2005). Lopes et al. subsequently also demonstrated the delivery of P20 across the skin using WLR (Trp-Leu-Arg-Arg-Ile-Lys-Ala-Trp-Leu-Arg-Arg-Ile-Lys-Ala-Trp-Leu-Arg-Arg-Ile-Lys-Ala) peptide. In general, covalent conjugation of the cargo peptides to the delivery peptides achieved better penetration as compared to co-application (Lopes et al. 2008). The coupling of small peptide cargoes (glycine-lysine-histidine) to TAT CPP similarly resulted in an increased absorption compared to the peptide cargoes alone (Lim et al. 2003). Kumar et al. have identified a T2 CPP (Leu-Val-Gly-Val-Phe-His) that is able to deliver not only hydrophilic cargoes such as 5-fluorouracil and rhodamine 123 hydrochloride but also hydrophobic molecules such as fluorescein isothiocyanate across the skin. Intercellular lipid pathway has been suggested to be the main route involved in the delivery (Kumar et al. 2012). In addition, hydrophobic molecules have also been delivered across the skin. Rothbard et al. reported the delivery of a cyclosporine A peptide conjugated with polyarginine-7 CPP. The particular CPP was able to deliver cyclosporine A well into the dermal compartment of both the mouse and human skin (Rothbard et al. 2000). Wender et al. have shown delivery of biotin on mouse skin into epidermis by using transporter-9 conjugated with biotin (Wender et al. 2002). Cohen-Avrahami et al. have demonstrated enhanced delivery of sodium diclofenac through porcine skin using penetratin. The peptide was able to disrupt the arrangement of lipids from hexagonal to liquid in the SC layer (Cohen-Avrahami et al. 2012). Carmichael et al. have demonstrated that molecules as big as 150 kDa (botulinum neurotoxin type A) can also be delivered transdermally across the skin by using a synthetic peptide (Ala-Cys-Ser-Ser-Ser-Pro-Ser-Lys-His-Cys-Gly). Although the exact mechanism has not been elucidated, intrafollicular transport has been suggested to be the route of skin penetration (Carmichael et al. 2010). Peptides have similarly been used as delivery agents for vaccination, especially for skin-associated cancers. Schutze-Redelmeier et al. have shown the delivery of an antigenic peptide ($OVA_{257–264}$- Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu) attached to an antennapedia transduction sequence (ANTP; Arg-Gln-Ile-Lys-Ile-Trp-Phe-Gln-Asn-Arg-Arg-Met-Lys-Trp-Lys-Lys), a CPP, in the tape-stripped skin of mice. The delivery of this antigenic peptide in conjugation with ANTP resulted in enhanced penetration of the antigen as compared to the antigen alone (Schutze-Redelmeier et al. 2004). Chen et al. have demonstrated the use of cyclic peptide (Ala-Cys-Ser-Ser-Ser-Pro-Ser-Lys-His-Cys-Gly) to deliver a large unconjugated, hydrophilic protein, insulin,

transdermally in rats. Systemic circulation of this hormone was achieved by creating a transient opening in the skin barrier (Chen et al. 2006). Hou et al. demonstrated the use of arginine-rich intracellular delivery (AID) peptides for the delivery of non-conjugated green fluorescent protein (GFP) across mouse skin. The addition of oleic acid as chemical enhancer further increased the permeation rate of the proteins (Hou et al. 2007).

22.5.2 Delivery of Nucleotides Across the Skin

The use of traditional viral vectors for the delivery of oligonucleotides such as small interfering ribonucleic acid can be potentially carcinogenic and lead to immune rejection by the host cells. CPPs have emerged as a potential alternative to viral vectors and are capable of bypassing the problem of poor availability and poor clinical efficacy normally associated with viral vectors (Veldhoen et al. 2008). CPPs are also potentially more effective and less toxic than viral vectors. Delivery of oligonucleotides by CPPs has been achieved mostly through non-covalent conjugation techniques and has been shown to be successful in the delivery of both plasmid deoxyribonucleic acid and small interfering ribonucleic acid across various cells and tissues. CPPs also score over other non-viral delivery vectors such as cationic liposomes and polymers in terms of safety and efficacy both in vivo and in vitro. TAT peptides have been shown to form complexes with deoxyribonucleic acid which are taken up by different cells through the process of endocytosis. In addition, both antisense oligonucleotides and small interfering ribonucleic acid have been delivered using CPPs (Sandgren et al. 2002). Topical application of small interfering ribonucleic acid has been associated with the treatment of skin diseases like atopic dermatitis. Hsu and Mitragotri have recently demonstrated the delivery of small interfering ribonucleic acid across the SC and also into the viable epidermal and dermal layers using a SPACE peptide (Ala-Cys-Thr-Gly-Ser-Thr-Gln-His-Gln-Cys-Gly). Both the enhanced partitioning into the SC and the ability to impact keratin structure have been proposed as the mechanisms for delivery (Hsu and Mitragotri 2011).

22.5.3 Delivery of Nanoparticles

Nanoparticles span a broad range of sizes from 10 to 100 nm and comprise variety of materials including metals, oxides, semiconductor quantum dots, and carbon-based materials. Their small sizes and high surface-to-volume ratio make them ideal for use in the field of therapeutic and biomedical applications. These properties also enable nanoparticles to access otherwise inaccessible physiological compartments, and their large surface area enables them to carry large payloads per unit volume. Peptides have been employed to add further functionality to the growing field of nanoparticle-mediated drug delivery. Conjugation of peptides with nanoparticles has resulted in the generation of constructs with a wide variety of functionalities. Most importantly, peptides facilitate the delivery of nanoparticles significantly as compared to their passive transport. Peptides can impart high valency to nanoparticles while still restricting the size of peptide-nanoparticle conjugate. They represent the most promising means of noninvasively delivering nanoparticles into cells (Crombez et al. 2008; Lane 2011). As a result, several peptides such as TAT, Arg-Gly-Asp, neuropeptide, and rabies virus-derived peptide have been used to deliver nanoparticles (liposomes, gold particles, dendrimers, quantum dots, and micelles) into cells for applications ranging from intracellular labeling and imaging, drug delivery, gene silencing, and expression (Torchilin 2008). The targeted cells include human and murine fibroblasts, Michigan Cancer Foundation-7 (MCF-7) human breast cancer cells, endothelial cells, and neuronal cells. CPPs have also been used to deliver nanoparticles for the treatment of allergic contact dermatitis, psoriasis, and fungal, bacterial, and viral infections (Delehanty et al. 2010; Desai et al. 2010). Kang et al. studied the role of a CPP in enhancing the permeation of oregonin for the treatment of atopic dermatitis in

mice. Oregonin was formulated into both elastic liposomes and a conventional cream and applied onto the mouse skin. Separate experiments were also done where TAT peptide was added with these formulations. It was found that the addition of a TAT peptide to elastic liposomes or a conventional cream resulted in enhanced penetration of oregonin across mouse skin than that achieved by liposomes or cream alone (Kang, Eum et al. 2010). Patlolla et al. were able to achieve enhanced penetration of both the fluorescent dye 1, 1′- dioctadecyl-3, 3, 3′, 3′- tetramethylindodicarbocyanine perchlorate ('DiD'-oil) and celecoxib with TAT-coated nanoparticles across the rat skin. The permeation was found to be time dependent, and a deeper penetration was achieved as compared to the nanoparticles alone or nanoparticles coated with a control peptide (Patlolla et al. 2010).

22.6 Concluding Remarks

There continues to be a growing interest in the design and use of peptides as permeation enhancers for transdermal drug delivery. However, all studies reported to date are on animal (mouse, rat, or pig) skin. Given key biological and morphological differences between human and animal skin, the efficacy of peptide vectors in delivering cargoes across human skin still remains to be seen.

The size of peptide vectors is critical as it determines the in vivo stability, efficacy, and immunogenicity. Most peptides reported thus far are typically 10–15 amino acids long and in the appropriate size range. The amino acid composition of the peptide vectors is also important. Histidine and basic amino acids such as arginine have been found to be key constituents of effective delivery vectors (Kumar et al. 2012)

An additional key consideration is the conjugation between the peptide vector and the cargo. Most studies have reported delivery of the cargo as a covalent conjugate with the peptide vector, although a few have reported co-delivery of the two (Lopes et al. 2005, 2008; Hou et al. 2007). Only one study has systematically looked at the effects of conjugation. Lopes et al. demonstrated that greater skin penetration efficiency was achieved through conjugation as compared to co-delivery (Lopes et al. 2008). It is clear that further optimization is necessary to achieve therapeutic doses of cargoes while maintaining their stability and efficacy post-conjugation schemes. Achieving effective delivery without conjugation of the cargoes to peptide vectors would be ideal to avoid the costs involved in the conjugation and purification steps and to truly realize the benefits of using peptides as transdermal delivery agents. It also remains to be seen if there exists a synergy between peptides along with either chemical or physical penetration enhancers. Systematic studies to this end are yet to emerge.

In conclusion, peptides represent an interesting and potentially impactful class of permeation enhancers. Their success will depend on their ability to safely and reversibly permeabilize the skin for the delivery of hydrophilic drugs of large MW at therapeutically relevant concentrations for systemic use.

References

Akimoto T, Nagase Y (2003) Novel transdermal drug penetration enhancer: synthesis and enhancing effect of alkyldisiloxane compounds containing glucopyranosyl group. J Control Release 88:243–252

Allman R, Cowburn P et al (2000) *In vitro* and *in vivo* effects of a cyclic peptide with affinity for the αvβ3 integrin in human melanoma cells. Eur J Cancer 36(3):410–422

Asbill CS, El-Kattan AF et al (2000) Enhancement of transdermal drug delivery: chemical and physical approaches. Crit Rev Ther Drug Carrier Syst 17(6):621–658

Bardan A, Nizet V et al (2004) Antimicrobial peptides and the skin. Expert Opin Biol Ther 4(4):543–549

Bauerova K, Matusova D et al (2001) Chemical enhancers for transdermal drug transport. Eur J Drug Metab Pharmacokinet 26(1–2):85–94

Berti J, Lipsky J (1995) Transcutaneous drug delivery: a practical review. Mayo Clin Proc 70:581–586

Bos JD, Meinardi MHM (2000) The 500 dalton rule for the skin penetration of chemical compounds and drugs. Exp Dermatol 9(3):165–169

Bouwstra, JA (1997) The skin, a well organized membrane. Colloids Surf. A 123–124:403–413

Carmichael NME, Dostrovsky JO et al (2010) Peptide-mediated transdermal delivery of botulinum

neurotoxin type A reduces neurogenic inflammation in the skin. Pain 149(2):316–324
Chen YP, Shen YY et al (2006) Transdermal protein delivery by a coadministered peptide identified via phage display. Nat Biotechnol 24(4):455–460
Cohen-Avrahami M, Libster D et al (2012) Penetratin-induced transdermal delivery from H-II mesophases of sodium diclofenac. J Control Release 159(3):419–428
Crombez L, Morris MC et al (2008) Peptide-based nanoparticle for ex vivo and in vivo dug delivery. Curr Pharm Des 14(34):3656–3665
Delehanty JB, Boeneman K et al (2010) Peptides for specific intracellular delivery and targeting of nanoparticles: implications for developing nanoparticle-mediated drug delivery. Ther Deliv 1(3):411–433
Desai P, Patlolla RR et al (2010) Interaction of nanoparticles and cell-penetrating peptides with skin for transdermal drug delivery. Mol Membr Biol 27(7):247–259
Draize JH, Woodard G et al (1944) Methods for the study of irritation and toxicity of substances applied to the skin and mucous membranes. J. Pharmacol. Exp. Ther. 82:377–390
Fang JY, Hwang TL et al (2003) In vitro and in vivo evaluations of the efficacy and safety of skin permeation enhancers using flurbiprofen as a model drug. Int J Pharm 255(1–2):153–166
Finnin CB, Morgan TM (1999) Transdermal penetration enhancers: applications, limitations and potential. J Pharm Sci 88:955–958
Green BG (2000) Measurement of sensory irritation of the skin. Am. J. Contact Dermatitis 11:170–180
Hou YW, Chan MH et al (2007) Transdermal delivery of proteins mediated by non-covalently associated arginine-rich intracellular delivery peptides. Exp Dermatol 16(12):999–1006
Hsu T, Mitragotri S (2011) Delivery of siRNA and other macromolecules into skin and cells using a peptide enhancer. Proc Natl Acad Sci U S A 108(38):15816–15821
Kang MJ, Eum JY et al (2010) Facilitated skin permeation of oregonin by elastic liposomal formulations and suppression of atopic dermatitis in NC/Nga mice. Biol Pharm Bull 33(1):100–106
Kanikkannan N, Singh M (2002) Skin permeation enhancement effect and skin irritation of saturated fatty alcohols. Int J Pharm 248:219–228
Karande P, Jain A et al (2005) Design principles of chemical penetration enhancers for transdermal drug delivery. Proc Natl Acad Sci U S A 102(13):4688–4693
Kim YC, Ludovice PJ et al (2007) Transdermal delivery enhanced by magainin pore-forming peptide. J Control Release 122(3):375–383
Kligman AM (1965) Topical pharmacology and toxicology of dimethyl sulfoxide. 1. JAMA 193:796–804
Kumar S, Sahdev P et al (2012) Identification of a novel skin penetration enhancement peptide by phage display peptide library screening. Mol Pharm 9(5): 1320–1330
Lane ME (2011) Nanoparticles and the skin-applications and limitations. J Microencapsul 28(8):709–716
Lashmar UT, Hadgraft J et al (1989) Topical application of penetration enhancers to the skin of nude mice: a histopathological study. J. Pharm. Pharmacol. 41:118–122
Lee JK, Kim DB et al (2000) In vitro cytotoxicity tests on cultured human skin fibroplasts to predict skin irritation potential of surfactants. Toxicol. In Vitro 14:345–349
Lee WR, Pan TL et al (2008) Erbium: YAG laser enhances transdermal peptide delivery and skin vaccination. J Control Release 128(3):200–208
Lim JM, Chang MY et al (2003) Penetration enhancement in mouse skin and lipolysis in adipocytes by TAT-GKH, a new cosmetic ingredient. J Cosmet Sci 54(5):483–491
Lopes LB, Brophy CM et al (2005) Comparative study of the skin penetration of protein transduction domains and a conjugated peptide. Pharm Res 22(5):750–757
Lopes LB, Furnish E et al (2008) Enhanced skin penetration of P20 phosphopeptide using protein transduction domains. Eur J Pharm Biopharm 68(2):441–445
Namjoshi S, Benson HAE (2010) Cyclic peptides as potential therapeutic agents for skin disorders. Biopolymers 94(5):673–680
Nasrollahi SA, Taghibiglou C et al (2012) Cell-penetrating peptides as a novel transdermal drug delivery system. Chem Biol Drug Des 80(5):639–646
Oyston PCF, Fox MA et al (2009) Novel peptide therapeutics for treatment of infections. J Med Microbiol 58(8):977–987
Partidos C, Beignon AS et al (2003) Immunity under the skin: potential application for topical delivery of vaccines. Vaccine 21(7):776–780
Patlolla RR, Desai PR et al (2010) Translocation of cell penetrating peptide engrafted nanoparticles across skin layers. Biomaterials 31(21):5598–5607
Perlman F, Wolfe HF (1966) Dimethylsulfoxide as a penetrant carrier of allergens through intact human skin. J Allergy 38(5):299–307
Prausnitz M, Mitragotri S et al (2004) Current status and future potential of transdermal drug delivery. Nat Rev Drug Discov 3(2):115–124
Rothbard JB, Garlington S et al (2000) Conjugation of arginine oligomers to cyclosporin A facilitates topical delivery and inhibition of inflammation. Nat Med 6(11):1253–1257
Sandgren S, Cheng F et al (2002) Nuclear targeting of macromolecular polyanions by an HIV-Tat derived peptide – role for cell-surface proteoglycans. J Biol Chem 277(41):38877–38883
Schauber J, Gallo RL (2007) Expanding the roles of antimicrobial peptides in skin: alarming and arming keratinocytes. J Invest Dermatol 127(3):510–512
Schutze-Redelmeier MPM, Kong S et al (2004) Antennapedia transduction sequence promotes anti tumour immunity to epicutaneously administered CTL epitopes. Vaccine 22(15–16):1985–1991
Stoughton RB (1965) Dimethyl sulfoxide (DMSO) induction of a steroid reservoir in human skin. Arch Dermatol 91:657–660

Stoughton RB (1982) Enhanced percutaneous penetration with 1-dodecylazacycloheptan-2-one. Arch Dermatol 118(7):474–477

Takanashi Y, Higashiyama K et al (1999) Thiomenthol derivatives as novel percutaneous absorption enhancers. Drug Dev Ind Pharm 25:89–94

Torchilin VP (2008) Cell penetrating peptide-modified pharmaceutical nanocarriers for intracellular drug and gene delivery. Biopolymers 90(5):604–610

Veldhoen S, Laufer SD et al (2008) Recent developments in peptide-based nucleic acid delivery. Int J Mol Sci 9(7):1276–1320

Walters KA (1989) Penetration enhancers and their use in transdermal therapeutic systems. In: Hadgraft J, Guy RH (eds) Transdermal drug delivery. Developmental issues and research initiatives. Marcel Dekker, New York, pp 197–233

Wender PA, Rothbard JB et al (2002) Oligocarbamate molecular transporters: design, synthesis, and biological evaluation of a new class of transporters for drug delivery. J Am Chem Soc 124(45): 13382–13383

Zasloff M (2002) Antimicrobial peptides of multicellular organisms. Nature 415(6870):389–395

Application of Biomaterials in Percutaneous Absorption Enhancement

23

Liang Fang and Yang Chen

Contents

23.1 Introduction

Chemical penetration enhancers currently represent the most widely used method to facilitate the drug delivery across the skin barrier. Up to date, hundreds of structurally different chemicals have been synthesized and evaluated for their ability of penetration enhancement toward various drugs (Karande et al. 2005). However, the success of these chemical enhancers has been limited, owing to their common skin irritancy and little effect on the large hydrophilic molecules (Bos and Meinardi 2000). Therefore, the search continues for an ideal enhancer that can be widely used in clinic and laboratory investigations. Recently, a series of biomaterials has been identified that can effectively enhance the drug absorption through the skin, especially for those poorly permeable macromolecules, which are often circumscribed by conventional chemical enhancers.

In this chapter, we presented a brief overview of the investigations on biomaterials with the ability of penetration enhancement. The paper organization essentially follows the review published by us in 2013 (Chen et al. 2013).

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N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_23

23.2 Biomaterials Used as Transdermal Penetration Enhancers

23.2.1 Protein Transduction Domains

Protein transduction domains (PTDs), also known as cell-penetrating peptides (CPPs), can generally cross the biological membranes and facilitate the cell uptake of molecules without using any receptors and without causing any significant membrane damage. They are relatively short (less than 30 amino acids in length), water soluble, and amphipathic or cationic peptides (Nasrollahi et al. 2012). So far, many of them have been successfully used to mediate the intracellular delivery of plasmid deoxyribonucleic acid (DNA), oligonucleotides, small interfering ribonucleic acid (siRNAs), proteins and peptides, contrast agents, and various drugs (Koren and Torchilin 2012).

In transdermal drug delivery systems (TDDS), PTDs can be covalently linked to the drugs and enhance their penetration into and across the skin. Under the physiological pH within the skin tissue, the PTD-drug complex can release the active drug or remain unchanged to produce similar therapeutic effects as the free drug does. As an example, heptamer oligomer of arginine (R7) was first suggested as a potential PTD enhancer to increase the percutaneous absorption of cyclosporine A (Rothbard et al. 2000). Since then, the synthetic PTDs have increasingly been introduced in TDDS for enhanced delivery of different active therapeutic agents, as seen in Table 23.1.

Furthermore, modifications of pharmaceutical nanocarriers with PTDs may be used to increase the permeation of nanocarriers in the skin. It has been proved that the nanostructured lipid carrier (NLC) surface modified with TAT or polyarginine can effectively enhance the skin penetration of drugs (e.g., spantide II and ketoprofen) into deeper tissues in the skin, while the non-transduction peptide-modified NLCs exhibit no such effect (Desai et al. 2010; Shah et al. 2012).

However, conjugation with the therapeutic agents is largely limited for the application of PTDs in TDDS because the tailor-made synthetic processes of the conjugates should be established for individual molecules, and the degradation profile of the conjugates delivered into bodies should be carefully considered. Here, it is important to indicate that some of the PTDs can also be used as carrier, non-covalently bound to the drug to enhance drug absorption through the skin. For example, Hou et al. found that arginine-rich intracellular delivery (AID) peptides, such as TAT, R9, R9Z, and SR9, exhibited excellent enhancing ability in the delivery of green fluorescent protein in the animal skin without conjugation with the transported protein (Hou et al. 2007).

The mechanisms underlying the skin penetration enhancement induced by PTDs and their conjugates remain highly debated. Unlike the normal cell membrane, the outmost layer of the skin is composed of dead cells rich in keratin, and the endocytosis of these biomaterials which plays

Table 23.1 Examples of PTDs in transdermal drug delivery systems

PTDs	Sequences[a]	Active therapeutic agents	References
R7	RRRRRRR	Cyclosporine A	Rothbard et al. (2000)
TAT	GRKKRRQRRRPPQ	Glycine-lysine-histidine	Lim et al. (2003)
YARA	YARAAARQARA	Heat shock protein (P20)	Lopes et al. (2005)
NONA	RRRRRRRRR	Sodium diclofenac	Cohen-Avrahami et al. (2011)
SPACE	ACGSTQHQCG	siRNA	Hsu and Mitragotri (2011)
PEN	RQIKIWFQNRRMKWKK	Sodium diclofenac	Cohen-Avrahami et al. (2011)
RALA	RALA RALARALRALAR	Sodium diclofenac	Cohen-Avrahami et al. (2011)

[a]Abbreviations of amino acids in these PTD sequences have been defined as follows: *A* alanine, *C* cysteine, *G* glycine, *H* histidine, *K* lysine, *M* methionine, *N* asparagine, *P* proline, *Q* glutamine, *R* arginine, *S* serine, *T* threonine, *W* tryptophan, *Y* tyrosine

an essential role in transmucosal enhancement is not expected for the stratum corneum (SC). Therefore, the lipids and keratins in the SC may still be the main acting sites for biomaterial penetration enhancers.

23.2.2 Non-transduction Peptides

In addition to PTDs, drug delivery in the skin may also be improved by other non-transduction peptides.

A variety of peptides known to form pores in bacterial cell membranes is found in nature and has been extensively investigated due to their potential application as novel antibiotics. One of the most studied is magainin, which is originally isolated from the skin of the African clawed frog, *Xenopus laevis*. Magainin has 23 amino acid residues (GIGKFLHSAKKFGKAFVGEIMNS) and a net charge up to +4 (Zasloff 1987). Therefore, it can easily bind to the negatively charged lipid membranes by way of electrostatic interactions and form pores with the approximately estimated size of 1 nm (Matsuzaki et al. 1997). Studies showed that magainin could be used to make lipid bilayers in the SC leaky as well. However, because of the natural weak penetration ability of magainin, another chemical enhancer was needed to increase the skin penetration of magainin to synergistically increase the permeability of the skin. For example, magainin showed a huge enhancement of skin permeability only when used in combination with sodium lauryl sulfate and ethanol (Kim et al. 2007, 2008).

To avoid this limitation of magainin, a smaller (5–6 amino) skin penetration-enhancing peptide called T2 peptide (LVGVFH) has been developed. It was suggested that T2 peptide could significantly enhance the transdermal absorption of hydrophilic 5-fluorouracil without the aid of other skin penetration enhancers. The limitation of T2 peptide was that it was only effective on the skin which was pretreated with it first. Conjugation of T2 peptide with the drug molecules might address this problem and further improved its penetration enhancement efficacy (Kumar et al. 2012).

TD-1 (ACSSSPSKHCG) is the first synthetic peptide specially designed as an enhancer for transdermal drug delivery. It might create a transient opening on the skin barrier, enabling the drug passage through the SC and reach the systemic circulation. Studies revealed that TD-1 could increase the skin delivery of insulin, human growth hormone, and botulinum neurotoxin type A (Chen et al. 2006; Carmichael et al. 2010).

23.2.3 Chitosan and Its Derivatives

Chitosan is a nontoxic, biocompatible polymer that can enhance the drug absorption across mucosa epithelia. Based on the cationic nature of chitosan and the similarity of epithelial cells and the skin epidermis, namely, the fixed negative charges in the tight junction between their respective cells, it is therefore reasonable to speculate that chitosan and its derivatives can also be used to enhance the drug penetration through the skin. Permeation enhancement effect of chitosan has been demonstrated in the transdermal delivery of carvedilol (Sapra et al. 2008).

To seek more potent penetration enhancers and to improve the solubility of chitosans, *N*-trimethyl chitosans (TMCs) were synthesized and investigated for their permeation enhancement ability. It was revealed that TMCs enhanced the transdermal permeation of testosterone, and their enhancement activity significantly increased with the quaternization degree. A mechanistic study suggested that TMCs could alter the secondary structure of keratin, increase the water content in the SC, decrease human keratinocyte cell line (HaCaT cell membrane potentials), and enhance HaCaT cell membrane fluidity, which could lead to a more loose packing of the SC for skin penetration enhancement effect (He et al. 2008, 2009).

To avoid the drawbacks of high molecular weight and low solubility in water or organic vehicles, low molecular weight chitosans (LMWCs) have also been studied as penetration enhancers in the transdermal delivery of baicalin. When the molecular weight decreased from 5000 to 1000 Da, the enhancement factor significantly increased from 1.0 to 15.9. However, this

enhancement effect would decrease with the reduced degree of deacetylation. In addition, concentrations and pH could both affect the activity of LMWCs. For example, there was an optimal concentration range (0.5–1 % by weight) for CS80–1000 (CS with 80 % deacetylation degree and 1 KDa) to enhance the percutaneous absorption of baicalin, and its maximum enhancement effect was obtained at pH 7.5 (Zhou et al. 2010).

D-acetylglucosamine (β-D-GlcNAc) and D-glucosamine (β-D-$GlcNH_2$) are the two units in the copolymer structure of chitosans. There has been a study revealing that only the segment of β-D-$GlcNH_2$ is effective toward enhancing the drug permeation through the skin (Zhou et al. 2010).

In a recent study, a series of biomimetic chitosan derivatives, *N*-arginine chitosans (*N*-arginine CSs), has been synthesized by chemical coupling of chitosans and L-arginine, mimicking the arginine-rich PTD (e.g., the AID peptides) to obtain the penetration enhancement functions of typical PTDs. It was shown that chemical conjugation of arginine and chitosan had a significantly stronger effect than their corresponding physical mixture on the skin penetration of hydrophilic adefovir (Lv et al. 2011).

23.2.4 Metabolic Modulators

23.2.4.1 Lipid Synthesis Inhibitors

The lipids in the SC, consisting of ceramides, cholesterol, and fatty acids, play a key role in maintenance of the transdermal barrier function. A decrease in the amount of any of these critical lipid species may alter the molar ratio required for normal barrier function and break the skin barrier integrity. Therefore, the skin permeability can be enhanced by lipid metabolic intervention (Patil et al. 1996).

As shown in Fig. 23.1, the lamellar bodies generated during the keratinocyte differentiation are considered as the sources of these lipids. In addition to phospholipids, glucosylceramides, sphingomyelin, and cholesterol, lamellar bodies also contain lipid hydrolases, such as β-glucocerebrosidase, acidic sphingomyelinase, secretory phospholipase A2, and acidic/neutral lipases. Following the secretion of lamellar bodies, β-glucocerebrosidase converts glucosylceramides into ceramides, acidic sphingomyelinase converts sphingomyelin into ceramides, and phospholipases convert phospholipids into free fatty acids and glycerol (Feingold 2007, 2009). Application of fatty acid synthesis inhibitors (e.g., cerulenin and 5-(tetradecyloxy)-2-furancarboxylic acid),

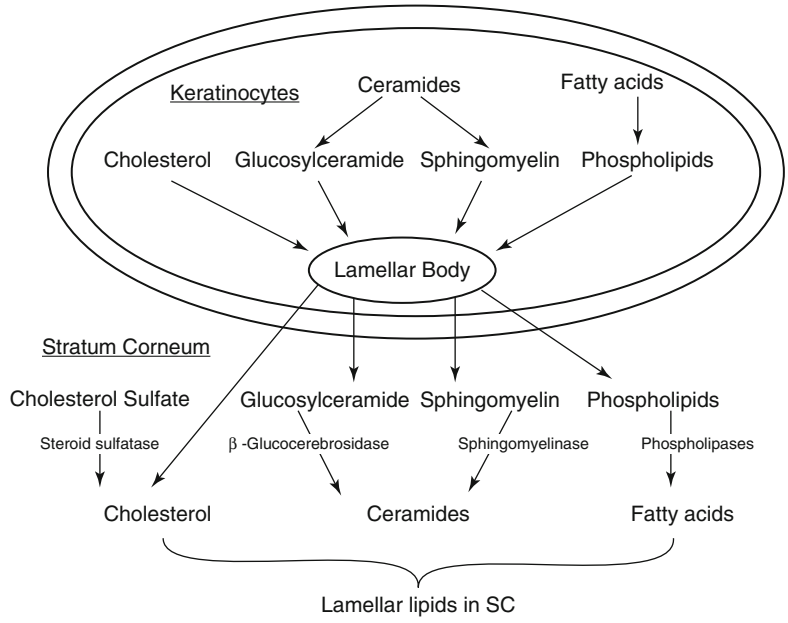

Fig. 23.1 The formation of SC intercellular lipids that provide the barrier to penetration of exogenous molecules into the skin

cholesterol synthesis inhibitors (e.g., atorvastatin and fluvastatin), or ceramide synthesis inhibitors (e.g., β-chloroalanine) on the organic solvent pretreated skin could delay the normalization of the lipid barrier function and enhance the transdermal delivery of different drugs, such as caffeine, lidocaine hydrochloride (Tsai et al. 1996), levodopa (Babita and Tiwary 2002, 2004a, b), and 5-fluorouracil (Babita et al. 2005). Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) experiments revealed that treatment of rat skin with lipid synthesis inhibitors only decreased the amount of lipids in the SC, without any effect on the lipid fluidization (Gupta et al. 2004).

23.2.4.2 Regulator of Tight Junction

Tight junctions (TJs) are cell-cell junctions that connect neighboring cells and control the paracellular passage of molecules. There is a plethora of data demonstrating that the barrier function of TJs can be modulated by various internal and external stimuli, such as chitosans which is commonly used to enhance the absorption of poorly permeable drugs (Gopalakrishnan et al. 2009).

In the human epidermis, there are various TJ proteins controlling the paracellular pathway of molecules across the skin, including occluding, claudin, and zonula occludens protein 1 (ZO-1). In the study of their assembly and function, AT1002 (H-FCIGRL-OH) which is a six-mer synthetic peptide has been identified as a potential TJ modulator to enhance the skin delivery of siRNA for skin disease treatment. AT1002 could inhibit the express of ZO-1 in the skin, and this effect might not induce serious skin damages since it could be easily recovered after skin washing (Uchida et al. 2011).

23.2.4.3 Trypsin

Trypsin is a proteolytic enzyme that has extensively been used in the in vitro epidermal separation and keratinocyte isolation. Li et al. first found that trypsin could be used as a percutaneous penetration enhancer that promoted the transdermal absorption of insulin (Li et al. 2009). Fluorescence microscopy examination revealed that trypsin increased the skin penetration of fluorescein isothiocyanate (FTIC)-insulin via the intercellular pathway and the hair follicular route. ATR-FTIR experiments indicated that trypsin altered the SC protein structure and thereby affected skin barrier properties resulting in the penetration-enhancing effect (Li et al. 2008).

23.2.5 Cyclodextrins

Cyclodextrins (CDs) are cyclic oligosaccharides with a hydrophilic outer surface and a somewhat lipophilic central cavity. The three major CDs, α-, β-, and γ- CD are respectively composed of six, seven, or eight (α-1,4) linked D-glucopyranose units. These parent CDs, as well as their various kinds of chemically modified derivatives, have been extensively researched for their topical use as formulation additives and transdermal absorption promoters (Matsuda and Arima 1999; Loftsson and Masson 2001).

The roles of CDs in the enhanced transdermal drug delivery can be summarized as follows. First, CDs can form hydrophilic inclusion complexes with lipophilic compounds in aqueous solution to improve drug solubility without changing their intrinsic ability to permeate the lipophilic barrier (Zi et al. 2008). Second, CDs are able to extract significant amounts of some lipophilic components from the skin (Legendre et al. 1995). Third, CDs have the potential to modify the drug release from polymeric systems (Arima et al. 1996). Forth, the skin irritancy of chemicals can be alleviated by CDs in the formulation (Ventura et al. 2006).

In addition, CDs can also be used as stabilizers in the formulation to reduce the drug decomposition in the formulation, protect against skin metabolism, and depress the loss of the volatile drug (Umemura et al. 1990; Lopez et al. 2000; Davaran et al. 2006).

23.2.6 Dendrimers

Dendrimers are new artificial macromolecules which have the structure like a tree, with a central core, interior branches, and terminal groups

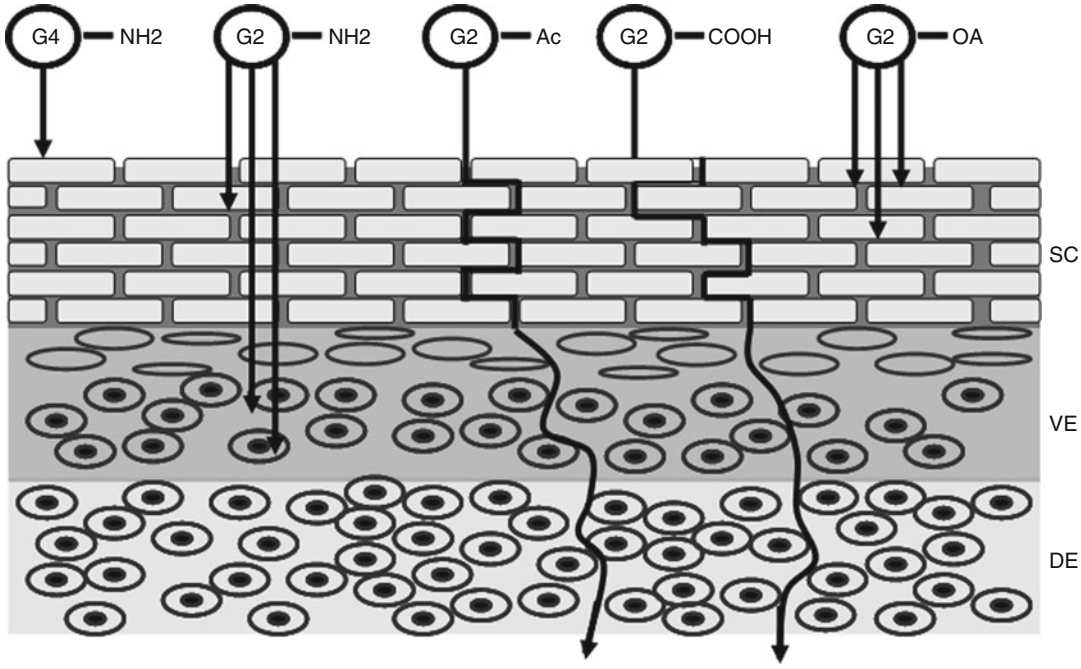

Fig. 23.2 Schematic representation of generation (G) four dendrimers. In terms of molecular size, PAMAM dendrimers can be classified as G 0–10, and their surface can be modified to be amine, carboxyl, acetyl, or hydroxyl terminated for different surface charge

which decorate the surface. Cavities inside the core and the interior branches can be modified to carry hydrophobic and hydrophilic drugs. So far, dendrimers with different functionalities have widely been used in biomedical, pharmaceutical, and biopharmaceutical fields. Among them, the most investigated one for transdermal drug delivery is the polyamidoamine (PAMAM) dendrimer. In terms of molecular size, PAMAM dendrimers (Fig. 23.2) can be classified as generation (G) 0–10, and their surfaces can be respectively modified to be amine, carboxyl, acetyl, or hydroxyl terminated for different surface charges (Nanjwade et al. 2009).

PAMAM dendrimers have demonstrated their enhancing ability in in vitro and in vivo transdermal penetration of various bioactives, such as indomethacin and 8-methoxypsoralen (Chauhan et al. 2003; Borowska et al. 2010). In addition, it was suggested that PAMAM dendrimer could also covalently conjugate with different NSAIDs (ketoprofen and diflunisal) to improve the drug permeation.

Molecular size and surface charge are two important factors that can affect the penetration-enhancing ability of PAMAM dendrimers. Generally, the small-sized dendrimer might be a more potent penetration enhancer than the larger ones for transdermal delivery of drugs, such as riboflavin and 5-fluorouracil (Venuganti and Perumal 2009; Filipowicz and Wołowiec 2011). In the transdermal delivery of 5-fluorouracil, the enhancing activity of PAMAM dendrimers with different surface charges ranked in the following decreasing order: G4-NH2 > G4-OH > G3.5-COOH (Venuganti and Perumal 2009). ATR-FTIR studies revealed that the cationic PAMAM dendrimers could more easily interact with the lipids in the SC and increase the drug penetration (Venuganti and Perumal 2009).

A recent mechanistic study suggested that different PAMAM dendrimers exhibited different penetration-enhancing ability in the skin, which was shown in Fig. 23.3. It was indicated that the smaller PAMAM dendrimers (G2-NH_2) penetrate into the skin layers more efficiently than the larger

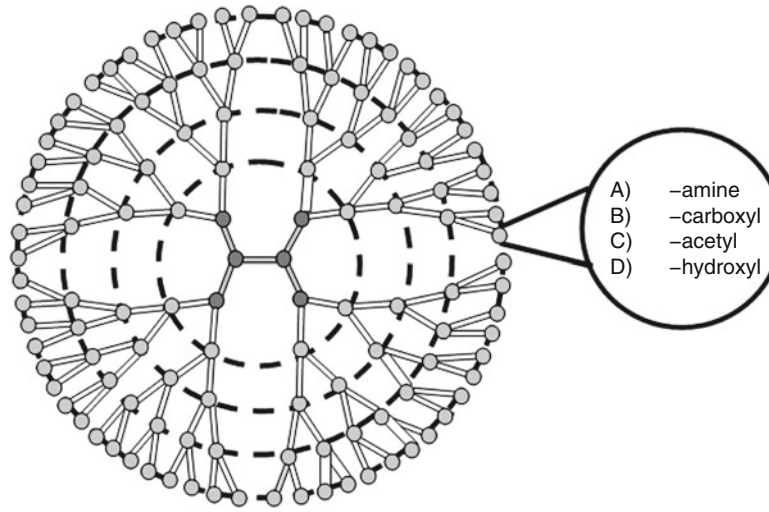

Fig. 23.3 Schematic illustration of skin penetration of PAMAM dendrimers with different molecular sizes and surface attachments, *SC* stratum corneum, *VE* viable epidermis, *DE* dermis

ones. More specifically, G2-NH_2 could be absorbed into the SC as well as the underlying viable epidermis, while G4-NH_2 only retained at the outmost layer of the SC. The study also revealed that surface modification by acetylation or carboxylation (G2-COOH) could significantly increase the penetration-enhancing ability of PAMAM dendrimers, likely through an intercellular pathway. In contrast, the amine-modified dendrimers, G2-NH_2, could interact with the cells in epidermal and dermal layers, enhance the cell internalization and skin retention, but significantly reduce the amount of drug permeation across the skin. In addition, conjugation of oleic acid to G2 dendrimers could change their partitioning behavior and potentially make their joint log P fall into an optimum range for the best skin permeability, resulting in the increased drug absorption and retention in the skin (Yang et al. 2012).

In addition to PAMAM dendrimers, dendritic PEGylated polyglycerol amine has also been used as a potential nanocarrier to enhance the percutaneous penetration, especially through the follicular route (Küchler et al. 2009).

Three different possible mechanisms of dendrimers were very recently reviewed and discussed by Y. Sun et al. (2012). First, dendrimers can act as a drug release modifier and boost the drug dissolution from the vehicle. Second, they can be used in nanoparticles to preferably penetrate the skin via the follicular route. Third, certain low-generation dendrimers may impair the SC barrier function, particularly in the presence of a potent vehicle (Sun et al. 2012).

Conclusion

Use of biomaterials as potential penetration enhancers is a new and developing method that has broad potential for improving the percutaneous penetration of a wide range of therapeutic agents. As a promising enhancement technology, it will continue to be a popular research topic in the investigation of drug delivery through the skin. At the same time, additional investigations are still needed to extend the insights into their potential skin irritancy, in vivo efficacy, as well as their enhancing mechanisms.

References

Arima H, Miyaji T, Irie T, Hirayama F, Uekama K (1996) Possible enhancing mechanism of the cutaneous permeation of 4-biphenylylacetic acid by beta-cyclodextrin derivatives in hydrophilic ointment. Chem Pharm Bull 44:582

Babita SG, Tiwary AK (2002) Role of sphingosine synthesis inhibition in transcutaneous delivery of levodopa. Int J Pharm 238:43

Babita K, Tiwary AK (2004a) Skin lipid synthesis inhibition: a possible means for enhancing percutaneous delivery of levodopa. Curr Drug Deliv 1:397

Babita K, Tiwary AK (2004b) Transcutaneous delivery of levodopa: enhancement by fatty acid synthesis inhibition. Mol Pharm 2:57

Babita K, Rana V, Tiwary AK (2005) Lipid synthesis inhibitors: effect on epidermal lipid conformational changes and percutaneous permeation of levodopa. AAPS PharmSciTech 6:E473

Borowska K, Laskowska B, Magoń A, Mysliwiec B, Pyda M, Wołowiec S (2010) PAMAM dendrimers as solubilizers and hosts for 8-methoxypsoralene enabling transdermal diffusion of the guest. Int J Pharm 398:185

Bos JD, Meinardi MM (2000) The 500 Dalton rule for the skin penetration of chemical compounds and drugs. Exp Dermatol 9:165

Carmichael NM, Dostrovsky JO, Charlton MP (2010) Peptide-mediated transdermal delivery of botulinum neurotoxin type A reduces neurogenic inflammation in the skin. Pain 149:316

Chauhan AS, Sridevi S, Chalasani KB et al (2003) Dendrimer-mediated transdermal delivery: enhanced bioavailability of indomethacin. J Control Release 90:335

Chen Y, Shen Y, Guo X, Zhang C, Yang W, Ma M, Liu S, Zhang M, Wen LP (2006) Transdermal protein delivery by a coadministered peptide identified via phage display. Nat Biotechnol 24:455

Chen Y, Wang ML, Fang L (2013) Biomaterials as novel penetration enhancers for transdermal and dermal drug delivery systems. Drug Deliv 20:199

Cohen-Avrahami M, Libster D, Aserin A, Garti N (2011) Sodium diclofenac and cell-penetrating peptides embedded in HII mesophases: physical characterization and delivery. J Phys Chem B 115:10189

Davaran S, Hanaee J, Rashidi MR, Valiolah F, Hashemi M (2006) Influence of poly (ethylene glycol)-g-cyclodextrin complexes on stabilization and transdermal permeation of ascorbic acid. J Biomed Mater Res A 78:590

Desai P, Patlolla RR, Singh M (2010) Interaction of nanoparticles and cell-penetrating peptides with skin for transdermal drug delivery. Mol Membr Biol 27:247

Feingold KR (2007) Thematic review series: skin lipids the role of epidermal lipids in cutaneous permeability barrier homeostasis. J Lipid Res 48:2531

Feingold KR (2009) The outer frontier: the importance of lipid metabolism in the skin. J Lipid Res 50:s417

Filipowicz A, Wołowiec S (2011) Solubility and in vitro transdermal diffusion of riboflavin assisted by PAMAM dendrimers. Int J Pharm 408:152

Gopalakrishnan S, Pandey N, Tamiz AP, Vere J, Carrasco R, Somerville R, Tripathi A, Ginski M, Paterson BM, Alkan SS (2009) Mechanism of action of ZOT-derived peptide AT-1002, a tight junction regulator and absorption enhancer. Int J Pharm 365:121

Gupta M, Mahajan A, Babita K, Gupta S, Tiwary AK et al (2004) Inhibition of skin sphingosine synthesis: enhanced percutaneous permeation of 5-fluorouracil. Pharmzie 59:212

He W, Guo X, Zhang M (2008) Transdermal permeation enhancement of N-trimethyl chitosan for testosterone. Int J Pharm 356:82

He W, Guo X, Xiao L, Feng M (2009) Study on the mechanisms of chitosan and its derivatives used as transdermal penetration enhancers. Int J Pharm 382:234

Hou YW, Chan MH, Hsu HR, Liu BR, Chen CP, Chen HH, Lee HJ (2007) Transdermal delivery of proteins mediated by non-covalently associated arginine-rich intracellular delivery peptides. Exp Dermatol 16:999

Hsu T, Mitragotri S (2011) Delivery of siRNA and other macromolecules into skin and cells using a peptide enhancer. Proc Natl Acad Sci U S A 108:15816

Karande P, Jain A, Ergun K, Kispersky V, Mitragotri S (2005) Design principles of chemical penetration enhancers for transdermal drug delivery. Proc Natl Acad Sci U S A 102:4688

Kim YC, Ludovice PJ, Prausnitz MR (2007) Transdermal delivery enhanced by magainin pore-forming peptide. J Control Release 122:375

Kim YC, Late S, Banga AK, Ludovice PJ, Prausnitz MR (2008) Biochemical enhancement of transdermal delivery with magainin peptide: modification of electrostatic interactions by changing pH. Int J Pharm 362:20

Koren E, Torchilin VP (2012) Cell-penetrating peptides: breaking through to the other side. Trends Mol Med 18:385

Küchler S, Radowski MR, Blaschke T, Dathe M, Plendl J, Haag R, Schäfer-Korting M, Kramer KD (2009) Nanoparticles for skin penetration enhancement – a comparison of a dendritic core-multishell-nanotransporter and solid lipid nanoparticles. Eur J Pharm Biopharm 71:243

Kumar S, Sahdev P, Perumal O, Tummala H (2012) Identification of a novel skin penetration enhancement peptide by phage display peptide library screening. Mol Pharm 9:1320

Legendre JY, Rault I, Petit A, Luijten W, Demuynck I, Horvath S, Ginot YM, Cuine A (1995) Effects of β-cyclodextrins on skin: implications for the transdermal delivery of piribedil and a novel cognition enhancing-drug, S-9977. Eur J Pharm Sci 3:311

Li Y, Quan Y, Zang L, Jin M, Kamiyama F, Katsumi H, Yamamoto A, Tsutsumi S (2008) Transdermal delivery of insulin using trypsin as a biochemical enhancer. Biol Pharm Bull 31:1574

Li Y, Quan Y, Zang L, Jin M, Kamiyama F, Katsumi H, Tsutsumi S, Yamamoto A (2009) Trypsin as a novel potential absorption enhancer for improving the transdermal delivery of macromolecules. J Pharm Pharmacol 61:1005

Lim JM, Chang MY, Park SG, Kang NG, Song YS, Lee YH, Yoo YC, Cho WG, Choi SY, Kang SH (2003) Penetration enhancement in mouse skin and lipolysis in adipocytes by TAT-GKH, a new cosmetic ingredient. J Cosmet Sci 54:483

Loftsson T, Masson M (2001) Cyclodextrins in topical drug formulations: theory and practice. Int J Pharm 225:15

Lopes LB, Brophy CM, Furnish E, Flynn CR, Sparks O, Komalavilas P, Joshi L, Panitch A, Bentley MV (2005) Comparative study of the skin penetration of protein transduction domains and a conjugated peptide. Pharm Res 22:750

Lopez RFV, Collett JH, Bentley MV (2000) Influence of cyclodextrin complexation on the in vitro permeation and skin metabolism of dexamethasone. Int J Pharm 200:127

Lv HX, Zhang ZH, Wang XP, Cheng QQ, Wang W, Huang XH, Zhou JP, Zhang Q, Hou LL, Huo W (2011) A biomimetic chitosan derivates: preparation, characterization and transdermal enhancement studies of N-arginine chitosan. Molecules 16:6778

Matsuda H, Arima H (1999) Cyclodextrins in transdermal and rectal delivery. Adv Drug Deliv Rev 36:81

Matsuzaki K, Nakamura A, Murase O, Sugishita K, Fujii N, Miyajima K (1997) Modulation of magainin 2-lipid bilayer interactions by peptide charge. Biochemistry 36:2104

Nanjwade BK, Bechra HM, Derkar GK, Manvi FV, Nanjwade VK (2009) Dendrimers: emerging polymers for drug-delivery systems. Eur J Pharm Sci 38:185

Nasrollahi SA, Taghibiglou C, Azizi E, Farboud ES (2012) Cell-penetrating peptides as a novel transdermal drug delivery system. Chem Biol Drug Des 80:639

Patil S, Singh P, Szolar-Platzer C, Maibach H (1996) Epidermal enzymes as penetration enhancers in transdermal drug delivery. J Pharm Sci 85:249

Rothbard JB, Garlington S, Lin Q, Kirschberg T, Kreider E, McGrane PL, Wender PA, Khavari PA (2000) Conjugation of arginine oligomers to dendrimers A facilitates topical delivery and inhibition of inflammation. Nat Med 6:1253

Sapra B, Jain S, Tiwary AK (2008) Transdermal delivery of carvedilol containing glycyrrhizin and chitosan as permeation enhancers: biochemical, biophysical, microscopic and pharmacodynamic evaluation. Drug Deliv 15:443

Shah PP, Desai PR, Channer D, Singh M (2012) Enhanced skin permeation using polyarginine modified nanostructured lipid carriers. J Control Release 161:735

Sun M, Fan A, Wang Z, Zhao Y (2012) Dendrimer-mediated drug delivery to the skin. Soft Matter 8:4301

Tsai JC, Guy RH, Thornfeldt CR, Gao WN, Feingold KR, Elias PM (1996) Metabolic approaches to enhance transdermal drug delivery. 1. Effect of lipid synthesis inhibitors. J Pharm Sci 85:643

Uchida T, Kanazawa T, Takashima Y, Okada H (2011) Development of an efficient transdermal delivery system of small interfering RNA using functional peptides, Tat and AT-1002. Chem Pharm Bull 59:196

Umemura M, Ueda H, Tomono K, Nagai T (1990) Effect of diethyl-beta-cyclodextrin on the release of nitroglycerin from formulations. Drug Des Deliv 6:297

Ventura CA, Tommasini S, Falcone A, Giannone I, Paolino D, Sdrafkakis V, Mondello MR, Puglisi G (2006) Influence of modified dendrimers on solubility and percutaneous absorption of celecoxib through human skin. Int J Pharm 314:37

Venuganti VVK, Perumal OP (2009) Poly(amidoamine) dendrimers as skin penetration enhancers: influence of charge, generation, and concentration. J Pharm Sci 98:2345

Yang Y, Sunoqrot S, Stowell C, Ji J, Lee CW, Kim JW, Khan SA, Hong S (2012) Effect of size, surface charge, and hydrophobicity of poly(amidoamine) dendrimers on their skin penetration. Biomacromolecules 13:2154

Zasloff M (1987) Magainins, a class of antimicrobial peptides from Xenopus skin-isolation, characterization of two active forms, and partial c-DNA sequence of a precursor. Proc Natl Acad Sci U S A 84:5449

Zhou X, Liu D, Liu H, Yang Q, Yao K, Wang X, Wang L, Yang X (2010) Effect of low molecular weight chitosans on drug permeation through mouse skin: 1. Transdermal delivery of baicalin. J Pharm Sci 99:2991

Zi P, Yang X, Kuang H, Yang Y, Yu L (2008) Effect of HPβCD on solubility and transdermal delivery of capsaicin through rat skin. Int J Pharm 358:151

Synergy Between Chemical Penetration Enhancers

24

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and Adrian C. Williams

Contents

24.1 Introduction

In transdermal delivery, penetration enhancers are chemicals which improve percutaneous absorption of other molecules. They do so typically by transiently permeabilising the stratum corneum, the outermost layer of the skin which forms the primary barrier to the ingress of exogenous chemicals. Penetration enhancers are particularly useful in facilitating the dermal absorption of active pharmaceutical ingredients. To date, over 300 penetration enhancers have been studied, some of which have been found to act in synergy. Synergy between penetration enhancers presents exciting opportunities for formulation scientists to further improve the bioavailability of transdermal formulations whilst minimising adverse effects and costs. The purpose of this chapter is to provide an overview of synergistic penetration enhancer combinations and to summarise mechanisms of synergy.

24.1.1 Defining Synergy

The term 'synergy' is derived from the Greek word, *sunergos*, which means 'working together' (*sun*-, together; *ergon*, work). In popular usage, synergy is defined as the 'interaction or cooperation of two or more organisations, substances, or other agents to produce a combined effect greater

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N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_24

than the sum of their separate effects' (Oxford Dictionaries 2010). However, in specialised usage, such as in the scientific literature, a distinction has long been drawn between positive and negative synergy. Positive synergy refers to synergy as it is popularly understood, i.e. magnification of the combinatorial effect. By a pseudo-mathematical metaphor, we may call this relationship 2+2=5. Conversely, negative synergy refers to attenuation of effect when multiple entities work together (again, pseudo-mathematically, 2+2=3).

Nonetheless, with regard to penetration enhancers, negative synergy has often been ignored, e.g. 'a true synergistic effect is achieved when the combination of penetration enhancers elicits a greater effect than the individual components used alone' (Møllgaard 1993). As the field has advanced in recent years, the definition has evolved to include negative synergy, complete with mathematical formulae to quantify the effect (see Sect. 24.1.3).

The distinction between positive and negative synergy is important to this discussion because, although positive synergy in penetration enhancement is desirable and rightly deserves our focus, negative synergy may crucially determine co-formulation compatibility and thus should not be ignored. Thus, synergy can be defined as 'the phenomenon whereby multiple penetration enhancers working together results in an enhancement in skin permeability that is greater or less than the sum of enhancements obtained from these penetration enhancers working independently'. Accordingly, we will discuss both positive and negative synergy between penetration enhancers.

24.1.2 Discovering Synergy

The ideal combination of synergistically acting penetration enhancers should be highly potent at enhancing percutaneous absorption but nonirritant. Discovering synergistic combinations of penetration enhancers with these properties presents a significant challenge, since such combinations are relatively rare and also due to technological limitations in their detection.

Synergistic combinations of penetration enhancers are difficult to predict. Thus, synergy has often been discovered serendipitously through experimentation. However, it is possible to discover synergy systematically by screening permutations of penetration enhancer mixtures. To do this effectively, a reasonably large library of candidate penetration enhancers is usually necessary, and the speed of the screening technique is of essence given the large number of possible permutations, multiplied by the need to validate these against specific permeants. To illustrate the enormity of the task, it has been estimated that testing random selections of binary penetration enhancer combinations from the ~300 known penetration enhancers, at a modest 25 compositions per pair, will involve the evaluation of approximately a million different formulations (Karande et al. 2006a).

Traditionally, discovery of synergy has used static Franz diffusion cells. However, the process is laborious and time-consuming, as it requires the measurement and comparison of steady-state flux of multiple permeants with various penetration enhancer combinations. Since skin permeation experiments typically take hours or days to complete, with 10–20 experiments running in parallel, the discovery process can be extremely slow. Various degrees of automation can be introduced to improve the speed and manageability of this process, e.g. by using a flow-through diffusion cell (Bronaugh and Stewart 1985; Addicks et al. 1987) or an automated in vitro dermal absorption (AIDA) system (Moody and Martineau 1990). Nonetheless, skin permeation experiments remain the rate-limiting step in these methods. This, coupled with the need for large numbers of skin samples and the time needed to achieve steady-state flux, makes these methods inefficient at screening large libraries of penetration enhancer combinations for synergy (Karande and Mitragotri 2002).

More recently, high-throughput screening methods have been developed to rapidly discover synergistic combinations of penetration enhancers. The in vitro skin impedance-guided high-throughput (INSIGHT) screening method can rapidly assess the potency of 100 penetration

enhancer mixtures per square inch of skin sample simultaneously in an array format (Karande et al. 2004). It uses electrical conductivity (by measuring its reciprocal, i.e. electrical resistance) across the skin as a surrogate measure of skin permeability. The screening method is estimated to be over 100 times more efficient than the static Franz diffusion cell. In one study, the authors screened a library of over 5000 penetration enhancer mixtures using INSIGHT. Of these mixtures, 2 % were found to be synergistic. The results reiterate the rarity of synergistic penetration enhancer combinations and the value of high-throughput screening technology in their discovery. Only the leading hits from INSIGHT screening were then validated by skin permeation experiments using the static Franz diffusion cell and further tested for safety and bioavailability. This greatly reduces the need for skin permeation experiments that are the bottleneck in the conventional approach. Since electrical conductivity of the skin usually increases with increasing skin permeability, and can be measured instantaneously, this also speeds up the screening process considerably. Moreover, skin permeability estimated this way can be interpreted independently of the mechanism of action of the penetration enhancers, which is often poorly understood. Nevertheless, caution must be exercised to take into account the physicochemical properties of the permeant for which the penetration enhancer formulation is intended (Jain et al. 2006).

24.1.3 Quantifying Synergy

Clearly, to quantify synergy between penetration enhancers, penetration enhancement must be assessed. In the scientific literature, penetration enhancement has been reported in a number of ways, giving rise to numerous parameters, variously termed the 'permeability enhancement' (Johnson et al. 1996), 'flux enhancement' (Johnson et al. 1996), 'enhancement factor' (Kadir et al. 1988; Saleem et al. 2010), 'enhancement ratio' (Goodman and Barry 1988; Karande et al. 2004; Wang et al. 2008), 'enhancement potential' (Aboofazeli et al. 2002; Arora et al. 2010), 'enhancement index' (Williams and Barry 1991) and 'penetration index' (Barakat 2010). Despite the varied terminology, calculation of penetration enhancement is straightforward, and the various terms listed above are interchangeable in most cases. Nevertheless, it is important to clearly define the parameter used when reporting penetration enhancement.

For a given penetration enhancer (or combination of penetration enhancers), penetration enhancement can be calculated by comparing skin permeability in the presence and absence of the penetration enhancer(s). Skin permeability is usually represented by the permeability coefficient (K_p). The enhancement ratio (E_r) is the most commonly used parameter in the scientific literature. E_r can be described by Eq. 24.1:

$$E_r = \frac{K_{p,\text{enhancer}}}{K_{p,\text{control}}} \tag{24.1}$$

In addition, E_d can be defined as the difference between the permeability coefficients (Eq. 24.2). This parameter corresponds to the 'enhancement potential' defined by Arora et al. (2010).

$$E_d = K_{p,\text{enhancer}} - K_{p,\text{control}} \tag{24.2}$$

In Eqs. 24.1 and 24.2, $K_{p,\text{ enhancer}}$ and $K_{p,\text{ control}}$ denote the permeability coefficient in the presence and absence of the penetration enhancer(s), respectively. Generally, penetration enhancers increase K_p; therefore, $E_r > 1$ and $E_d > 0$.

Comparison of flux, J, has also been used instead of K_p where the permeant concentration in the formulation (C) can be considered constant, e.g. when a saturated solution of the permeant is used (Johnson et al. 1996; Aboofazeli et al. 2002). This is because, according to Eq. 24.3 (Williams 2003), any change in J under such circumstances is attributed solely to a proportional change in K_p:

$$J = K_p C \tag{24.3}$$

Electrical conductivity across the skin has also been used instead of K_p as a surrogate measure of skin permeability in calculating E_r and E_d. Electrical conductivity usually correlates well with K_p determined using static Franz diffusion

cells, especially for hydrophilic permeants (Karande et al. 2006b). E_r and E_d obtained using electrical conductivity measurements have shown good correlation ($r^2 \sim 0.9$) with each other; thus, both measures carry the same information (Arora et al. 2010). However, as E_r presents the information as a 'fold change' over $K_{p,\ control}$, it is more susceptible than E_d to the intrinsic variability in $K_{p,\ control}$ across different skin samples. For brevity, E_r and E_d are referred to in the following discussion collectively as the 'permeability enhancement', E, without discriminating one from the other.

Synergy can be expressed in terms of the permeability enhancement when a combination of penetration enhancers is used, relative to the permeability enhancement when the same penetration enhancers are used independently of each other. For example, consider two penetration enhancers, A and B, which, when used separately at a concentration of Y % (w/v), give permeability enhancements of E_A^Y and E_B^Y, respectively. When A and B are used in combination at a total concentration (A + B) of Y % (w/v), the formulation yields a combined permeability enhancement of $E_{A+B}^{X,Y}$. If the weight fraction of A in the mixture is given by X (where $X < 1$), then synergy, S, can be calculated using Eq. 24.4 (Karande and Mitragotri 2009):

$$S = \frac{E_{A+B}^{X,Y}}{X \cdot E_A^Y + (1-X) \cdot E_B^Y} \tag{24.4}$$

Equation 24.4 represents synergy in a binary system, i.e. one that contains two penetration enhancers. In theory, synergistic interactions may occur between any number of penetration enhancers. For formulations containing more than two enhancers, Eq. 24.4 can be condensed to Eq. 24.5 (Arora et al. 2010):

$$S = \frac{E^Y}{\sum_{i=1}^{n} \left(X_i \cdot E_i^Y \right)} \tag{24.5}$$

In Eq. 24.5, E^Y represents the permeability enhancement resulting from the mixture, Y represents the total concentration (in % w/v) of all penetration enhancers in the mixture combined, n is the number of penetration enhancers in the mixture, X_i is the weight fraction of the i-th penetration enhancer in the mixture (where each penetration enhancer is an iteration in the iteration series, and i is an integer denoting the iteration index or counter in that series; $1 \leq i \leq n$) and E_i^Y is the permeability enhancement obtained independently with Y % (w/v) of the i-th penetration enhancer.

From Eqs. 24.4 and 24.5, positive synergy is indicated if $S > 1$. Conversely, if $S < 1$, then negative synergy as indicated. On the other hand, if $S = 1$, the enhancement effect is additive, i.e. there is no synergy.

24.2 Synergistic Combinations of Penetration Enhancers

Scientific reports of synergy between penetration enhancers are scarce, and among the reports available, research methods vary greatly. Due to the scarcity and heterogeneity of data, generalisation is difficult. For this reason, the following discussion will be presented as a collection of case studies. It should be read with the following caveats in mind:

Firstly, whilst it is possible to quantify synergy using Eqs. 24.4 and 24.5, most reports of synergy in existing literature have not adopted this quantitative approach. Therefore, synergy will be discussed in the qualitative or quantitative terms as reported by the original authors.

Secondly, some penetration enhancers may function as vehicles while simultaneously exerting some penetration-enhancing effects. For example, propylene glycol (PG) is both a co-solvent and a mild penetration enhancer. In this role, it is usually used in large quantities, which may fundamentally alter the composition of the vehicle and thus the thermodynamic activity (e.g. solubility) of the permeant. Most reports of synergy involve such 'penetration-enhancing vehicles', but the vehicle effects are not usually decoupled from permeability enhancement. For the purpose of this discussion, 'penetration-enhancing vehicles' are considered penetration enhancers in their own right.

24.2.1 Positive Synergy

24.2.1.1 Terpenes and Alcohols

Terpenes are plant-derived components found in essential oils. As a penetration enhancer, terpenes are commonly formulated in ethanol and/or PG. Such formulations have consistently demonstrated improved permeability enhancements over terpene or alcohol alone. There have been various reports of positive synergy between terpenes and both alcohols in the transdermal delivery of both hydrophilic and lipophilic molecules.

In vivo experiments performed in rats have demonstrated synergistic enhancement between 1,8-cineole and ethanol, as well as between *l*-menthol and ethanol, in the skin penetration of diclofenac sodium from a gel ointment (Obata et al. 1991). Similarly, a combination of *d*-limonene and ethanol promoted the percutaneous absorption of indomethacin from a gel ointment in rats (Okabe et al. 1989).

Yamane et al. (1995) reported that formulations of 1,8-cineole, (+)-limonene, nerolidol and menthone, saturated in 80 % PG, enhanced skin permeability to 5-fluorouracil (a small hydrophilic molecule) by 24-, 4-, 18- and 21-fold, respectively. The permeability enhancement by terpenes increased with increasing concentrations of PG in the PG-water co-solvent system. Thus, synergistic action between the penetration enhancers was evidenced by the dependence of terpene activity on PG concentration.

The mechanism of synergy between terpenes and PG remains unclear. PG does not itself disrupt stratum corneum lipids at normal skin temperature nor does it enhance terpene uptake into the stratum corneum. However, there is evidence to suggest that PG enhances the disruption of stratum corneum lipids by terpenes (Cornwell et al. 1996). On the other hand, differential scanning calorimetry (DSC) and partitioning studies have suggested different mechanisms of action for the terpenes. Evidence produced by Yamane et al. (1995) showed that 1-8-cineole, nerolidol and menthone, but not (+)-limonene, disrupted stratum corneum lipids at physiological skin temperature. The enhancement effect of (+)-limonene was attributed to phase separation of the terpene in stratum corneum lipids. These terpene activities, in conjunction with increased drug partitioning caused by PG, were thought to be responsible for the synergistic activity.

24.2.1.2 Azone and Alcohols

Azone, or 1-dodecylazacycloheptan-2-one, is a synthetic penetration enhancer. It permeabilises the stratum corneum probably by inserting itself within the lipid bilayers or through phase separation within the stratum corneum lipids (Williams and Barry 2004).

Goodman and Barry (1988) studied the effect of Azone, PG and their combination on the skin penetration of 5-fluorouracil. Azone (2 %, w/w) in PG increased skin permeability nearly 100-fold, compared to 8.6-fold by 3 % (w/v) Azone in an aqueous vehicle (saline containing Tween 20 at 0.1 %, w/v). PG by itself did not significantly alter skin permeability. DSC results suggested that Azone and PG affected different penetration pathways in the stratum corneum—Azone reduced the resistance of the intercellular pathway by disrupting intercellular lipids, whilst PG affected the intracellular pathway. However, how this modulation of the different pathways gave rise to the drastic increase in skin permeability is not clear.

Although the exact mechanism of synergy is not yet fully elucidated, others have shown that Azone is most effective when used in combination with PG. Príborský et al. (1987) found that, for the transdermal penetration of insulin, a low concentration of Azone (0.1 %) in combination with 40 % PG was most effective. On the other hand replacing PG with polyethylene glycol (PEG), another polar vehicle, inhibited the penetration-enhancing activity of Azone on metronidazole (Wotton et al. 1985). This was explained by the observation that PEG, unlike PG, did not penetrate the skin readily and thus was unable to increase partitioning of metronidazole into the skin by co-diffusion (as PG probably did). Interestingly, pretreatment of skin samples with Azone followed by administration of trifluorothymidine (the permeant) in a PG vehicle resulted in 50–100 % greater penetration

Table 24.1 The flux, rate constant and enhancement ratio of various tenoxicam formulations across hairless guinea pig skin

Formulation	Flux ($\mu g \cdot cm^{-2} \cdot h^{-1}$)	k^a ($mg \cdot cm^{-2} \cdot min^{-½}$)	E^b
PG (20 %)	1.74 ± 0.17	10.44 ± 0.48	1
OA (15 %)	10.49 ± 0.88	8.07 ± 0.28	6
PG-OA (20 %:15 %)	35.39 ± 2.69	8.96 ± 0.05	20

Source: Larrucea et al. (2001)
Flux and release rate data are mean ± standard error
[a]*k* denotes the rate constant for tenoxicam release from the gel
[b]*E* values denote the enhancement ratios and are derived by dividing the flux obtained with the respective formulations by that obtained with PG

enhancement compared to treatment with formulations containing both Azone and PG without pretreatment (Sheth et al. 1986). The observation that fluid uptake in stratum corneum samples immersed in 2 % (w/w) Azone in PG was at least twice that in PG alone (Goodman and Barry 1988) also supports the notion that Azone facilitates PG penetration into the skin.

24.2.1.3 Fatty Acids and Alcohols

Fatty acids have been used extensively to enhance the skin penetration of a range of lipophilic and hydrophilic permeants, usually in an alcohol-based vehicle. Fatty acids and alcohols together produce a two-component system comprising a polar solvent and a nonpolar molecule. Such blends of penetration enhancers appear to work particularly well for hydrophilic permeants (Møllgaard 1993). Positive synergy between fatty acids and alcohols in such mixtures is well documented.

Cooper (1984) explored various combinations of alcohols and fatty acids in facilitating the skin penetration of nonpolar permeants. Using salicylic acid as a model permeant, and saturated solutions of the permeant to ensure equal thermodynamic activity, the author observed that a 1:1 molar ratio combination of oleic acid (OA) and PG increased steady-state flux 20-fold, compared to OA and PG alone (which themselves showed similar levels of enhancement). The author also reported synergistic effects between combinations of other alcohols (diethylene glycol, PEG) and fatty acids, but noted that unsaturated fatty acids were more potent penetration enhancers than saturated fatty acids in these combinations. Comparison between the flux of salicyclic acid across human epidermis and a silicone (dimethyl polysiloxane) membrane revealed that the interaction of PG with the skin, rather than solubilisation of the permeant per se, was responsible for the enhancement effect of PG.

Johnson et al. (1996) investigated the influence of ethanol and linoleic acid on the transdermal permeation of corticosterone across human cadaver skin. Whilst 50 % (v/v) ethanol gave a 40-fold increase in flux over PBS, PBS saturated with linoleic acid did not significantly alter flux. The use of 50 % (v/v) ethanol saturated with linoleic acid resulted in a flux enhancement of 900-fold over the base vehicle, phosphate-buffered saline (PBS). This enhancement was 20-fold that obtained using 50 % (v/v) ethanol in PBS. Thus, synergy between ethanol and linoleic acid was apparent in the 50 % (v/v) ethanol solution saturated with linoleic acid. The authors noted that this formulation increased both skin permeability and the solubility of corticosterone over the base vehicle, whilst in the absence of linoleic acid, 50 % (v/v) ethanol increased corticosterone solubility but reduced skin permeability.

Furthermore, Larrucea et al. (2001) reported that the flux of tenoxicam (1 %) across rat skin, from a gel containing 15 % OA and 20 % PG, was enhanced markedly compared to gels containing either OA or PG. Data from the study are shown in Table 24.1. It is noteworthy that flux increased by nearly threefold with the OA-PG formulation, compared with the sum of flux obtained separately with either OA or PG. The release kinetics of tenoxicam from the gel across a cellulose nitrate membrane was determined using static Franz diffusion cells. The release rates were similar for all gels and in fact slightly

Table 24.2 Flux of nicardipine formulated with various penetration enhancers across hairless guinea pig skin

Formulation	Flux[a] ($\mu g \cdot cm^{-2} \cdot h^{-1}$)	E_{PG}[b]	E_{OA}[b]	E_{DMI}[b]
PG	0.58 ± 0.40	–	–	–
OA	2.59[c]	–	–	–
DMI	1.39 ± 1.13	–	–	–
PG-OA (90 %:10 %, v/v)	115.15 ± 39.05	199	45	–
PG-OA-DMI (80 %:10 %:10 %, v/v)	471.37 ± 252.24	814	182	339

Source: Aboofazeli et al. (2002)
[a]Flux data are mean ± standard deviation
[b]*E* values represent flux obtained with solvent blend divided by flux obtained with the neat solvent indicated by the subscript
[c]Standard deviation unavailable

higher from the gel containing PG but not OA. Therefore, the flux was not limited by tenoxicam release from the gel. Rather, the penetration-enhancing effect of OA was found to be dependent on the concentration of PG. From these observations, it is clear that PG modulated the penetration enhancing of OA; thus, the two components worked together synergistically.

Synergy between OA, PG and dimethyl isosorbide (DMI) in a binary or ternary mixture has also been reported (Squillante et al. 1998a; Aboofazeli et al. 2002). Aboofazeli et al. (2002) compared the flux enhancements of saturated nicardipine solutions in PG-OA-DMI (80 %:10 %:10 %, v/v), PG-OA (90 %:10 %, v/v) and each neat solvent alone, through hairless guinea pig skin. Flux enhancements were calculated as the flux obtained with each solvent blend over that obtained with the respective neat solvents. The data are summarised in Table 24.2. The use of solvent blends resulted in fluxes 45- to >800-fold greater than the use of neat solvents. The ternary mixture (PG-OA-DMI) also gave greater flux enhancements over the binary mixture (PG-OA). Considering PG as the primary vehicle in these mixtures, the addition of OA and DMI had a marginal effect on the solubility of nicardipine compared to neat PG, yet it resulted in disproportionate enhancements in the flux of nicardipine from these mixtures. Similarly, Squillante et al. (1998a, b) reported remarkable flux enhancements for nifedipine through hairless mouse skin using a PG-OA-DMI (84 %:10 %:6 %, molar ratio) mixture optimised to achieve a balance between high flux and a short lag time.

Funke et al. (2002) compared the steady-state flux of the lipophilic molecules, estradiol and an estradiol analogue, through hairless mouse skin, in the presence of various compositions of PG, lauric acid and DMI. In this study, in contrast with other studies (Squillante et al. 1998a; Aboofazeli et al. 2002), DMI was regarded as an inert vehicle. Also, unsaturated solutions of the permeants, at 2 % (w/v), were used. Lauric acid (10 %, v/v in DMI) and PG alone exhibited similar levels of flux enhancement—both improved the steady-state flux by 10-fold for estradiol and by 20-fold for its analogue, compared with DMI. A combination of PG-lauric acid (90 %: 10 %, v/v) further enhanced steady-state flux by 22-fold over that achieved by lauric acid (10 %, v/v in DMI) or PG alone. Using this formulation, the steady-state flux through the intact skin was comparable to the steady-state flux through the skin from which the stratum corneum had been removed to allow unhindered permeation. Alternative combinations of PG with DMSO, or DMI with other penetration enhancers (DMSO, dodecanol), did not significantly improve flux. By measuring the steady-state flux of lauric acid and PG while varying their concentrations in the mixture, the authors determined that the steady-state flux of lauric acid and PG was dependent on the concentration of each other and that they mutually enhanced their permeation through the skin.

The exact mechanism of synergy between fatty acids and alcohols remains unclear in most cases. However, since alcohols are good solvents for lipophilic molecules and readily penetrate the skin, they may solubilise fatty acids to allow the

latter to co-permeate more readily. This is supported, for example, by the observation that the flux of nitroglycerin in aqueous ethanol (<70 %, v/v) through the skin was directly related to its solubility in ethanol and depended linearly on concomitant ethanol flux (Berner et al. 1989). Evidence also suggests that PG may enhance skin permeability by solvating keratin in corneocytes, which may enhance intracellular transport of permeants, in addition to enhanced intercellular transport by other lipid-disrupting penetration enhancers (Goodman and Barry 1988).

Additionally, it has been suggested that PG may prolong the retention of OA in the stratum corneum, thus potentiating the effect of OA on stratum corneum lipid disruption (Goodman and Barry 1988). Increased permeation of fatty acids and their lipid-disrupting action on the skin barrier may in turn instigate more rapid alcohol penetration to further propagate the effect. Indeed, Taguchi et al. (1999) showed that fatty acids enhanced PG distribution in the skin. Alcohols may also prevent hydrophilic permeants from forming hydrogen bonds within the skin, e.g. by displacing water molecules from binding and nonbinding sites (thus dehydrating it) or by competing with the permeants for hydrogen bonding sites (Goodman and Barry 1988). In the presence of a lipid-disrupting penetration enhancer such as OA, this effect should be significant only if the stratum corneum lipid structure has been disrupted by the penetration enhancer and is no longer limiting the permeant's rate of diffusion across the skin (Williams and Barry 1989).

24.2.1.4 Pyrrolidones

Pyrrolidones are chemicals containing a lactam ring. Pyrrolidone derivatives such as 2-pyrrolidone, *N*-methyl-2-pyrrolidone (MP) and 1-lauryl-2-pyrrolidone (LP) have been investigated as penetration enhancers. These are polar, aprotic and colourless solvents at room temperature.

Sasaki et al. (1990) studied the skin penetration of phenolsulfonphthalein (phenol red) under the influence of MP and LP across rat skin. Phenol red was prepared in isopropyl myristate (IPM) containing up to 2 mmol L^{-1} of MP and/or LP. In isolation, MP alone had low penetration-enhancing properties, whereas LP had high penetration-enhancing properties but was limited by a prolonged lag time. The steady-state flux obtained with MP-LP (at 2 mmol L^{-1} of each penetration enhancer) was 1.8-fold the steady-state flux obtained independently with MP and LP combined. The mixture also produced a shorter lag time (0.10 h) compared to MP (0.62 h) or LP (2.14 h) alone. The enhanced steady-state flux was maintained when the concentration of MP was reduced by 75 %, and the short lag time was maintained even with a 95 % reduction in MP concentration. Mechanistic studies revealed that LP enhanced skin penetration of MP, while MP facilitated the accumulation of LP in the skin.

24.2.1.5 Esters and Fatty Acids

The choline ester, lauroyl choline (LC), has been investigated as a penetration enhancer for transdermal, oral and rectal drug delivery (Alexander and Fix 1989; Loftsson et al. 1989). LC (2 %, w/v) has produced positive synergy when used with OA (2 %, v/v) formulated in a neat PG vehicle, in the skin penetration of 17β-estradiol and acyclovir (Loftsson et al. 1989). Whereas the addition of LC and OA resulted in a 6.9-fold and 1.3-fold increase in the permeability of hairless mouse skin to 17β-estradiol over PG alone, addition of both LC and OA to the PG vehicle yielded a 14-fold increase in skin permeability over PG alone. This combined enhancement was nearly twice the sum of independent enhancements attributed to LC-PG and OA-PG. The synergistic effect between LC and OA was even more pronounced in the delivery of acyclovir across hairless mouse skin. Whilst OA-PG enhanced skin permeability to acyclovir by 139-fold over PG alone, no detectable amount of acyclovir penetrated the skin when administered with LC-PG. However, LC-OA-PG enhanced skin permeability to acyclovir 404-fold over PG alone or nearly 3 times the sum of the independent effects derived from LC-PG and OA-PG. However, in the same study, a similar co-formulation of LC (5 %, w/v) and OA (5 %, v/v) in PG yielded an additive rather than synergistic effect on the skin penetration of nitroglycerin. It is also not clear from this study whether the apparent synergy between LC and OA can exist independently of PG.

24.2.1.6 Surfactants

Two of the most potent synergistic penetration enhancer combinations identified using the INSIGHT screening method were sodium lauryl ether sulfate (SLES; 0.35 %, w/v) in combination with 1-phenylpiperazine (PPZ; 0.15 %, w/v), and N-lauroyl sarcosine (NLS; 0.6 %, w/v) in combination with sorbitan monolaurate (Span® 20, S20; 0.4 %, w/v). These penetration enhancer mixtures were tested for their ability to enhance the permeation of inulin (a hydrophilic macromolecule) through porcine skin, using static Franz diffusion cells. SLES-PPZ and NLS-S20 yielded permeability enhancements of approximately 80-fold ($S=3.5$) and 30-fold ($S=2.5$), respectively, while exhibiting low irritation potentials (Karande et al. 2004).

SLES, NLS and S20 are well-established penetration enhancers in their own right (Aioi et al. 1993; López et al. 2000; Trabaris et al. 2012). SLES and NLS (as a sodium salt) are anionic surfactants, whereas S20 is a nonionic surfactant. The lesser known penetration enhancer, PPZ, is a member of the piperazine (nitrogen-containing ring) family of chemicals. Other derivatives of PPZ have been investigated as penetration enhancers, and PPZ itself has been used alone as a penetration enhancer for oral delivery (Whitehead et al. 2008). However, very little information is available about its mechanism of action as a penetration enhancer except that it purportedly enhances permeation through the paracellular route (Whitehead and University of California 2007).

It is interesting that among the most potent synergistic penetration enhancer mixtures identified using the INSIGHT screening method are three surfactants. Surfactants have multiple documented mechanisms of action. A main function of surfactants is the solubilisation of lipophilic molecules. Thus, in the skin, these surfactants may solubilise and disrupt stratum corneum lipids. Indeed, SLES and the closely related sodium lauryl sulfate (SLS) have both been shown to extract human epidermal lipids in vitro (Bahl 1985). Surfactants also form micelles above their critical micelle concentrations. SLS and SLES have been shown to permeabilise the skin when used above their critical micelle concentrations (Lu and Moore 2012); it has been suggested that micelles thus formed may additionally act as carriers to transport permeants across the stratum corneum (Moore et al. 2003). In the case of NLS-S20, the penetration enhancers formed aggregates suggestive of micelles in the formulation, although whether these aggregates were responsible for permeabilising the skin remains to be investigated (Karande et al. 2007).

24.2.1.7 Ureas and Alcohols

Some derivatives of urea (1-dodecylurea, 1,3-didodecylurea and 1,3-diphenylurea) have shown positive synergy with PG when used as saturated solutions (i.e. at approximately 2.6 mg mL^{-1}, 0.7 mg mL^{-1} and 15 mg mL^{-1}, respectively). Williams and Barry (1989) applied 5-fluorouracil and the urea-based penetration enhancers, both as saturated solutions, in liquid paraffin or PG. PG alone or the urea analogues (including urea itself) in liquid paraffin had no significant enhancing effect on the skin penetration of 5-fluorouracil. However, when administered in PG, the urea analogues (but not urea itself) enhanced skin permeability to 5-fluorouracil by sixfold compared to either PG alone or the urea analogues in liquid paraffin. Penetration enhancement was attributed mainly to increased diffusivity of the skin (which increased by ninefold), as partitioning of the hydrophilic 5-fluorouracil from the formulation into the skin was reduced by a factor of about 0.7 with the use of PG. PG may however potentiate the effect of the urea-based penetration enhancers, which are lipophilic in nature, by increasing their partitioning into the skin.

24.2.1.8 Surfactants and Alcohols

NLS and ethanol have demonstrated positive synergy in enhancing skin penetration of fluorescein, a hydrophilic and moderately large molecule (Kim et al. 2008). In isolation, ethanol and NLS showed little (threefold in the case of ethanol) or negligible effect on skin permeability to fluorescein, the electrical resistance across the skin or the structural organisation within the stratum corneum. However, in combination, the penetration

Table 24.3 Skin penetration of scutellarin across rat skin

Enhancer	J^a (μg·cm^{-2}·h^{-1})	T_l^b (h^{-1})	K_p^c (cm·h^{-1})	E^d
Ethanolamine	55.10 ± 6.65	1.86 ± 0.85	17.50 ± 1.80	7.11
Azone	42.40 ± 4.61	1.72 ± 0.29	0.18 ± 0.02	5.64
Menthol	27.80 ± 2.04	3.51 ± 0.67	3.73 ± 0.27	3.28
Ethanolamine + Azone	24.90 ± 2.03	3.31 ± 0.17	4.81 ± 0.39	3.01
Ethanolamine + menthol	29.80 ± 3.19	1.38 ± 0.32	16.30 ± 1.75	4.26

Source: Wang et al. (2008)
Data are mean ± standard error
[a] J steady-state flux
[b] T_l lag time
[c] K_p permeability coefficient
[d] E enhancement ratio, calculated as the ratio of cumulative amount of scutellarin penetrated over 12 h in the presence of the enhancer to the cumulative amount penetrated in its absence

enhancer mixture led to a 24-fold enhancement in fluorescein delivery. The optimal composition of this penetration enhancer mixture was 3 % (w/v) NLS (range studied: 0–3 %, w/v) and 25–50 % (v/v) ethanol (range studied: 0–100 %, v/v). Mechanistic studies revealed that NLS led to significant fluidisation of stratum corneum lipids and induced conformational changes in stratum corneum proteins, whereas ethanol extracted stratum corneum lipids. The authors suggest that stratum corneum lipid extraction by ethanol may increase NLS permeation into the stratum corneum, thus potentiating its effects on stratum corneum lipid fluidity and stratum corneum protein conformation.

24.2.1.9 Sulfoxides and Alcohols

Positive synergy between dimethyl sulfoxide (DMSO) and PG in enhancing percutaneous insulin absorption has been described (Príborský et al. 1987). Insulin, being a macromolecule, does not normally penetrate the skin to any appreciable degree. Indeed, in the absence of PG and DMSO, insulin penetration across newborn pig skin was extremely low and was undetectable in some instances using the enzyme-linked immunosorbent assay employed. PG (20 %) and 5 % DMSO independently enhanced percutaneous absorption of insulin by approximately three- and sixfold, respectively. In comparison, a mixture of 20 % PG and 5 % DMSO resulted in a nearly 70-fold enhancement over non-enhanced insulin penetration or approximately 7 times the sum of the independent enhancements brought about by 20 % PG and 5 % DMSO. The base vehicle in this case comprised PG and sodium citrate buffer (pH 3.0). The authors did not comment on the mechanism of synergy.

24.2.2 Negative Synergy

24.2.2.1 Amines and Azone

Wang et al. (2008) reported negative synergy between ethanolamine and Azone, as well as between ethanolamine and menthol (Sect. 24.2.2.2), in a vehicle comprising IPM-ethanol (4:1, w/w). In this study, ethanolamine was used at a 1:1 molar ratio to the permeant, scutellarin, which was used in saturation. Azone was used at 5 % (w/w). The enhancement ratio was calculated from the cumulative amount of scutellarin penetrated across rat skin over 12 h in the presence or absence of the penetration enhancer(s). The results are summarised in Table 24.3. Ethanolamine and Azone were both effective penetration enhancers when used in isolation. However, in combination, ethanolamine and Azone decreased steady-state flux and increased the lag time, both by approximately twofold, compared to either penetration enhancer alone. This significantly reduced the amount of scutellarin penetrated and thus the enhancement ratio. The permeability coefficient was also reduced by over threefold compared to ethanolamine alone. In this instance, it was proposed that the amines formed ion pairs with the permeant. Such ion pairs altered the physicochemical properties of the permeant, such as lipophilicity, making them more

favourable for skin penetration. However, the authors did not discuss potential vehicle effects or possible mechanisms of the synergy.

24.2.2.2 Amines and Terpenes

In the same study described in Sect. 24.2.2.1, Wang et al. (2008) also reported that a combination of ethanolamine and menthol significantly reduced steady-state flux and the enhancement ratio compared to ethanolamine alone (Table 24.3). Meanwhile, the lag time was shortened, whilst the permeability coefficient appeared to be unaffected. Menthol was used at 5 % (w/w) in this study.

Conclusions

Synergy between penetration enhancers can be harnessed to greatly improve percutaneous absorption without necessitating a large increase in the dose of penetration enhancers used. However, the choice of combination will depend on various formulation considerations, including the mechanism of synergy and the physiochemical properties of the permeant. In designing a formulation containing multiple penetration enhancers, positive as well as negative synergy should be considered to ensure optimal potency, safety and compatibility. However, there are few reports of negative synergy in the existing literature compared to positive synergy. This is probably attributable to some degree to reporting bias arising from the traditional focus on positive synergy. Better documentation of synergy (e.g. more quantitative reporting covering negative as well as positive synergy) will clearly be beneficial. The molecular mechanisms underlying most synergistic combinations of penetration enhancers have also not been fully elucidated. It is interesting to note that, judging from the case studies summarised in this chapter, the majority of synergistic actions reported in the existing literature involve alcohols that are often used as vehicles. This may or may not indicate significant vehicle effects. In any case, a better understanding of the underlying molecular mechanisms may help in the selection of synergistic combinations of penetration enhancers.

References

Aboofazeli R, Zia H, Needham TE (2002) Transdermal delivery of nicardipine: an approach to in vitro permeation enhancement. Drug Deliv 9:239–247

Addicks WJ, Flynn GL, Weiner N (1987) Validation of a flow-through diffusion cell for use in transdermal research. Pharm Res 4:337–341

Aioi A, Kuriyama K, Shimizu T, Yoshioka M, Uenoyama S (1993) Effects of vitamin E and squalene on skin irritation of a transdermal absorption enhancer, lauroylsarcosine. Int J Pharm 93:1–6

Alexander J, Fix JA (1989) Enhancement of absorption of drugs from gastrointestinal tract using choline ester salts. US Patent 4,822,773

Arora A, Kisak E, Karande P, Newsam J, Mitragotri S (2010) Multicomponent chemical enhancer formulations for transdermal drug delivery: more is not always better. J Control Release 144:175–180

Bahl M (1985) ESCA studies on skin lipid removal by solvents and surfactants. J Soc Cosmet Chem 36:287–296

Barakat NS (2010) Evaluation of glycofurol-based gel as a new vehicle for topical application of naproxen. AAPS PharmSciTech 11:1138–1146

Berner B, Mazzenga GC, Otte JH, Steffens RJ, Juang RH, Ebert CD (1989) Ethanol: water mutually enhanced transdermal therapeutic system II: skin permeation of ethanol and nitroglycerin. J Pharm Sci 78:402–407

Bronaugh RL, Stewart RF (1985) Methods for in vitro percutaneous absorption studies IV: the flow-through diffusion cell. J Pharm Sci 74:64–67

Cooper ER (1984) Increased skin permeability for lipophilic molecules. J Pharm Sci 73:1153–1156

Cornwell PA, Barry BW, Bouwstra JA, Gooris GS (1996) Modes of action of terpene penetration enhancers in human skin; differential scanning calorimetry, small-angle X-ray diffraction and enhancer uptake studies. Int J Pharm 127:9–26

Funke AP, Schiller R, Motzkus HW, Günther C, Müller RH, Lipp R (2002) Transdermal delivery of highly lipophilic drugs: in vitro fluxes of antiestrogens, permeation enhancers, and solvents from liquid formulations. Pharm Res 19:661–668

Goodman M, Barry BW (1988) Action of penetration enhancers on human skin as assessed by the permeation of model drugs 5-fluorouracil and estradiol. I. Infinite dose technique. J Invest Dermatol 91:323–327

Jain A, Karande P, Mitragotri S (2006) High throughput screening of transdermal penetration enhancers: opportunities, methods and applications. In: Smith EW, Maibach HI (eds) Percutaneous penetration enhancers. CRC/Taylor & Francis, Boca Raton, pp 319–334

Johnson ME, Mitragotri S, Patel A, Blankschtein D, Langer R (1996) Synergistic effects of chemical enhancers and therapeutic ultrasound on transdermal drug delivery. J Pharm Sci 85:670–679

Kadir R, Stempler D, Liron Z, Cohen S (1988) Penetration of adesanosine into excised human skin from binary vehicles: the enhancement factor. J Pharm Sci 77: 409–413

Karande P, Mitragotri S (2002) High throughput screening of transdermal formulations. Pharm Res 19:655–660
Karande P, Mitragotri S (2009) Enhancement of transdermal drug delivery via synergistic action of chemicals. Biochim Biophys Acta 1788:2362–2373
Karande P, Jain A, Mitragotri S (2004) Discovery of transdermal penetration enhancers by high-throughput screening. Nat Biotechnol 22:192–197
Karande P, Jain A, Mitragotri S (2006a) Insights into synergistic interactions in binary mixtures of chemical permeation enhancers for transdermal drug delivery. J Control Release 115:85–93
Karande P, Jain A, Mitragotri S (2006b) Relationships between skin's electrical impedance and permeability in the presence of chemical enhancers. J Control Release 110:307–313
Karande P, Jain A, Arora A, Ho MJ, Mitragotri S (2007) Synergistic effects of chemical enhancers on skin permeability: a case study of sodium lauroylsarcosinate and sorbitan monolaurate. Eur J Pharm Sci 31:1–7
Kim Y-C, Park J-H, Ludovice PJ, Prausnitz MR (2008) Synergistic enhancement of skin permeability by N-lauroylsarcosine and ethanol. Int J Pharm 352: 129–138
Larrucea E, Arellano A, Santoyo S, Ygartua P (2001) Combined effect of oleic acid and propylene glycol on the percutaneous penetration of tenoxicam and its retention in the skin. Eur J Pharm Biopharm 52:113–119
Loftsson T, Somogyi G, Bodor N (1989) Effect of choline esters and oleic acid on the penetration of acyclovir, estradiol, hydrocortisone, nitroglycerin, retinoic acid and trifluorothymidine across hairless mouse skin in vitro. Acta Pharm Nord 1:279–286
López A, Llinares F, Cortell C, Herráez M (2000) Comparative enhancer effects of Span20 with Tween20 and Azone on the in vitro percutaneous penetration of compounds with different lipophilicities. Int J Pharm 202:133–140
Lu G, Moore DJ (2012) Study of surfactant-skin interactions by skin impedance measurements. Int J Cosmet Sci 34:74–80
Møllgaard B (1993) Synergistic effects in percutaneous enhancement. In: Walters KA, Hadgraft J (eds) Pharmaceutical skin penetration enhancement. M. Dekker, New York, pp 229–242
Moody RP, Martineau PA (1990) An automated in vitro dermal absorption procedure: I. Permeation of (14) C-labelled N, N-diethyl-m-toluamide through human skin and effects of short-wave ultraviolet radiation on permeation. Toxicol In Vitro 4:193–199
Moore PN, Puvvada S, Blankschtein D (2003) Challenging the surfactant monomer skin penetration model: penetration of sodium dodecyl sulfate micelles into the epidermis. J Cosmet Sci 54:29–46
Obata Y, Takayama K, Machida Y, Nagai T (1991) Combined effect of cyclic monoterpenes and ethanol on percutaneous absorption of diclofenac sodium. Drug Des Discov 8:137–144
Okabe H, Takayama K, Ogura A, Nagai T (1989) Effect of limonene and related compounds on the percutaneous absorption of indomethacin. Drug Des Deliv 4: 313–321
Oxford Dictionaries (2010) Synergy. In: Oxford dictionaries. Oxford University Press. http://oxforddictionaries.com/definition/english/synergy. Accessed 13 Sept 2012
Príborský J, Takayama K, Nagai T, Waitzová D, Elis J (1987) Combination effect of penetration enhancers and propylene glycol on in vitro transdermal absorption of insulin. Drug Des Deliv 2:91–97
Saleem M, Bala S, Liyakat, Aeajaz A (2010) Effect of different carriers on in vitro permeation of meloxicam through rat skin. Indian J Pharm Sci 72:710–718
Sasaki H, Kojima M, Nakamura J, Shibasaki J (1990) Enhancing effect of combining two pyrrolidone vehicles on transdermal drug delivery. J Pharm Pharmacol 42:196–199
Sheth NV, Freeman DJ, Higuchi WI, Spruance SL (1986) The influence of Azone, propylene glycol and polyethylene glycol on in vitro skin penetration of trifluorothymidine. Int J Pharm 28:201–209
Squillante E, Maniar A, Needham T, Zia H (1998a) Optimization of in vitro nifedipine penetration enhancement through hairless mouse skin. Int J Pharm 169:143–154
Squillante E, Needham T, Maniar A, Kislalioglu S, Zia H (1998b) Codiffusion of propylene glycol and dimethyl isosorbide in hairless mouse skin. Eur J Pharm Biopharm 46:265–271
Taguchi K, Fukushima S, Yamaoka Y, Takeuchi Y, Suzuki M (1999) Enhancement of propylene glycol distribution in the skin by high purity cis-unsaturated fatty acids with different alkyl chain lengths having different double bond position. Biol Pharm Bull 22: 407–411
Trabaris M, Laskin JD, Weisel CP (2012) Effects of temperature, surfactants and skin location on the dermal penetration of haloacetonitriles and chloral hydrate. J Expo Sci Environ Epidemiol 22:393–397
Wang M, Fang L, Ren C, Li T (2008) Effect of ion-pairing and enhancers on scutellarin skin permeability. J Pharm Pharmacol 60:429–435
Whitehead KA, University of California (2007) Safe and effective methods for improving the oral delivery of macromolecules. University of California, Santa Barbara
Whitehead K, Karr N, Mitragotri S (2008) Safe and effective permeation enhancers for oral drug delivery. Pharm Res 25:1782–1788
Williams AC (2003) Transdermal and topical drug delivery from theory to clinical practice. Pharmaceutical Press, London
Williams AC, Barry BW (1989) Urea analogues in propylene glycol as penetration enhancers in human skin. Int J Pharm 56:43–50
Williams AC, Barry BW (1991) The enhancement index concept applied to terpene penetration enhancers for human skin and model lipophilic (oestradiol) and hydrophilic (5-fluorouracil) drugs. Int J Pharm 74:157–168
Williams AC, Barry BW (2004) Penetration enhancers. Adv Drug Deliv Rev 56:603–618

Wotton PK, Møllgaard B, Hadgraft J, Hoelgaard A (1985) Vehicle effect on topical drug delivery. III. Effect of Azone on the cutaneous permeation of metronidazole and propylene glycol. Int J Pharm 24:19–26

Yamane MA, Williams AC, Barry BW (1995) Terpene penetration enhancers in propylene glycol/water co-solvent systems: effectiveness and mechanism of action. J Pharm Pharmacol 47:978–989

Toxicological Aspects of Chemical Penetration Enhancers

25

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25.1 Introduction

Dermal delivery is perhaps one of the oldest methods of drug administration. In ancient times cutaneous wounds and other skin problems were treated with animal fats and resins. The dermal as well as the transdermal drug delivery concept is based on the ability of certain substances to increase the cutaneous permeability. The skin is the largest organ of the body in mammals, and it is also the first line of defense against foreign materials. For this reason not all substances can reach the body system through intact skin. The skin is composed of three layers, epidermis being the outermost layer, which is supported by a nourishing layer of fibroelastic tissue called the dermis, and a variable deep layer, composed mainly of adipose tissue called the hypodermis or subcutis. The epidermis is composed of several layers (called strata) of epithelial cells. The *stratum corneum* is the most external stratum of the epidermis. This is composed of a thick lamella of keratin and keratinized cells embedded in lipid matrix,

N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8_25

which function as a practically impermeable barrier that makes it difficult for dermal and transdermal delivery of drugs. The goal of a penetration enhancer is to increase the permeability of the *stratum corneum* without altering the physical integrity of this layer. In order to meet this goal, chemical penetration enhancers have been used, which must have specific characteristics, such as being pharmacologically inert, nonirritant, nonimmunogenic (non-allergenic or non-sensitizing), and nontoxic to the skin or to the body system as a whole. Although all the mentioned aspects are important, the main objective of this chapter is to describe some of the reported toxicological features for a few of the currently most utilized skin penetration enhancers. Among the most used penetration enhancers are the following chemical classes: terpenes, fatty acids, fatty alcohols, alcohols, glycols, laurocapram (Azone®), sulfoxides, pyrrolidones, and surfactants.

25.2 Terpenes

Terpenes are natural substances contained in plant essential oils. These compounds are designated by the general formula $(C_5H_8)_n$. However, these nonaromatic compounds may also include oxygen atoms. This class of substances may provide the right balance between safety and penetration-enhancing ability (Ahad et al. 2009 and Narishetty and Panchagnula 2004a). The mechanism of action of terpenes is based either on their ability to increase the *stratum corneum* lipid fluidity or on the perturbation of the barrier integrity of the *stratum corneum* (Gao and Singh 1998). Some potential mechanisms by which terpenes operate is by disrupting the bilayer lipids in the cellular membrane of keratinocytes or by their interaction with intracellular proteins (Williams and Barry 2012). The terpenes are widely used in the pharmaceutical and food industry, mainly as medicines, flavoring, and fragrance agents. Based on its penetration-enhancing ability, eucalyptol (1,8-cineole) has been reported to be one of the most potent terpenes (Ahad et al. 2009 and Narishetty and Panchagnula 2004a). Cineole's major use in the pharmaceutical industry is as a flavoring in expectorants and cough syrups. It is used in the cosmetic industry as a flavoring in toothpastes and mouthwashes, as well as in perfumery. It is also used in the food industry (e.g., chewing gum and ice cream) (Medi et al. 2006). This substance was the most promising chemical penetration enhancer among terpenes when tested in vitro across rat skin for propanolol, indomethacin, imipramine, urea, and zidovudine (Amnuaikit et al. 2005; Ogiso et al. 1995; Narishetty and Panchagnula 2004b; Jain et al. 2002). Cineole may induce several systemic reactions in humans when ingestion or exposure to vapors occurs. Among the reactions that have been reported in humans is transient coma with symptoms including epigastric burning, nausea, vomiting, vertigo, ataxia, muscle weakness, occasional cyanosis, respiratory edema, miosis, and convulsions (Gosselin et al. 1976). Other effects include fever, albuminuria, painful urination, hematuria, transient renal lesions, and respiratory failure with death as final outcome (Gosselin et al. 1976). Fatalities have occurred with oral doses as low as 3.5 mL given neat. However, recovery has occurred after an oral dose as high as 30 mL (Gosselin et al. 1976). The probable lethal oral dose in humans is 50–500 mg/kg (between 1 teaspoon and one ounce for a 70 kg person). Exposure to vapor causes irritation to the human eye, which is perceptible at 175 ppm in air, and is reported to be unpleasant at 720–1,100 ppm. Slight transient damage to corneal epithelium may be induced by direct contact with cineole (Grant 1986). Apparently nonlethal doses of this terpene induce hepatic microsomal enzymes (Gosselin et al. 1976). In general, 1,8-cineole was of low to moderate acute oral toxicity in rodents, causing central nervous system and respiratory effects and inducing increased hepatic enzyme activity and bile secretion. According to De Vincenzi et al. (2002), the mice are less susceptible than rats to the toxicity of 1,8-cineole. This author found that toxicity may be induced in rats dosed orally with this terpene at 600 mg/kg, while a similar toxic profile may be induced in mice when dosed orally at 1200 mg/kg (De Vincenzi et al. 2002). The design of this study did not allow the establishment of a no observable effect level (NOEL) or no observable adverse effect level

(NOAEL) for cineole in mice or rats. The oral LD_{50} of eucalyptol in rats is 2480 mg/kg. In mice the lowest published lethal dose (LD_{50}) was established at 50 mg/kg when dosed subcutaneously, and LD_{50s} of 100 and 2500 mg/kg were observed when dosed by intramuscular or oral routes, respectively (NTP-PAFA 2006). In dogs the LD_{50} was established at 1500 mg/kg when administered subcutaneously (NTP 1991). In rats, eucalyptol induces increased levels of glucuronyl transferase with no changes in histopathology such as no observed effects on the size of liver cell endoplasmic reticulum. The activity of other liver enzymes, including P450 cytochromes CYP3A1, CYP3A2, CYP2B1, CYP2B2, methoxyresorufin O-demethylase (MROD), benzyloxyresorufin O-debenzylase (BROD), and pentoxyresorufin O-depenylase (PROD), was increased in male rats when dosed orally at 400 mg/kg of eucalyptol (NTP-PAFA 2006). In a different study, performed also in rats, 1,8-cineole increased the production of β-2 microglobulin in the kidney of male rats when administered orally for 28 days at a dose of 500 mg/kg (Kristiansen and Madsen 1995). The genotoxic potential of 1,8-cineole was assessed in AMES (bacterial reverse mutation test or *Salmonella*/microsome test) (Haworth et al. 1983), chromosomal aberration, and sister chromatid exchange tests (Galloway et al. 1987), with negative genotoxicity for the first two and positive results for the last test. Cineole was not carcinogenic when dosed at 12 g/kg for 8 weeks in a mouse primary lung tumor model (Stoner et al. 1973.). Cineole showed teratogenic effects in rats when dosed at 2 g/kg during gestation days 19–22 (NTP-PAFA 2006).

Regardless of the potential systemic toxic effects of eucalyptol (1,8-cineole), this terpene was not irritant when applied in solution to the skin of human volunteers or when applied to rabbit skin. However, nasal irritation and rapid heartbeat occurred in some children after its direct instillation into the nostrils. Cineole also induced allergic skin reactions in several turpentine-sensitive subjects (BIBRA 1991). These reactions may be explained by the association of 1,8-cineole-induced itching in mice with the mast cell degranulation, which possibly involves opioidergic and adenosinergic mechanisms (Santos and Rao 2002). In summary, 1,8-cineole is well tolerated when applied onto the skin, but it may cause low to moderate systemic effects when ingested or administered via subcutaneous or intramuscular injection.

L-Menthol is another terpene that is widely used as a flavoring, disinfectant, and cooling compound in confectionary products, liquors, chewing gums, toothpastes, cosmetics, and common cold ointments for human purposes (OECD-SIDS 2003). In countries such as Canada, l-menthol is registered for control of mites in apiculture (OECD-SIDS 2003).

L-menthol is a food ingredient generally recognized as safe (GRAS), and not much monitoring of its toxicological potential is performed. The most common routes of consumer exposure to l-menthol are the oral and dermal; mainly when mentholated products are used. Other routes of exposure are inhalation from mentholated cigarettes or mentholated cleaning products. Maximum usage levels in food, cosmetic, and pharmaceutical products have been established. The recommended levels for the addition of l-menthol to products are up to 2 % for oral care products, up to 4 % for pharmaceuticals, up to 0.45 % to cigarettes, up to 0.3 % to tobacco, and up to 1 % to perfumed products (OECD-SIDS 2003).

In humans the l-menthol is well absorbed by the oral route. The absorption through skin is slower than oral absorption. It appears that absorption by inhalation is also very efficient (OECD-SIDS 2003). The toxicological potential of l-menthol has been studied in humans and in laboratory species. In rats, skin irritation, such as slight subepidermal edema and swelling in collagen fibers, was observed at 5 % l-menthol formulations (Narishetty and Panchagnula 2004b). In humans, the ingestion of high doses of l-menthol may cause abdominal pain, convulsions, nausea, vomiting, vertigo, ataxia, drowsiness, and coma (OECD-SIDS 2003). Some of these symptoms were observed in children ingesting about 200–250 mg/kg. The symptoms were fully reversible by 4 days post exposure (OECD-SIDS 2003). The ingestion of

8000–9000 mg of l-menthol by three volunteers (corresponding dose of approximately 120 mg/kg) caused a cold burning sensation in the mouth, throat, and esophagus; a cold sensation in mucous membranes of the nose and on the skin of the hand and feet; and fatigue (OECD-SIDS 2003). Mild abdominal discomfort was reported when l-menthol was dosed at 20 mg/kg (OECD-SIDS 2003). Adverse neurological effects (central nervous system or CNS) were observed in a 13-year-old boy exposed by to approximately 200 mg of menthol by inhalation (O'Mullane et al. 1982). Similar effects were reported in a woman after smoking 80 mentholated cigarettes for 3 months. This woman showed insomnia, unsteady gait, mental confusion, depression, vomiting, and cramp in the legs (Luke 1962). Reflex apnea was induced in children after administration of l-menthol in their nostrils. The clinical manifestations were laryngospasm, spasm of the glottis or instant collapse, dyspnea, apnea, unconsciousness, cyanosis, and hyperextended extremities (OECD-SIDS 2003). It is possible that these symptoms are not a result of a direct poisoning effect, but a reflex reaction of the trigeminal nerve (OECD-SIDS 2003).

In laboratory animals, the liver weights of rats dosed orally with menthol at a dose of 200 mg/kg for 28 days were increased, and a non-dose-related vacuolization of hepatocytes was also reported. No toxicological effects were observed at doses lower than 200 mg/kg. However, it was not possible to establish a NOEL in this study. Therefore, the 200 mg/kg should be the established NOAEL in this study, based on the described hepatic effects (OECD-SIDS 2003). Rats exposed for 71–79 days to l-menthol vapors presented pulmonary and tracheal irritation, but no toxicological systemic effects (OECD-SIDS 2003). In subchronic studies (lasting for 13 weeks) performed in rats and mice, this compound did not induce any alterations in organ weights when dosed up to 937/998 mg/kg in male/female rats or up to 3913/4773 mg/kg in male/female mice. A slight increase in severity of spontaneous interstitial nephritis was reported after microscopic examination of kidneys from the male rats dosed at the highest dose level. Reduction in body weight gain was the only effect observed in mice when dosed at the highest dose. The NOAELs derived from these studies were 937 and 998 mg/kg for the male and female rats, respectively. The NOAELs in mice were 1956 and 2386 mg/kg in males and females, respectively (OECD-SIDS 2003).

A chronic study (103 weeks) in rats dosed orally with l-menthol (about 188 and 375 mg/kg/day) reported minimal test article-related effects that included a slight increase in spontaneous chronic nephritis in male rats at both dose levels and a slightly reduced body weight in females. The NOAELs in this study were 375 mg/kg in the males and 188 mg/kg in the females (OECD-SIDS 2003). A NOAEL of 667 mg/kg was established in a chronic study performed in mice dosed orally for 103 weeks with d-/l-menthol at dose levels of 334 and 667 mg/kg (OECD-SIDS 2003).

Menthol has been reported to be non-genotoxic and noncarcinogenic based on results from standard genotoxicity and carcinogenicity tests. No information exists regarding the potential of l-menthol to induce reproductive toxicity. However, this compound proved to be non-teratogenic when administered orally to species such as rat, rabbit, mouse, and hamster at doses ranging from 185 to 425 mg/kg, which are not maternally toxic doses (OECD-SIDS 2003). All menthol isomers present a similar safety-toxicology profile.

In summary, terpenes are penetration enhancers with an acceptable safety profile when administered onto the skin or when administered at low-dose levels orally or by inhalation in adults and individuals provided they are not allergic to terpenes or to the delivered compounds. Based on results of some studies reported above, special care is needed when children or susceptible individuals are exposed to these compounds by inhalation or by ingestion of pure compound.

25.3 Fatty Acids

Fatty acids consist of long hydrocarbon chains with a terminal carboxyl group. Lauric acid, myristic acid, palmitic acid, linolenic acid, and oleic acid are some of the fatty acids that have

been used to increase the percutaneous absorption of hydrophilic and lipophilic drugs. Oleic acid is perhaps the most popular of these long-chain fatty acids. Oleic acid is an 18-carbon *cis*-mono unsaturated fatty acid. This review will be focused on the toxicological potential of some of the fatty acids used as penetration enhancers.

Increased cutaneous penetration of a wide variety of drugs has been demonstrated when oleic acid is used as an enhancer. Among those drugs are 5-fluorouracil, salicylic acid, tamoxifen, estradiol, and progesterone (Gao and Singh 1998). Although extensive efforts to define the mechanism of action of this penetration enhancer have been devoted, it has not been possible to fully describe how oleic acid can increase the permeability of the *stratum corneum*. However, based on existing reports, it is clear that the penetration-enhancing effect of this enhancer on human skin is in part due to the interaction with and the modification of lipid domains in the *stratum corneum*, as would be expected for a long-chain fatty acid with a *cis* configuration (Barry 1991). This interaction and modification of lipids in the *stratum corneum* consequently increases the drug flux in the epidermis (Barry 1991). Touitou and collaborators (2002) demonstrated by scanning electron microscopy that skin treated with 10 % oleic acid in ethanolic solution induced the generation of pores on the surface of epidermal keratinocytes (Touitou et al. 2002).

The potential toxicity of oleic acid and other fatty acids has also been studied. Erythema and edema are the most common effects reported after cutaneous administration of lauric acid, palmitic acid, myristic acid, stearic acid, or oleic acid in 5 % (w/v) alcohol solutions (Liebert 1987). Boelsma and collaborators (1996) reported that oleic acid induced skin irritation and inflammatory infiltrate as consequence of cytokine activation (Boelsma et al. 1996). Other effects in the skin are follicular epidermal hyperplasia produced after daily (6 times per week for 1 month) topical application of undiluted commercial grade oleic acid in mice (Liebert 1987). Myristic acid induced thinning of collagen fibers and dermal lymphocytic-histiocytic infiltration after topical administration in depilated skin of rabbits (Liebert 1987). Local cutaneous edema was induced by stearic acid in rats after topical application of a cosmetic containing 2 % of this acid. The same effect was observed in rabbits receiving 20 applications of 2 mL/kg of 2 % stearic acid (Liebert 1987). The topical application of lauric acid in intact and abraded skin of rabbits induced erythema at 72 h posttreatment and also induced blanching and coriaceous tissue in the abraded areas.

Studies to assess photosensitization have been conducted using variable concentrations (from 25 to 100 %) of stearic acid topically applied to guinea pigs. No photosensitizing effect was found at any of the dose levels tested (Liebert 1987).

The potential of oleic acid to induce skin comedones was evaluated. Very large comedones were induced by UVA- irradiated and nonirradiated oleic acid in New Zealand white rabbits. In this study, the lipid peroxide concentration was positively correlated with the degree of comedones formation (Liebert 1987).

Studies have been conducted to assess the potential of fatty acids to induce ocular irritation. No irritation or minimal ocular irritation was produced in rabbits after ophthalmic administration of 0.1 mL of oleic acid. The ocular instillation of commercial grade lauric acid caused corneal opacity, mild conjunctivitis, and iritis during the first 72 h post dosing. However, this effect was not observed when the same acid was administered as an 8.7 % aqueous dilution in rabbits or as 1 % aqueous preparation of a soap containing 1.95 % of the acid. No ocular irritation was induced by administration of commercial grade palmitic or stearic acid in rabbits, while slight conjunctival irritation was induced by administration of commercial grade myristic acid in the same animal species (Liebert 1987).

In studies administering oleic acid by ways different than topical administration, other effects associated to the test article were reported. The subcutaneous administration of oleic acid at volumes from 0.25 to 0.5 mL for 400 days had no adverse effects in the growth of treated albino mice. However, the life duration of both males and females was lower than the life duration in control animals. Nonclinical toxicity values have been established for oleic acid in different

laboratory species. An oral LD_{50} of 74 g/kg was established in rats. The LD_{50} is reduced to 2.4 mg/kg when dosed intravenously (IV) in the same species. The IV LD_{50} in mice is 230 mg/kg, while the dermal LD_{50} in guinea pig is higher than 3000 mg/kg (HSDB 2008). Acute exposure to doses as high as 21.5 mL/kg of oleic acid and up to 10 g/kg of commercial grade lauric, palmitic, and myristic acids by oral gavage to rats resulted in no deaths and no significant findings at necropsy (HSDB 2008). Severe pulmonary damage (mainly pulmonary edema) and death were induced by IV injection of oleic acid at 42 mg/kg in rats (HSDB 2008). In dogs, the repeated injection of oleic acid at 0.09 g/kg over a period of 1–3 months induced significant pulmonary changes, including thrombosis, and cellular necrosis. In a different study using dogs, severe pulmonary edema was induced following an IV injection of 60 mg/kg of oleic acid (HSDB 2008).

The subchronic and perchronic oral exposure to oleic acid caused deterioration on health and subsequent death after 17 weeks of continuous oral treatment of rabbits at a dose of 4.5 g/kg/day, while normal growth and health was reported in rats orally dosed with 7.5 g/kg/day for up to 24 weeks (HSDB 2008).

The genotoxic and carcinogenic potential of oleic acid has also been assessed by several authors. All the published results report a negative genotoxic potential of oleic acid when tested in bacterial and mammal systems. No carcinogenic or potentiating carcinogenic effect was identified when mice or rats were dosed orally with oleic acid or with a combination of this acid with a tumorogenic substance such as benzo(a)pyrene. Therefore, it was concluded by different authors that oleic acid is not tumorogenic (HSDB 2008).

No evidence of maternal or fetal toxicity (teratogenicity) was identified in a reproductive toxicity assessment of oleic acid dosed topically in rats. Although the oral administration of this enhancer did not affect the fertility of male rats, apparently it impaired the reproductive capacity in females by interfering with parturition and mammary gland development when dosed at 7.5 g/kg/day in diet for 16 weeks in Sprague-Dawley rats (HSDB 2008).

In summary fatty acids have a good safety profile when administered topically onto the skin or even into the eye, causing only minimal or slight transient irritation. No severe systemic effects have been reported when oleic acid is administered orally; however, severe pulmonary effects can be induced by intravenous administration of these compounds.

25.4 Fatty Alcohols, Ethanol, and Glycols

25.4.1 Fatty Alcohols

Fatty alcohols are substances used as penetration enhancers for several drugs. Studies using different fatty alcohols have been performed in order to assess their penetration potential using melatonin as a permeant through porcine and human skin in vitro (Williams and Barry 2012). Fatty alcohols from octanol to myristyl alcohol have been evaluated. However, the skin irritation induced by saturated fatty alcohols has not been studied widely (Kanikkannan and Singh 2002). In one of the few published reports, Kanikkannan and collaborators assessed the skin irritation potential of several fatty alcohols in rat hairless skin. Using erythema as a skin irritation indicator, lauryl alcohol proved to be the most irritant of the tested fatty alcohols, which included myristyl alcohol, tridecanol, decanol, undecanol, nonanol, and octanol (Kanikkannan and Singh 2002). However, lauryl alcohol induced a lower transepidermal water loss (TEWL) than other fatty alcohols such as myristyl alcohol and undecanol. The authors concluded that octanol and nonanol were less irritant than the other fatty alcohols tested when used to increase the permeation of melatonin in hairless rat skin in vivo (Kanikkannan and Singh 2002).

25.4.2 Ethanol

Ethanol is commonly used in many transdermal formulations and is often the solvent of choice for use in patches and other products with direct exposure to skin. Ethanol can act as a cutaneous

penetration enhancer through several potential mechanisms of action. Among those mechanisms is the volatile capacity of the ethanol, which may remove some of the lipid fraction from the *stratum corneum* when used at high concentrations, and for prolonged times, this mechanism may improve drug flux through skin. The solvent features of ethanol can increase the solubility of the drug in vehicle, increasing penetration of the drug. Moreover, it is also possible that the rapid permeation of ethanol or its evaporative loss from the donor phase modifies the thermodynamic activity of the drug in the formulation (Williams and Barry 2012). Ethanol has been used to enhance the penetration of several compounds such as levonorgestrel, estradiol, hydrocortisone, imipramine hydrochloride, naloxone, zidovudine, and 5-fluoroacil in rat skin and of estradiol in human skin in vivo (Panchagnula et al. 2001; Jain et al. 2002; Williams and Barry 2012). Although several reports exist in the scientific literature regarding the safety of ethanol, there is no up-to-date risk assessment of ethanol application on the skin. The main concern of topical ethanol applications for human health is its potential carcinogenic effect because there is ambiguous evidence for the carcinogenicity of ethanol when orally consumed in alcoholic beverages. However, there is no evidence which associates the topical administration of ethanol with an increased risk of cutaneous cancer (Lachenmeier 2008). Even though there is no evidence which links the cutaneous administration of ethanol with increased risk for cancer in skin, other factors such as synergy with other chemicals should be taken into consideration. Therefore, each formulation containing ethanol should be evaluated for its carcinogenic potential.

The use of ethanol is associated with skin irritation or contact dermatitis, especially in individuals with an aldehyde dehydrogenase (ALDH) deficiency. It has been demonstrated that relatively low blood concentrations of ethanol and its metabolite acetaldehyde may be measured after regular cutaneous application of ethanol. However, those measurable levels are below the toxicological threshold. Percutaneous toxicity after application of ethanol through lacerated skin has been reported to be possible, especially in children (Lachenmeier 2008). Some side effects of the transdermal patches using ethanol as permeation enhancer have been reported. Among those side effects are cutaneous intolerance (manifested by signs such as erythema) and allergic contact dermatitis, which in some regard have been associated with ethanol as one of the causal agents. However, in some of the reported cases, the combination of effects between the different components of the dosed preparations cannot be ruled out. Therefore, it is inconclusive whether ethanol or other agents in the products that come in contact with skin were the real causes for the side effects observed (Heard et al. 2006; Heard and Screen 2008). In other studies, it was not possible to detect changes in the TEWL after the topical administration of ethanol (Loffler et al. 2007; Kramer et al. 2002).

In an exceptional review published by Lachenmeier and collaborators in 2008, the authors draft a list of conclusions regarding the safety of topical administration of ethanol. Among those conclusions the authors mention the following: (A) Topically applied ethanol on un-lacerated human skin will not cause acute or systemic toxic effects, which can only occur if applied on damaged skin; especially in children. (B) Adverse effects of topically applied ethanol may include skin irritations to allergic contact dermatitis. (C) Ethanol and its metabolite acetaldehyde are potentially carcinogenic for humans; however, only limited evidence supports the carcinogenicity of mouthwashes and a complete lack of data about the carcinogenicity of all other groups of products. (D) Further concerns include the permeation-enhancing capabilities of ethanol, which could lead to an increased absorption of other components of topically applied formulations. (E) Safety assessments of ethanol in any form of application must include the carcinogenic and genotoxic properties of ethanol and its metabolite acetaldehyde.

In summary, ethanol is a compound that is widely used as penetration enhancer for several drugs. Although there are some concerns regarding the potential carcinogenic effect of topical administration of ethanol, no evidence which

could demonstrate this effect has been found. Therefore, the main proved concerns regarding potential toxicity of topical administration of ethanol are those related to local skin or eye irritation.

25.4.3 Glycols (Propylene Glycol (PG) and Polypropylene Glycol (PPG))

The most known compound among the glycols is propylene glycol (PG), whose activity is thought to result from solvation of α-keratin within the *stratum corneum* (Ahad et al. 2009). PG has been used as a stand-alone penetration enhancer and also widely used as a vehicle for penetration enhancers and shows synergistic action when used with, for example, oleic acid (Williams and Barry 2012). However, even though several pharmaceutical formulations contain unsaturated fatty acids such as oleic acid and PG, the general use of this combination is limited due to the high prevalence of dermal side effects including *stratum corneum* lipid extraction and damage to viable epidermal cells, which presumably occurs because of the acidic nature of the fatty acids (Sintov et al. 1999; Touitou et al. 2002).

The toxicology of PGs and PPGs has been assessed in several studies. Sax and collaborators in 1979 (CIR 1994) reported an acute oral LD_{50} of 21 g/kg for PG in five female Fischer strain rats, while in 1976 Bartsch reported an acute oral LD_{50} of 25 mL/kg in 10 male and female Sprague-Dawley rats (CIR 1994). In the Sprague-Dawley rat, the LD_{50} of PG is reduced to 13 g/kg or to 6.2 mL/kg after intraperitoneal or intravenous administration, respectively (CIR 1994). In specific pathogen-free NMRI (SPF-NMRI) mice, the LD_{50} reported by Bartsch and collaborators is 6.4 mL/kg (CIR 1994).

Significant decreases in the levels of fibrinogen, albumin, and globulin in plasma were detected in Wistar strain rats after the acute oral administration of PG at 9.66, 19.32, or 38.64 mmol/kg. These results suggest that PG may affect the hepatic function in either the synthesis or the secretion of proteins in the treated rats (CIR 1994). Lethality has also been reported in horses. After an accidental oral administration of 3.8 L of PG to a 400–500 kg male horse, signs of ataxia, pain, salivation, and excessive sweating were observed within 10–15 min post dosing. All signs, with exception of ataxia, resolved within 5 min. However, the next day, the animal became increasingly ataxic and died of apparent respiratory arrest. Death occurred approximately 28 h after exposure. No macroscopic changes were observed at necropsy. There was a PG concentration of 9,000 mg/L in serum and 7,500 mg/L in renal fluids (combined blood and urine). Histological changes included moderate myocardial perivascular edema with dilation of lymphatics and moderate pulmonary edema characterized by proteinaceous material in alveoli and in some of the bronchioles. Hepatic lesions consisted of scattered single-cell hepatocytic necrosis and minimal acute suppurative pericholangitis. Peracute renal infarcts characterized by multiple linear areas of coagulative tubular necrosis were also observed (CIR 1994).

For polypropylene glycols (PPGs), the acute toxicity of PPGs of various molecular weights was evaluated in Sherman strain rats. The mean LD_{50} values were 2.91 g/kg for polypropylene glycol 425 (PPG 425), 2.15 g/kg for polypropylene glycol 1025 (PPG 1025), and 9.76 g/kg for polypropylene glycol 2025 (PPG 2025), respectively. The animals treated with any of the mentioned PPGs showed sluggishness, prostration, tremors, convulsions, and rapid death. At necropsy, the following macroscopic changes were observed: minor pulmonary hemorrhage, congestion in the liver and spleen, and renal ischemia (CIR 1994). In a different study, male rats received different oral doses of 10 % aqueous PPG 1200 and the resulting LD_{50} was 640 mg/kg. Additional studies assessing the oral toxicity on PPGs of various molecular weights have reported LD_{50} values in rats ranging from 0.5 to more than 40 g/kg.

The acute oral toxicity of PPG 1200 was tested in dogs. A subconvulsant effect was observed after administration of 50 mg/kg (CIR 1994). In guinea pigs the PPG 1200 LD_{50} value for males and females was 1,320 mg/kg.

Additional acute oral toxicity studies in guinea pig have reported LD_{50} values ranging from 1.5 to 17 g/kg (CIR 1994).

The acute toxicity for PPGs using parenteral administration has also been calculated. Shaffer and collaborators in 1951 reported LD_{50} values of 0.46 g/kg for PPG 425, 0.23 g/kg for PPG 1025, and 4.47 g/kg for PPG 2025 when dosed via intraperitoneal route to Sherman rats. The same authors reported LD_{50} values of 0.41 g/kg for PPG 425, 0.12 g/kg for PPG 1025, and 0.71 g/kg for PPG 2025 when the compounds were administered via intravenous into Sherman rats (CIR 1994). Death was observed in all treated groups regardless of the route of administration. Death was preceded by tremors, prostration, frothing at mouth, and audible rales (CIR 1994).

Studies in mice involving intraperitoneal dosing of various PPGs reported LD_{50} values of 700 mg/kg, 195 mg/kg, 12 mg/kg, and 3,600 mg/kg for PPG 400, PP G750, PPG 1200, and PPG 2000, respectively (CIR 1994).

In dogs, the intravenous administration of an aqueous solution of PPG 400 at 10–20 mg/kg resulted in tremors with convulsions. Convulsions were also observed in dogs dosed IV with PPG 750 at 8–15 mg/kg. Convulsions and death were observed in dogs treated IV with 20 mg/kg of PPG 750. The IV administration of PPG 1200 elicits tremors or convulsions when dosed at 7 or 15 mg/kg, respectively, while convulsions and death were observed in dogs dosed at 20 mg/kg with the same compound. No adverse effects were observed in dogs dosed IV with PPG 2000 at 100 mg/kg (CIR 1994).

The assessment of the acute toxicity of PPG 1200 when injected IM into dogs resulted in subconvulsant effects or mild convulsions in dogs treated with 45 or 60 mg/kg, respectively (CIR 1994).

The subchronic toxicity of PG was assessed in rats dosed IV for three consecutive days with 100 % PG at 0.75, 1.5, or 3 mL/kg. Slight hyperemia of the intestinal mucosa was the most prominent finding in animals dosed with the lowest or the highest dose. Similar changes were reported in dogs dosed with 100 % PG at 1.5 mL/kg (CIR 1994). Cats dosed with 41 % PG in dry feed developed moderate polyuria and polydipsia. These changes are consistent with renal excretion of PG, which acts as an osmotic diuretic. Other signs in these cats were decreased activity, mental depression, and slight to moderate ataxia, which may be related to the metabolism of PG to D-lactate (CIR 1994).

No significant adverse effects were reported in subchronic oral studies using rats dosed with PG. The most significant effects observed were related to the urinary system and were characterized by hematuria (CIR 1994).

The acute dermal toxicity of PG in rabbits was 20,800 mg/kg. PG is essentially nonirritating to the skin and mildly irritating to the eyes. Several studies support that PG is not a skin sensitizer (CIR 1994; OECD-SIDS 2001). Repeated exposures of rats to PG in drinking water or feed did not result in adverse effects at levels up to 10 % in water (estimated at about 10 g/kg/day) or 5 % in feed (dosage reported as 2.5 g/kg/day) for periods up to 2 years. In cats, two studies of at least 90 days duration show that a species-specific effect of increased Heinz bodies was observed (NOAEL = 80 mg/kg/day; LOAEL = 443 mg/kg/day), with other hematological effects (decrease in number of erythrocytes and erythrocyte survival) reported at higher doses (6–12 % in diet, or 3.7–10.1 g/cat/day). PG did not cause fetal or developmental toxicity in rats, mice, rabbits, or hamsters (NOAELs range from 1.2 to 1.6 g/kg/day in four species). No reproductive effects were found when PG was administered at up to 5 % in the drinking water (reported as 10.1 g/kg/day) of mice. PG was not genotoxic as demonstrated by a battery of in vivo (micronucleus, dominant lethal, chromosome aberration) and in vitro (bacterial and mammalian cells and cultures) studies. No increase in tumors was found in all tissues examined when PG was administered in the diet of rats (2.5 g/kg/day for 2 years) or applied to the skin of female rats (100 % PG; total dose not reported; 14 months) or mice (mouse dose estimated at about 2 g/kg/week; lifetime). These data support lack of carcinogenicity for PG (OECD-SIDS 2001).

In summary, after an analysis of a series of laboratory studies (some of those described above), the Cosmetic Ingredient Review (CIR)

expert panel issued several conclusions regarding the safety of PG. Among the studies performed were acute, subchronic and short-term animal studies, which suggested little toxicity beyond slight growth and body weight decreases. Little ocular or skin irritation and no sensitization were observed in experimental animals. Small increases in fetal malformations were seen in mice injected subcutaneously with PG, but a continuous breeding reproduction study in mice showed no reproductive toxicity following oral administration. The compound was also negative in a wide range of mutagenesis studies, and no carcinogenicity potential was detected in mice and rat studies. Clinical data in humans showed skin irritation and sensitization reactions to PG in normal subjects at concentrations as low as 10 % under occlusive conditions and dermatitis patients at concentrations as low as 2 %. In these studies, test concentrations ranged from 2 to 100 %. Reactions were observed at concentrations as low as 10 % in predictive tests and as low as 2 % in provocative tests. Based on these results the general conclusion of the experts was, "A concentration limit for propylene glycol is considered necessary." It was decided that the 50 % concentration is safe for the use in cosmetics. Moreover, a more recent review by another panel agreed with what had been concluded earlier. This review panel stated: "True allergic reactions to propylene glycol are uncommon and the clinical significance has probably been overestimated". Additional studies have been performed after these reviews. In two repeated insult patch tests, no indication of allergy was noted upon challenge. The incidence of irritation responses was 1 in 104 tested individuals. These findings support the conclusion issued by the FDA's experts, in regard to the safety of the use of PG in cosmetics up to concentrations of 50 % (CIR 1994).

25.5 Azone® (Laurocapram)

Azone® (1-dodecylazacycloheptan-2-one, laurocapram), along with its derivates, is probably the most investigated penetration enhancers and is effective at low concentrations for both lipophilic and hydrophilic drugs but suffers from toxicity problems. The enhancing effect of laurocapram (Azone®) is attributed to different mechanisms, such as insertion of its dodecyl group into the intercellular lipidic bilayer, increase of the motion of the alkylic chains of lipids, and fluidization of the hydrophobic regions of the lamellate structure (Lopez-Cervantes et al. 2006). It is thought that the penetration-enhancing effects of Azone® are exerted by interacting with the intercellular lipids of the *stratum corneum* causing an increase in their fluidity. The effects on the lipid bilayer are considered to be partially due to its C12 alkyl chain, which helps it insert among the acyl chain of lipids in the bilayers of the intercorneocyte space (Lopez-Cervantes et al. 2006). The enhanced fluidity of intercellular lipids is thought to facilitate the diffusion of the drug molecules through the hydrocarbon chains of the lipid bilayer in the *stratum corneum* (Lopez-Cervantes et al. 2006).

In humans, pure Azone® is poorly absorbed through skin, and the limited amount of Azone® absorbed is cleared rapidly by the kidneys (Wiechers et al. 1987). There have been several studies to assess the irritant potential of Azone®. The results of those studies are contrasting, some reporting that Azone® is a moderate irritant and others reporting that is not irritating (Barry and Bennett 1987; Lopez-Cervantes et al. 2006). In rats, it has been demonstrated that Azone® causes a minimal skin irritation and enhances the TEWL values (Fang et al. 2003a). It is possible that the skin irritation caused by the Azone® is the main reason for not using it as a penetration enhancer in transdermal systems.

Toxicological studies reveal a low toxicity for laurocapram, and for some derivates, a relationship exists between toxicity and the number of carbons in the alkyl chain. Laurocapram is minimally absorbed through the skin and rapidly eliminated from circulation in humans (Lopez-Cervantes et al. 2006). In cutaneous studies in rats, it was demonstrated that morphological microscopic changes in the skin after treatment with Azone® were located in the epidermis. Proliferation was observed in the superficial layer of the skin. The epidermis had increased numbers

of neutrophils and lymphocytes, indicating the inflammation of keratinocytes (Fang et al. 2003b). Laurocapram proved to be safe after cutaneous administration to humans in combination with methotrexate (Sutton et al. 2001; Demierre et al. 2003).

In summary, the safety of laurocapram administered by ways other than cutaneous administration has not been completely assessed. The assessment of the toxic potential of cutaneous administration of Azone® has generated contrasting results, reporting Azone® as a nonirritant in some reports and other results reporting this compound as a moderate skin irritant.

25.6 Sulfoxides

25.6.1 Dimethyl Sulfoxide (DMSO)

DMSO has been widely investigated as a percutaneous penetration enhancer for a wide range of drugs (Medi et al. 2006). Although DMSO was shown to be an effective percutaneous penetration enhancer, the toxicity problems associated with its use prevented the widespread use of DMSO in transdermal systems.

Several studies to assess the toxicity of DMSO have been reported. The acute LD_{50} levels have been calculated in mice and rats. The intravenous LD_{50} level at 24 h is 11 g/kg in mice, while the levels after intraperitoneal administration are 20.1 and 13.7 g/kg for mice and rat, respectively. LD_{50} levels after subcutaneous administration are 16.0 g/kg in the mouse and 13.7 g/kg in the Wistar rat. It was not possible to establish an acute oral LD_{50} in mice and rat. The maximum oral dose never fatal in mice was $\geq$14 g/kg and in rat was $\geq$15.0 g/kg, while the minimum dose always fatal was 14.0 g/kg in mice and 15 g/kg in Wistar rat (Caujolle et al. 1966). The most characteristic symptoms observed after administration of DMSO were catatonia of the tail (Straub's reaction) and hypothermia in the mouse. In the rat the main symptom was profuse lacrimal secretion. However, the toxic effects were observed only in doses close to the lethal dose when parenterally administered.

Smith and collaborators reported the assessment of the toxic potential of DMSO after dermal administration to rats (Smith et al. 1967). The authors immersed rats up to their necks in various concentrations of DMSO, not observing immediate effects. However, within 24 h, most of the animals treated with undiluted DMSO were dead, as were some animals dipped in 80 % DMSO-water. The single-dose dermal LD_{50} in mice and rats was estimated to be equal to, or less than, 50 and 40 g/kg, respectively (Smith et al. 1967).

Brown and collaborators painted undiluted DMSO onto five male hairless mice twice a week for 30 weeks with no discernible effect. Moreover, the same authors applied undiluted DMSO daily for 28 days to the clipped backs of guinea pigs and found no gross or microscopic evidence of damage (Brown et al. 1963).

In a series of experiments, DMSO was applied to shaved skin in the back of dogs and monkeys at doses of 3.3–33 g/kg/week for 6 months. The compound was used at concentrations from 60 to 100 %. The skin became transiently reddened and warm from the application, particularly with the undiluted DMSO. Furfuraceous and membranous desquamation of the epidermis began within 3 weeks and persisted throughout to the end of dosing period. There were no changes in behavior, body weight, hematology, blood chemistry, or urinalysis. No obvious changes were observed in organ weights, and some test article-related effects were seen at gross and microscopic examination at the site of administration in the skin. Other microscopic effects observed in the skin after dermal administration of DMSO to rats were hyperkeratosis, parakeratosis, and focal ulcerations, whereas to dogs and monkeys, it produced only desquamation (Smith et al. 1967). Changes observed by other authors when 90 % DMSO was applied to the trunk of 20 human patients were erythema in some patients and mild scaling and diffuse erythematous dermatitis in others. When DMSO was administered at doses double the ones described above, some individuals showed irritation. Skin biopsies revealed the presence of mild perivascular lymphocytic infiltration, moderate acanthosis, absence of the granular layer, and a parakeratotic, thickened cornified

layer. These changes partially resemble some changes observed in rats that were dipped into DMSO (Kligman 1965).

Regarding the ocular administration of DMSO, Brown and coworkers administered undiluted DMSO into the conjunctival sac of rabbits and noted no adverse effects (Brown et al. 1963). Similarly, using the same species, Smith and collaborators dosed undiluted and diluted 25 and 60 % DMSO (Smith et al. 1967). These authors found that DMSO caused a dose-related edema and erythema of the orbital tissues when administered at 60 and 100 % solutions. While the undiluted compound also produced lacrimation and drooping of the lower eyelid (Smith et al. 1967).

The assessment of the teratogenic potential of DMSO was assessed in rats, rabbits, and chickens. The results of those assessments revealed that the toxicity of DMSO is discreet, showing considerable tolerance in the species tested in single- or repeated-dose studies with duration of up to 30 days of administration of DMSO. The teratogenic effects of DMSO were demonstrated in chick embryo and in mammals (mice, rabbit, and rat). The main teratogenic effects in both mammals and chicken are effects on nervous system (anencephalia and microphalia), skeletal malformations, and celosomia (Smith et al. 1967).

The single- and repeated-dose experiments that were performed to assess the toxic potential of DMSO revealed some important properties of DMSO. First, DMSO is well absorbed following administration by all routes. This compound possesses a high local toxicity, characterized by local tissue irritation and destruction after dosing by most of the routes of administration. Since the LD_{50}s are all in the terms of grams per kilogram of body weight, DMSO possesses low systemic toxicity. Finally, the toxicological effects induced by DMSO are similar in all tested species.

In summary, although some adverse effects have been observed after a single- or repeated-dose administration of DMSO, those effects have not prevented the use of DMSO in the formulation compositions used in clinical development and veterinary products.

25.7 Pyrrolidones

A range of pyrrolidones and other structurally related compounds have been investigated as potential penetration enhancers in human skin. The most studied analogues of naturally occurring pyrrolidone carboxylic acid are 2-pyrrolidone (2P) and N-methyl-pyrrolidone (NMP). It has been demonstrated that 2P increases the skin permeability by enhancing the diffusivity of the drugs through polar routes of the skin (Southwell and Barry 1983). Consequently, as is the case for other enhancers, such as Azone®, pyrrolidones apparently are more active with hydrophilic rather than with lipophilic molecules (Williams and Barry 2012).

Regarding mechanism of action, pyrrolidones act well upon the *stratum corneum*. Apparently they act by altering the solvent nature of the membrane, and pyrrolidones have been used to generate "drug reservoirs" within the skin membranes. Those reservoirs allow the slow or sustained release of the permeant from the *stratum corneum*. However, as with other penetration enhancers, the clinical use of pyrrolidones is often prevented due to reported adverse reactions, which are mainly referred to as skin irritation.

Pyrrolidones have been used as permeation promoters for numerous molecules including hydrophilic and lipophilic permeants. Among those molecules are: nalone, 5-fluoroacil, hydrocortisone, betamethasone, indomethacin, luteinizing hormone-releasing hormone, mannitol, metronidazole, naloxone, progesterone, nitroglycerine, and sulfaguanidine (Williams and Barry 2012; Ahad et al. 2009; Medi et al. 2006).

Regarding safety, it has been reported that in humans, NMP and 2P cause skin irritation while increasing the bioavailability of betamethasone-17-benzoate (Ahad et al. 2009). In an in vivo vasoconstrictor bioavailability study, pyrrolidones caused erythema in some patients, although this effect had a relatively short duration. Also a toxic hygroscopic contact to N-methyl-2-pyrrolidone has been reported (Williams and Barry 2012).

In a toxicological evaluation of eprinomectin (Longrange Merial®), an injectable parasiticide approved for its use in cattle in the United States

and other countries, the toxic potential of NMP was assessed in several repeated dose studies. NMP was included in this product as an excipient (FDA-CVM 2001). The literature reports the carcinogenic potential of NMP in mice, but not in rats as described in the freedom of information document for NADA 141–3227, and was conducted to investigate the human food safety issues of the excipient. The NOEL identified in a 1 week oral gavage repeat-dose study in mice was 1000 mg/kg/day based on proliferative effects at 3000 g/kg/day (FDA-CVM 2001).

In subchronic studies the NOEL identified in a 90-day repeat oral dose study in mice was 167 g/kg/day based on the increased liver weight and increased incidence of centrilobular hepatocellular hypertrophy observed at 417 mg/kg/day. A similar study using Wistar rats dosed with the compound in the diet showed a NOEL of 40 mg/kg/day, based on a decrease of body weights, an increase in the thyroid weight, and changes in the chemical properties of urine (FDA-CVM 2001).

The NOEL obtained from a 90-day repeat oral dose study in dogs was 79 mg/kg/day based on the lack of body weight gain of the highest dose group (250 mg/kg/day) (FDA-CVM 2001).

After the analysis of the results from the subchronic and chronic studies performed to assess the toxic potential of NMP, the most appropriate toxicity study for determining the human health protective value of NMP residues in edible tissues was the 90-day oral toxicity study in mice. The NOEL of NMP for this study was 167 mg/kg/day (FDA-CVM 2001).

No teratogenic effects were observed in Sprague-Dawley rats dosed with up to 237 mg/kg/day. The NOEL identified in a developmental toxicity study in New Zealand white rabbits was 55 mg/kg/day with respect to maternal toxicity and 175 mg/kg/day with respect to developmental toxicity (FDA-CVM 2001).

In a multigeneration rat reproduction study with N-methyl-2-pyrrolidone, the NOEL for reproductive and developmental effects was established as 160 mg/kg/day of the compound. The 160 mg/kg/day dose level was established as the parental, reproductive, and developmental NOEL in this study (FDA-CVM 2001).

In summary, pyrrolidones and specifically NMP may cause skin erythema and irritation after cutaneous administration. When orally administered, this compound may be teratogenic at doses higher than 160 mg/kg/day and may have adverse effects on the body weight, thyroid weight, and liver, in which microscopic changes were observed in the hepatocellular areas, having hepatocellular hypertrophy.

25.8 Surfactants

As happens with other classes of skin penetration enhancers, surfactants are found in many existing therapeutic, cosmetic, and agrochemical preparations. Surfactants are usually added to formulations in order to facilitate the solubilization of lipophilic active ingredients. The surfactants have the ability of solubilize lipids within the *stratum corneum*. Surfactants are typically composed of a lipophilic alkyl or aryl fatty chain, together with a hydrophilic head group. These enhancers can be anionic or cationic. Anionic surfactants include sodium lauryl sulfate (SLS), while cationic surfactants include cetyltrimethyl ammonium bromide (Williams and Barry 2012). There is a wide use of nonionic surfactants in topical formulations as solubilizing agents. Among those, the polysorbates (ethoxylated sorbitan esters) are very common. Tween® 80 was reported to accelerate hydrocortisone and lidocaine penetration, and Tween® 20 improved the permeation of 5-fluoroacyl across hairless mouse skin (Kogan and Garti 2006).

Surfactants generally have low toxicity, and most have been shown to enhance the flux of materials permeating through biological membranes. The cationic surfactants are claimed to be more potent than anionic surfactants. Both types of surfactants have potential for damaging human skin; sodium lauryl sulfate (an anionic surfactant) is a powerful irritant for human skin (Ahad et al. 2009). Nonionic surfactants tend to be widely recognized as safe (Kogan and Garti 2006).

Turkoglu and Sakr investigated the irritation potential of sodium laureth sulfate alone and in

combination with lauryl glucoside, polysorbate 20, and cocamidopropyl betaine in 13 human subjects (Turkoglu and Sakr 2001). The highest irritation potential was observed with the composition containing sodium laureth sulfate, lauryl glucoside, and cocamidopropyl betaine together. Among the subformulations, cocamidopropyl betaine showed the highest irritation grade. It was concluded that the irritation potential of surfactants was related to the total surfactant concentration as well as to the chemical structure of the surfactant molecules (Ahad et al. 2009). Recently, new low-irritant surfactants based on caprylocaproyl macroglycerides for microemulsions as drug delivery vehicles for topical application were studied (Kogan and Garti 2006).

In general surfactants may be irritant for skin; however, regardless of the minimal adverse effects reported, surfactants should be considered of low toxicity or generally safe.

Conclusion

In the drug development process, it is important to perform a thorough assessment of the toxic potential of each of the components of a drug formulation. This review of the toxicology of several penetration enhancers provides a summary of the reported safety assessment for those enhancers. However, it is our responsibility as toxicologists to evaluate the potential toxicological effect that certain enhancers may have as components of formulations intended to be used in humans or in animals. The main toxicological effects, LD_{50}s, and NOELs/NOAELs described in this review are summarized in Table 25.1.

Table 25.1 Summary of Toxicological Effects of Chemical Penetration Enhancers

Penetration enhancer	Toxic syndrome/lowest LD_{50}/lowest NOEL reported/species tested and way of administration	Reference
Terpenes		
1,8-Cineole (eucalyptol)	In humans: transient coma, epigastric burning, nausea, vertigo, ataxia, muscle weakness, occasional cyanosis, respiratory edema, miosis, convulsions, fever, albuminuria, hematuria, painful urination, respiratory failure It also induced allergic skin reactions and tachycardia	Gosselin et al. (1976), BIBRA (1991), Santos and Rao (2002), NTP, PAFA (2006), NTP Repository (1991), Galloway et al. (1987), Stoner et al. (1973), De Vincenzi et al. (2002)
	Possible lethal dose in human: 50–500 mg/kg	
	LD_{50} 2480 mg/kg rat, oral	
	LD_{50} 2500 mg/kg mouse, oral	
	LD_{50} 100 mg/kg mouse, IM	
	LD_{LO} 1500 mg/kg dog, SC	
	Genotoxicity negative and carcinogenicity negative (at 12 g/kg for 8 weeks in mouse primary lung tumor model)	
	Teratogenic at 2 g/kg rat, oral	
	Central nervous system and respiratory effects and increased hepatic enzyme activity with bile secretion in rodents	

Table 25.1 (continued)

Penetration enhancer	Toxic syndrome/lowest LD_{50}/lowest NOEL reported/ species tested and way of administration	Reference
L-Menthol	In human: high oral doses may cause abdominal pain, convulsions, nausea, vomiting, vertigo, ataxia, drowsiness, coma	OECD-SIDS (2001, 2003), O'Mullane et al. (1982)
	250–250 mg/kg caused some of the signs above in children	
	120 mg/kg caused cold burning sensation in the mouth, esophagus, nasal mucosa, skin in humans	
	20 mg/kg caused mild abdominal discomfort	
	NOAEL 200 mg/kg increased hepatic weight, rat, 28 days, oral	
	NOAEL 937 mg/kg reduction body weight, male rat, 13 weeks, oral	
	NOAEL, 1956 mg/kg; male mice, 13 weeks, oral	
	NOAEL 188 mg/kg, rat, 103 weeks, oral	
	NOAEL 667 mg/kg, mice, 103 weeks, oral	
	Genotoxicity negative and carcinogenicity negative	
	Non-teratogenic at up to 425 mg/kg rat, mouse, rabbit, oral	
Fatty acids		
Oleic acid	Several species: erythema and edema, skin irritation, inflammatory infiltrate, follicular epidermal hyperplasia after cutaneous administration in 5 % (w/v)	Liebert (1987), Boelsma et al. (1996), HSDB (2008)
	Induction of comedones in rabbit skin	
	No ocular irritation in rabbits after ophthalmic administration of 0.1 mL oleic acid	
	LD_{50} 74 mg/kg, rat, SC	
	LD_{50} 2.4 mg/kg, rat, IV	
	LD_{50} higher than 3000 mg/kg, guinea pig, dermal	
	Rat: severe pulmonary damage and death, IV injection at 42 mg/kg	
	Dog: significant pulmonary changes, thrombosis, cellular necrosis, at 0.09 g/kg IV for 1–3 months	
	Severe pulmonary edema at 60 mg/kg IV in dogs	
	Genotoxicity negative, carcinogenicity negative (rat and mouse)	
	Reproductive effects in females (affect parturition and mammary gland development) at 7.5 g/kg/day in diet for 16 weeks, Sprague-Dawley rats	
Myristic acid	Rabbit: thinning collagen fibers and lymphocytic-histiocytic infiltration	Liebert (1987), HSDB (2008)
	Rabbit: slight conjunctival irritation after administration of commercial grade myristic acid	
	No acute toxicity in rats with up to 10 g/kg oral dose in rat	

(continued)

Table 25.1 (continued)

Penetration enhancer	Toxic syndrome/lowest LD_{50}/lowest NOEL reported/ species tested and way of administration	Reference
Stearic acid	Rats: local cutaneous edema after topical application 2 % solution	Liebert (1987)
	Rabbits: cutaneous edema after 20 applications 2 mL/kg 2 % solution	
	No photosensitizing, 100 % stearic acid, guinea pigs, topical	
	No ocular irritation in rabbits after administration of commercial stearic acid	
Lauric acid	Rabbits: erythema 72 h posttopical treatment	Liebert (1987), HSDB (2008)
	Rabbit: corneal opacity, mild conjunctivitis, and iritis after ophthalmic administration of commercial grade lauric acid. No effects when administered at a 8.7 % solution	
	No acute toxicity in rats with up to 10 g/kg oral dose in rat	
Fatty alcohols		
Octanol/nonanol	Octanol and nonanol are less irritant than decanol and undecanol in hairless rat skin in vivo	Kanikkannan and Singh (2002)
Ethanol	Associated to skin irritation or contact dermatitis in individuals with aldehyde dehydrogenase deficiency	Lachenmeier (2008)
	Application of ethanol on intact human skin does not cause acute systemic toxic effects	
	Ethanol and its metabolites are potentially carcinogenic for humans addicted to alcoholic beverages	
Glycols		
PG	LD_{50} 21 g/kg, Fischer rat, oral	CIR (1994)
	LD_{50} 13 g/kg, Sprague-Dawley rat, IP	
	LD_{50} 6.2 mL/kg, Sprague-Dawley rat, IV	
	LD_{50} 6.4 mL/kg SPF-NMRI mouse	
	PG may affect hepatic function (decreased fibrinogen, albumin, and globulin in plasma at 9.66, 19.32, or 38.64 mmol/kg in Wistar rat)	
	Horses: lethal when dosed at 3.8 L into a 400–500 kg horse. Ataxia, pain, salivation, excessive sweating, respiratory arrest, and death. Histology: myocardial perivascular edema, pulmonary edema, lymphangiectasia, suppurative pericholangitis, and renal infarcts	
	LD_{50} dermal 20.8 g/kg. No irritant to skin or eyes	
	No adverse effects in rats dosed 10 g/kg/day in water or 2.5 g/kg/day in feed for 2 years in rats	
	NOEL 80 mg/kg/day and LOAEL 443 mg/kg/day for 90 days in cats. Increased Heinz body formation	CIR (1994)
	Developmental NOELs from 1.2 to 1.6 g/kg/day in rat, rabbit, hamster, or mice	
	No reproductive effects at 10.1 g/kg/day in mice	
	Genotoxicity negative/carcinogenicity negative at up to 2.5 g/kg/day for 2 years in rats (oral drug in diet)	
	Carcinogenicity negative at up to 2 g/kg/week dermal application for lifetime (mice) or for 14 weeks (rat)	

Table 25.1 (continued)

Penetration enhancer	Toxic syndrome/lowest LD_{50}/lowest NOEL reported/ species tested and way of administration	Reference
PPGs	LD_{50} 2.91 g/kg (PPG425), 2.15 g/kg (PPG1025), 9.76 g/kg (PPG2025), Sherman rat, oral. Signs: sluggishness, prostration, tremors, convulsions, sudden death	CIR (1994)
	LD_{50} 640 mg/kg (PPG1200), rat, oral	
	Guinea pig: LD_{50} (PPG1200) 1.320 g/kg, oral	
	LD_{50} 0.46 g/kg (PPG425), 0.23 g/kg (PPG1025), 4.47 g/kg (PPG2025), Sherman rat, IP	
	LD_{50} 0.41 g/kg (PPG425), 0.12 g/kg (PPG1025), 0.71 g/kg (PPG2025), Sherman rat, IV	
	LD_{50} 700 mg/kg (PPG400), 195 mg/kg (PPG750), 12 mg/kg (PPG1200), 3600 mg/kg (PPG2000), mouse, IP	
	Dog: acute oral toxicity PPG1200 at 50 mg/kg with subconvulsant effect	
	Dog: tremors and/or convulsions with PPG400 at 10–20 mg/kg IV, PPG750 at 8–15 mg/kg IV, PPG1200 at 7–15 mg/kg IV. Death with PPG750 at 20 mg/kg IV, PPG1200 at 20 mg/kg IV	
	No adverse effects with 100 mg/kg PPG2000 in dogs dosed IV	
Azone (laurocapram)		
	Minimal skin irritation in rats	Fang et al. (2003a, b)
	Proliferation of epidermis and neutrophilic-lymphocytic infiltration of epidermis after cutaneous administration in rats	
Dimethyl sulfoxide (DMSO)		
	LD_{50} 11 g/kg in mice	Kligman (1965), Brown et al. (1963)
	LD_{50} 20.1 g/kg mice, IP; LD_{50} 16 g/kg, mice, SC	
	Maximum oral never fatal dose 14.0 g/kg, mice	
	Dermal application to rats induced: hyperkeratosis, parakeratosis, focal ulcerations	
	LD_{50} 13.7 g/kg rat, IP and SC	
	Maximum oral never fatal dose 15 g/kg, rat	
	Dog and monkey: 3.3–33 g/kg in shaved skin caused, reddened skin	
	Rabbits: no adverse effects after ocular application of undiluted DMSO	
	Teratogenic in chick embryo, mice, rabbit, rat after 30 days of dosing	

(continued)

Table 25.1 (continued)

Penetration enhancer	Toxic syndrome/lowest LD_{50}/lowest NOEL reported/ species tested and way of administration	Reference
Pyrrolidones		
NPM	Cause skin irritation	Ahad et al. (2009), Williams and Barry (2012), FOI NADA 141-327
	Cause skin erythema in some patients	
	NOEL 1000 mg/kg/day for 1 week, mice, oral. Based on proliferative effects at 3000 mg/kg/day.	
	NOEL 167 mg/kg/day for 90 days, mice and rat, oral. Based on increased liver weight and increased incidence of centrilobular hepatocellular hypertrophy at 417 mg/kg/day	
	NOEL 40 mg/kg/day for 90 days, drug in diet, Wistar rat	
	NOEL 79 mg/kg/day for 90 days, oral, dog. Based on lack of body weight gain at 250 mg/kg/day	
	NOEL 55 mg/kg/day, oral, rabbit. Based on maternal toxicity, and NOEL 175 mg/kg/day based on developmental toxicity	
	No teratogenic in Sprague-Dawley rat at up to 237 mg/day/kg	
	NOEL 160 mg/kg/day in a multigeneration reproductive toxicology in rat. This was a NOEL for parental and fetal toxicity	
Surfactants		
	Generally have low toxicity	Kogan and Garti (2006)
	The highest irritation potential was observed with combination of laureth sulfate, lauryl glucoside, and cocamidopropyl betaine	

References

Ahad A, Aqil M, Kohli K et al (2009) Chemical penetration enhancers: a patent review. Expert Opin Ther Pat 19(7):969–988

Amnuaikit C, Ikeuchi I, Ogawara K et al (2005) Skin permeation of propanolol from polymeric film containing terpene enhancers for transdermal use. Int J Pharm 289(1-2):167–178

Barry BW (1991) The LPP theory of permeation. In: Bronaugh RL, Maibach HI (eds) In vitro percutaneous absorption: principles, fundamentals and applications. CRC Press, Boca Raton, pp 165–183

Barry BW, Bennett SJ (1987) Effect of penetration enhancers on the permeation of mannitol, hydrocortisone, and progesterone through human skin. J Pharm Pharmacol 39:535–546

BIBRA WG (1991) Eucalyptol: 8

Boelsma E, Tanojo H, Bodde HE et al (1996) Assessment of the potential irritancy of oleic acid on human skin: evaluation in vitro and in vivo. Toxicol In Vitro 10:729–742

Brown VK, Robinson J, Stevenson DE (1963) A note on the toxicity and solvent properties of dimethyl sulfoxide. J Pharm Pharmacol 15:688–692

Caujolle FME, Caujolle DH, Cross SB et al (1966) Limits of toxic and teratogenic tolerance of dimethyl sulfoxide. Ann N Y Acad Sci 141(1):110–126

CIR (1994) Final report on the safety assessment of propylene glycol and polypropylene glycols. J Am Coll Toxicol 13(6):437–491

De Vincenzi MD, Silano M, De Vicenzi A (2002) Constituents of aromatic plants: eucalyptol. Fitoterapia 73:269–275

Demierre MF, Vachon L, Ho V et al (2003) Phase 1/2 pilot study of methotrexate-laurocpram topical gel for the treatment of patients with early-stage mycosis fungoides. Arch Dermatol 139:624–628

Fang JY, Hung CF, Chiu HC et al (2003a) Efficacy and irritancy of enhancers on the in vitro and in vivo percutaneous absorption of curcumin. J Pharm Pharmacol 55:593–601

Fang JY, Hwang TL, Fang CL et al (2003b) In vitro and in vivo evaluations of the efficacy and safety of skin permeation enhancers using flurbiprofen as a model drug. Int J Pharm 255:153–166

FDA-CVM (2001) Freedom of information summary-original new drug application NADA 141-327 (Longrange). C. f. V. Medicines. FDA-CVM, Washington, DC

Galloway S, Armstrong M, Reuben C et al (1987) Chromosome aberration and sister chromatid exchanges

in Chinese hamster ovary cells: Evaluations of 108 chemicals. Environ Molec Mutagen 10(Suppl 10): 1–176

Gao S, Singh J (1998) Effect of oleic acid: ethanol and oleic acid: propylene glycol on the in vitro percutaneous absorption of 5-fluoroacil and tamoxifen and the macroscopic barrier property of porcine epidermis. Int J Pharm 165:45–55

Gosselin RE, Hodge HC, Smith RP et al (1976) Clinical toxicology of commercial products. Williams & Wilkins, Baltimore

Grant WM (1986) Toxicology of the eye. Charles C Thomas Publisher, Springfield

Haworth S, Lawlor T, Mortelmans K et al (1983) Salmonella mutagenicity test results for 250 chemicals. Environ Molec Mutagen 5(Suppl 1):3–142

Heard CM, Screen C (2008) Probing the permeation enhancement of mefenamic acid by ethanol across full-thickness skin, heat-separated epidermal membrane and heat-separated dermal membrane. Int J Pharm 349:323–325

Heard CM, Kung D, Thomas CP (2006) Skin penetration enhancement of mefenamic acid by ethanol and 1,8-cineole can be explained by the "pull" effect. Int J Pharm 321:167–170

HSDB (2008) Oleic acid 112-80-1 HSDB number 1240, HSDB Hazardous Substances Data Bank

Jain AK, Thomas NS, Panchagnula R (2002) Transdermal drug delivery of imipramine hydrochloride. I. Effect of terpenes. J Control Release 79(1-3):93–101

Kanikkannan N, Singh M (2002) Skin penetration enhancement effect and skin irritation of saturated fatty alcohols. Int J Pharm 248:219–228

Kligman AM (1965) Topical pharmacology and toxicology of dimethyl sulfoxide. JAMA 193:796–804, 923–928

Kogan A, Garti N (2006) Microemulsions as transdermal drug delivery vehicles. Adv Colloid Interface Sci 123–126:369–385

Kramer A, Berning T, Kampf G (2002) Clinical double-blind trial on the dermal tolerance and user acceptability of six alcohol-based hand desinfectants for hygenic hand desinfection. J Hosp Infect 51:114–120

Kristiansen E, Madsen C (1995) Induction of protein droplet (α2μ-globulin) nephropathy in male rats after short-term dosage with 1,8-cineole and *l*-limonene. Tox Letters 80:147–152

Lachenmeier DW (2008) Safety evaluation of topical applications of ethanol on the skin and inside the oral cavity. J Occup Med Toxicol 3(26)

Liebert MA (1987) Final report on the safety assessment of oleic acid, lauric acid, palmitic acid, myristic acid, and stearic acid. J Am Coll Toxicol 6(3):321–401

Loffler H, Kampf G, Schmermund D et al (2007) How irritant is alcohol? Br J Dermatl 157:74–81

Lopez-Cervantes M, Marquez-Mejia E, Cazares-Delgadillo J et al (2006) Chemical enhancers for the absorption of substances through the skin: Laurocapram and its derivates. Drug Devel Indust Pharm 32:267–286

Luke E (1962) Addiction to mentholated cigarettes. Lancet 1:110–111

Medi BM, Singh S, Singh J (2006) Assessing efficacy of penetration enhancers. CRC Press Taylor & Francis Group, Boca Raton

Narishetty ST, Panchagnula R (2004a) Transdermal delivery of zidovudine: effect of terpenes and their mechanism of action. J Control Release 95(3):367–379

Narishetty ST, Panchagnula R (2004b) Transdermal delivery system for zidovudine: in vitro, ex vivo and in vivo evaluation. Biopharm Drug Dispos 25(1):9–20

NTP (1991) 1,8-Cineol. U. NTP, NTP Chemical Repository:1–6

NTP-PAFA (2006) Eucalyptol 470-8-6. PAFA, NTP-NIH:1–3

O'Mullane NM, Joyce P, Kamath SV et al (1982) Adverse CNS effects of menthol-containing olbas oil. Lancet 319(8281):1121

OECD-SIDS (2001) SIDS Initial Assessment Report for 11th SAM. O. SIDS, UNEP Publications:1–86.

OECD-SIDS (2003) SIDS Initial Assessment Report for SIAM 16. O. SIDS. OECD, Paris, pp 1–59

Ogiso T, Iwaki M, Paku T (1995) Effect of various enhancers on transdermal penetration of indomethacin and urea, and relationship between penetration parameters and enhancement factors. J Pharm Sci 84:482–488

Panchagnula R, Salve PS, Thomas NS, Jain AK, Ramarao P (2001) Transdermal delivery of naloxone: effect of water, propylene glycol, ethanol and their binary combinations on permeation through rat skin. Int J Pharm 219(1-2):95–105

Santos FA, Rao VSN (2002) Possible role of mast cells in cineole-induced scratching behavoir in mice. Food Chem Toxicol 40:1453–1457

Sintov A, Ze'evi A, Uzan R et al (1999) Influence of pharmaceutical gel vehiclescontaining olein/sodium oleate combinations on hairless mouse skin, a histological evaluation. Eur J Pharm Biopharm 49:299–303

Smith ER, Hadidian Z, Mason MM (1967) The single-and repeated-dose toxicity of dimethyl sulfoxide. Ann N Y Acad Sci 141(1):96–109

Southwell D, Barry BW (1983) Penetration enhancers for human skin: mode of action of 2-pyrrolidone and dimethylformamide on partition and diffusion of model compounds water, *n*-alcohols, and caffeine. J Invest Dermatol 80:507–514

Stoner GD, Shimkin MB, Kniazeff AJ et al (1973) Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumour response in strain A mice. Cancer Res 33:3069–3085

Sutton L, Swinehart JM, Cato A et al (2001) A clinical study to determine the efficacy and safety of 1% methotrexate/Azone (MAZ) gel applied topically once daily in patients with psoriasis vulgaris. Int J Dermatol 40:464–467

Touitou E, Godin B, Karl Y et al (2002) Oleic acid, a skin penetration enhancer, affects Langerhans cells and corneocytes. J Controld Release 80:1–7

Turkoglu M, Sakr A (2001) Evaluation of irritation potential of surfactant mixtures. Int J Cos Sci 21:371–382

Wiechers JW, Drenth BF, Jonkman JH et al (1987) Percutaneous absorption and elimination of the penetration enhancer Azone in humans. Pharm Res 4:519–523

Williams AC, Barry BW (2012) Penetration enhancers. Adv Drug Deliv Rev 64(Suppl Dec 2012):128–137

Index

N. Dragicevic, H.I. Maibach (eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*,
DOI 10.1007/978-3-662-47039-8