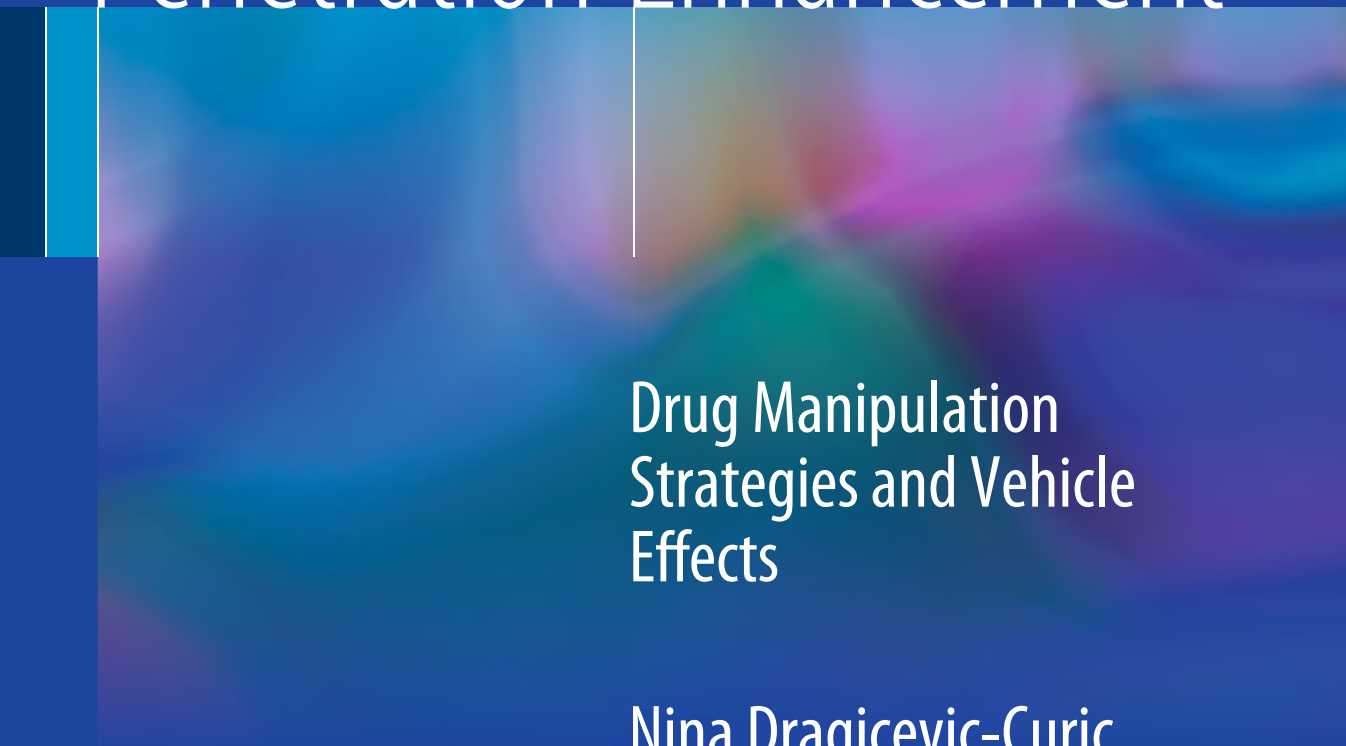
Percutaneous Penetration Enhancers

Chemical Methods in Penetration Enhancement



Drug Manipulation Strategies and Vehicle Effects

Nina Dragicevic-Curic
Howard I. Maibach
Editors

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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

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Part VI Gels as Vehicles for Skin Delivery

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Part I

The Skin

Skin Deep: The Basics of Human Skin Structure and Drug Penetration

1

Keng Wooi Ng and Wing Man Lau

Contents

1.1 Introduction

The skin is the heaviest organ of the human body, on average accounting for 10 % of the body mass and covering nearly 2 m^2 of the body surface area (McGrath et al. 2004; Williams 2003). It defines the boundary between the body and its surroundings, thus allowing vital bodily functions to occur within a controlled physiological environment. However, the skin is more than just a physical partition; rather, it provides an important *interface* through which we interact with the world. One such interaction takes the form of substance exchange between the body and the surrounding environment. Substance exchange is usually finely regulated by the skin, which possesses some exceptional properties to enable it to carry out this function. As a result, the skin is highly selective as to what it lets into, or out of, the body and at what rate. This presents a challenge to drug delivery across the skin into the body, as the molecules in question are likely to be poorly absorbed due to low skin permeation.

The overarching subject of this book is on skin permeation enhancement. Two key concepts, i.e. the properties of the skin and molecular transport through the skin, are fundamental to a full understanding of the subject. In this introductory chapter, we describe the basic properties of the skin and its functions, as well as the mechanisms of skin penetration and permeation, and relate them to the challenges that may be encountered in

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attempts to deliver molecules through the skin. The coverage is intentionally brief, since the aim is to provide a basic understanding of the subject area to prepare the reader for more advanced discussions in later chapters of this book. Also, although this chapter describes human skin, it is worth noting that many structural and functional parallels can be drawn between human skin and that of certain other animals. This caveat underpins the use of certain animal skin as in vitro models for human skin research, as will be apparent in some of the later chapters.

1.2 The Skin

1.2.1 Structure of the Skin

Human skin is a stratified epithelium, each tissue layer consisting of different cell types that perform distinct functions. It can be broadly divided into the overlying epidermis, dermis and underlying hypodermis (or subcutis) (Fig. 1.1). The epidermis can further be subdivided, from the outside to the inside, into the stratum corneum (horny layer), stratum granulosum (granular layer), stratum spinosum (prickle cell layer) and stratum basale (basal layer also called the stratum germinativum). The stratum basale and stratum spinosum are collectively known as the Malpighian layer. An additional layer, the stratum lucidum (clear layer) can be observed on parts of the body with thickened skin, such as the palm and sole of the foot. However, the stratum lucidum is often not considered a distinct epidermal layer but the lower part of the stratum corneum. In addition, there are appendageal features including hair follicles and sweat ducts that traverse various skin layers.

1.2.1.1 Stratum Corneum: The Primary Barrier

The stratum corneum is the outermost layer of the skin. It is typically 10–20 μm thick and composed of 10–15 layers of corneocytes (Agache 2004a; Williams 2003). Corneocytes are nonliving cells derived from terminally differentiated keratinocytes that have originated from the deeper layers of the epidermis. Morphologically, corneocytes are flattened and elongated, measuring about 0.2 μm thick and 40–60 μm wide (Kashibuchi et al. 2002). Corneocytes have a cornified envelope in place of a plasma membrane, which is surrounded by a lipid coat. They lack nuclei and cytoplasmic organelles but are filled with keratin filaments and are interspersed in a lipid-enriched extracellular matrix that also contains protein/peptide components (Elias 2012). This organisation of the stratum corneum is commonly referred to as the 'brick and mortar' model (Elias 1983; Michaels et al. 1975), where the corneocytes are likened to bricks and the extracellular matrix analogous to the mortar in a brick wall (Fig. 1.3). Corneocytes are connected by corneodesmosomes and are continuously shed from the skin surface via desquamation.

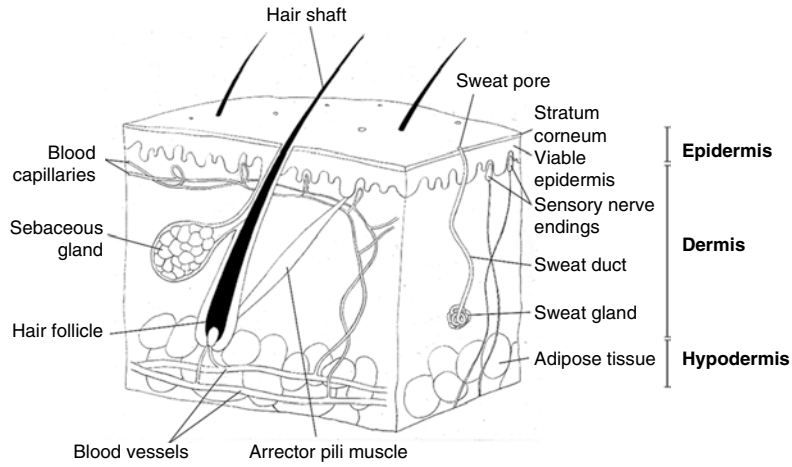

Fig. 1.1 A diagrammatic representation of the structure of human skin in cross section. The epidermis is composed of the stratum corneum and the viable epidermis. Diagram is not to scale

Since diffusion across the stratum corneum is considered as the major pathway of skin permeation (see Sect. 1.3), the structural properties of this layer have been extensively studied to elucidate its barrier function. Significant effort has been focused on elucidating the role of stratum corneum lipids, since the lipid-enriched extracellular matrix is the only continuous domain in the stratum corneum and thus likely to be pivotal to its barrier function (Bouwstra et al. 2002). This is supported by empirical data that demonstrate skin penetration predominantly through the extracellular matrix (Labouta et al. 2011), the marked reduction in the skin's barrier function following stratum corneum lipid extraction (Sweeney and Downing 1970), and that chemicals that disrupt stratum corneum lipid organisation enhance skin permeation (Williams and Barry 2004).

Through freeze-fracture electron microscopy, Breathnach et al. (1973) first observed that the extracellular space in the stratum corneum was composed of a 'laminated material'. It is now understood that stratum corneum lipids are organised into layers (lamellae) that run parallel to the flat plane of the corneocytes. According to the widely accepted Landmann model of skin barrier lipid morphogenesis (Landmann 1986), stratum corneum lipids are initially secreted by lamellar bodies found in keratinocytes and later fuse to form continuous lipid lamellae in the extracellular space. This model is supported by evidence of lipid membrane extrusion and fusion in the stratum corneum under electron microscopy (Madison et al. 1987). An alternative model, the membrane folding model, postulates that the extracellular lipid lamellae forms from single, coherent lipid structures through a non-fusogenic process (Norlén 2001a).

Ceramides, cholesterol and free fatty acids are the main constituents of the extracellular matrix, present in approximately equimolar proportions (McGrath et al. 2004). Of these, ceramides account for approximately 50 % of stratum corneum lipids by mass (Law et al. 1995). Eleven classes of ceramides, encompassing 342 individual ceramide species, have been identified in the human stratum corneum (Masukawa et al. 2008). Each ceramide molecule consists of a sphingoid moiety (sphingosine, phytosphingosine, 6-hydroxysphingosine or dihydrosphingosine) containing a polar head group and a hydrocarbon chain and another hydrocarbon chain derived from a fatty acid or fatty acid ester moiety (Fig. 1.2). The polar head groups

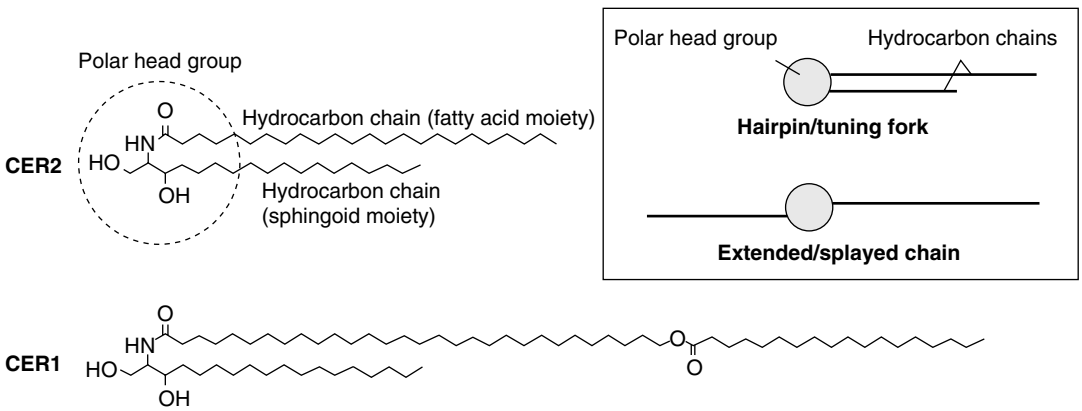

Fig. 1.2 Chemical structures of two stratum corneum ceramides and their possible conformations. Ceramide 2 (CER2) is shown as a typical ceramide consisting of a polar head group (*dotted circle*) and two hydrocarbon chains derived from a fatty acid and a sphingoid moiety, respectively. In CER2, the hydrocarbon chain derived from the fatty acid is 24 carbon atoms long, exemplifying the typical hydrocarbon chain length for this portion of ceramide molecules. Ceramide 1 (CER1) is shown to illustrate its exceptionally long fatty acid ester-derived hydrocarbon chain compared to the typical ceramide molecule (CER2). The *inset* shows two possible molecular conformations of ceramides, the 'hairpin' or 'tuning fork' conformation, where hydrocarbon chains point in the same direction, and the extended or 'splayed chain' conformation, where the hydrocarbon chains point in opposite directions

of ceramides can form lateral hydrogen bonds when organised in lattice structures within lipid bilayers. The hydrocarbon chains are mostly saturated, with few exceptions, and exhibit chain length distribution (Bouwstra and Gooris 2010; Masukawa et al. 2008). Notably, those ceramides containing a fatty acid ester moiety have an exceptionally long hydrocarbon chain. To illustrate this, the typical ceramide contains a total of 38–54 carbon atoms, whereas fatty acid ester-containing ceramides contain a markedly larger number (66–72) of carbon atoms (Masukawa et al. 2008). Like ceramides, the free fatty acids found in the human stratum corneum are mostly saturated. Cholesterol fluidises the stratum corneum lipid bilayers at skin temperature (Zbytovská et al. 2008). There also exists cholesterol sulphate, typically at 2–5 % weight ratio of total stratum corneum lipids, which appears to facilitate the formation of the lipid lamellae and stabilises the stratum corneum by inhibiting enzymatic degradation of corneodesmosomes (Sato et al. 1998).

The precise molecular arrangement of stratum corneum lipids within the extracellular matrix remains a subject of intense investigation. Under electron microscopy with ruthenium tetroxide fixation, the extracellular lipid matrix displays characteristic, alternating 'broad-narrow-broad' lucent bands (Madison et al. 1987; Swartzendruber et al. 1989). This trilamellar motif, with a repeat distance (periodicity) of approximately 13 nm, is known as the long periodicity phase (LPP). This is in contrast with the short periodicity phase (SPP), which has a periodicity of approximately 6 nm but has not been observed in some animal species. For this reason, the LPP is considered to be more important for the skin's barrier function and thus has been the focus of most investigations into stratum corneum lipid organisation. X-ray diffraction analysis has revealed that the extracellular lipids are packed into hexagonal or orthorhombic lattices along the plane parallel with the lamellae, orthorhombic packing being denser than hexagonal. Based on these and other supporting observations, a number of molecular models have been proposed to elucidate stratum corneum lipid organisation:

- The model proposed by Swartzendruber et al. (1989) describes a repeat unit (Landmann unit) of the extracellular lipid lamellae comprising two lipid bilayers. Ceramides in each apposing bilayer assume the 'splayed-chain' conformation (Fig. 1.2) and, in so doing, contribute hydrocarbon chains to form a lipid monolayer between the bilayers that holds the two bilayers together.
- The domain mosaic model (Forslind 1994) describes an intracellular lipid matrix where polar lipids are segregated in crystalline domains surrounded by liquid crystalline 'grain borders'. It is postulated that the liquid crystalline 'grain borders' provide a diffusion pathway for hydrophobic molecules to penetrate the skin.
- The 'sandwich' model (Bouwstra et al. 2000, 2001, 2002) describes alternating crystalline and liquid phases within the LPP. The repeating unit in this model comprises three lipid layers, namely, a middle, narrow liquid phase sandwiched between two adjacent, broad crystalline phases. Ceramides are packed into a crystalline lattice within the broader phases, but the long linoleate moieties in ceramide 1 and ceramide 4 protrude beyond the thickness of the crystalline phases into the space between the crystalline phases to form the narrow liquid phase with cholesterol. It is postulated that the liquid phase represents the main permeation pathway within the LPP. In this model, ceramides are typically depicted in the 'hairpin' (or 'tuning fork') conformation (Fig. 1.2).
- The single gel phase model (Norlén 2001b) describes the extracellular lipid matrix as a single and coherent lamellar gel phase, with no phase separation.
- The model proposed by Hill and Wertz (2003) describes three lipid layers of equal thickness within the LPP. According to this model, the broad-narrow-broad motif observed under electron microscopy with ruthenium tetroxide fixation is an artefact. It is suggested that unsaturated linoleate or sphingosine moieties are asymmetrically distributed in the central lamellae but not the outer lamellae, resulting in the reduction of a greater amount of the

fixative agent and hence an apparently narrower central band.
- The model presented by McIntosh (2003) has lamellae composed of twin lipid bilayers, with an asymmetric distribution of cholesterol and ceramide 1 in apposing monolayers of each lipid bilayer.
- The model of Schröter et al. (2009) suggested that the SPP is formed from lipid bilayers composed of short-chain ceramides, with the long-chain ceramide 1 spanning multiple lipid bilayers. Cholesterol is distributed homogenously within the lipid bilayers.

More recent work has shown that ceramides are fully extended in the stratum corneum, with the two hydrocarbon tails of each ceramide molecule pointing in opposite directions centred on the polar head of the sphingoid moiety (Iwai et al. 2012).

1.2.1.2 Viable Epidermis

Excluding the stratum corneum, the rest of the epidermis is composed of nucleated cells and therefore collectively referred to as the viable epidermis. The viable epidermis is typically 50–100 μm thick (Gentilhomme and Neveux 2004) and devoid of blood capillaries and sensory nerve endings. It is composed primarily of keratinocytes (95 %), with the remainder being Langerhans cells, melanocytes and Merkel cells. Keratinocytes arise from the stratum basale and undergo progressive differentiation whilst migrating towards the stratum corneum. Keratinocyte differentiation is characterised by increasing keratinisation (formation of intracellular networks of keratin fibres), the formation of the lamellar bodies that secrete stratum corneum lipids and the loss of intracellular organelles and nuclei. The process culminates in the formation of corneocytes in the stratum corneum. Keratinocyte differentiation serves to maintain the stratum corneum by replenishing stratum corneum lipids and corneocytes lost via desquamation.

1.2.1.3 Dermis

The dermis, typically ≥1 mm thick (Agache 2004b; Williams 2003), comprises the bulk of the skin and is responsible for its elasticity and strength. It is composed principally of fibroblasts in an extracellular matrix of structural proteins, mainly collagen and elastin. It also contains a range of immune cells including macrophages and dermal dendritic cells. The dermis can be subdivided into the upper papillary dermis and the lower reticular dermis, which can be distinguished microscopically from each other by the thinner and looser packing of collagen fibres in the papillary dermis. The papillary dermis contains papillae that interdigitate with the basal layer of the epidermis at the dermo-epidermal junction. The dermis contains hair follicles, sweat glands, sebaceous glands, sensory nerve endings, lymphatic vessels and blood capillaries which extend to the dermal side of the dermo-epidermal junction. This allows nutrient and oxygen delivery to, as well as waste removal from, the avascular epidermis to occur by diffusion across the dermo-epidermal junction.

1.2.1.4 Hypodermis

The hypodermis is the innermost layer of the skin. However, its absence is notable in some lean skin, such as that on the eyelid. The hypodermis is composed mainly of subcutaneous fat. Embedded in this skin layer are larger lymphatic and blood vessels.

1.2.2 Functions of the Skin

The primary function of the skin is to separate the internal physiological environment of the body from the external non-physiological environment. To put it plainly, it serves to 'keep the insides in, and the outsides out' (Williams 2003). The skin barrier is physical, chemical and immunological in nature. The physical barrier is provided primarily by the stratum corneum, that is to say, traversing the stratum corneum is usually the rate-limiting step in substance exchange between the body and the environment via the skin. This physical barrier is responsible for regulating not only the ingress of exogenous materials but also preventing excessive water loss from the body. The chemical barrier is known as the 'acid

mantle'. The skin owes its chemical barrier function to the acidic (pH 4–6) nature of the skin surface which protects the body in two ways. Firstly, it confers selective antimicrobial properties to the skin by maintaining the natural skin microflora, which live optimally in an acidic environment, whilst arresting the growth of pathogenic microorganisms which thrive in alkaline environments. Secondly, it helps maintain the integrity of the stratum corneum barrier since many skin enzymes pivotal to stratum corneum lipid homeostasis (e.g. β-glucocerebrosidase and sphingomyelinase) have a pH optima within this pH range (Bowser and Gray 1978; Takagi et al. 1999). Sebaceous glands in the skin, which secrete sebum, perform a similar function. Following its secretion to the skin surface, sebum forms a greasy film on the skin, which waterproofs the skin to maintain hydration and suppleness. Sebum also contains antimicrobial constituents.

The skin is also an immune-competent organ. A range of immune cells including Langerhans cells, dermal dendritic cells and macrophages are found in the skin (Zaba et al. 2008). These cells conduct immune surveillance and defend the body against invading microorganisms. They are antigen-presenting cells capable of priming naïve T lymphocytes to elicit a primary immune response against newly encountered antigens. This is an important role of the skin considering a compromised skin barrier is a common route of pathogen entry into the body. There is also an increasing body of evidence that supports a role for some skin dendritic cell subsets in inducing immune tolerance (Romani et al. 2012), which is equally important for maintaining immune homeostasis.

Moreover, the skin has an important role in thermoregulation, allowing thermal energy to be dissipated or conserved. Thermoreceptors in the skin detect heat and cold; they provide sensory input to the hypothalamus, which then invokes a range of thermoregulatory mechanisms to achieve temperature homeostasis. Adipose tissue in the hypodermis insulates the body from cold and prevents excessive heat loss from the body. Body hairs on the skin provide additional insulation by trapping a thin layer of air on the skin surface. This effect is maximised by the erecting of hairs, via constriction of the arrector pili muscle. Perspiration secreted through sweat pores on the skin surface helps reduce body temperature by dissipating heat from the body through the evaporation of water in sweat. Blood vessels in the skin dilate or constrict to adjust the blood flow and heat loss across the large skin surface area. These thermoregulatory mechanisms work in concert to help maintain a constant core body temperature of about 37 °C.

Apart from heat and cold, sensory nerve endings in the dermis detect touch, vibrations and pain. These sensations are critical to other functions of the body, such as locomotion and coordination. The ability to sense pain alerts us of danger and is crucial to survival.

Furthermore, the skin carries out important metabolic functions. Adipocytes in the hypodermis store excess energy in the form of subcutaneous fat, which can be mobilised rapidly during energy deprivation. The epidermis is the primary site of vitamin D synthesis in the body (Bikle 2011). The process, photolysed by ultraviolet irradiation, produces a precursor for vitamin D in the stratum spinosum and stratum basale, which is then converted into vitamin D by keratinocytes.

The skin additionally serves an excretory function, as minerals and other organic wastes are released through the skin dissolved in sweat. The hypodermis also provides mechanical protection to inner organs by cushioning the body against physical shock.

1.3 Drug Permeation Through the Skin

The skin is a selectively permeable barrier. As such, different drugs permeate through the skin at different rates. The rate of drug permeation is expressed as the flux (J), i.e. the amount of drug permeated per unit area, per unit time (usually μg cm^{-2} h^{-1}). The flux is determined by (a) the permeability of the skin to the permeant and (b) the concentration gradient (ΔC) of the permeant across the skin (usually μg ml^{-1}), according to Eq. 1.1:

$$J = K_p \cdot \Delta C \tag{1.1}$$

In Eq. 1.1, skin permeability is defined by the permeability coefficient, K_p (usually cm h^{-1}). Assuming passive drug absorption, the permeability coefficient is a combined measure of the partition coefficient (P, which depicts how readily the permeant partitions from the formulation into the skin), the diffusion coefficient (D, which measures how readily the permeant diffuses through the skin) and the diffusional path length (h), according to Eq. 1.2:

$$K_p = \frac{P \cdot D}{h} \tag{1.2}$$

The processes of partitioning and diffusion (and thus skin permeability, according to Eq. 1.2) are highly dependent on the physicochemical properties of the permeant, such as molecular mass and hydrophilicity. As a general rule, molecules that permeate the skin most readily have a molecular mass of <500 Da and are moderately hydrophilic, with an octanol-water partition coefficient (log $P_{\text{octanol–water}}$) of 1–3. The quantitative relationship between skin permeability (defined by K_p), molecular mass (MW) and hydrophilicity (defined by log $P_{\text{octanol–water}}$) is widely described using Eq. 1.3 (Potts and Guy 1992):

$$\log K_p = 0.71 \cdot \log P_{\text{octanol–water}} - 0.0061 \cdot \text{MW} - 2.74 \tag{1.3}$$

Other factors that may influence skin permeation include hydrogen bond activity, molecular volume, melting point and solubility. Other mathematical models have been devised to relate the role of these parameters to skin permeation (Magnusson et al. 2004; Moss et al. 2002).

1.3.1 Permeation Pathways

A molecule can permeate through the skin via either the transepidermal pathway (diffusing across the skin layers) or the appendageal pathway (through hair follicles or sweat ducts) (Fig. 1.3). The combined flux of these two pathways determines the overall observed flux across the skin.

1.3.1.1 Transepidermal Pathway

In the transepidermal pathway, the permeant traverses the intracellular and/or extracellular spaces, from the epidermis to the dermis and hypodermis. The molecule may do so either transcellularly or intercellularly. The transcellular route requires that the permeant traverse the alternating layers of cells and extracellular matrix. This involves a sequence of partitioning and diffusion into alternating hydrophilic and lipophilic domains. The cells and substances that comprise the hydrophilic or lipophilic domains vary between skin layers, but generally the interiors of cells are more hydrophilic than the extracellular matrix. In the intercellular route, the permeant navigates the tortuous path within the extracellular matrix, without traversing the cells. Small hydrophilic molecules generally favour the transcellular route over the intercellular route and vice versa for lipophilic molecules.

1.3.1.2 Appendageal Pathway

The appendageal (or shunt) pathway encompasses permeation through hair follicles (the transfollicular route) or sweat ducts. The transfollicular route has gained significant research interest in recent years and is covered in a separate chapter (Chap. 5).

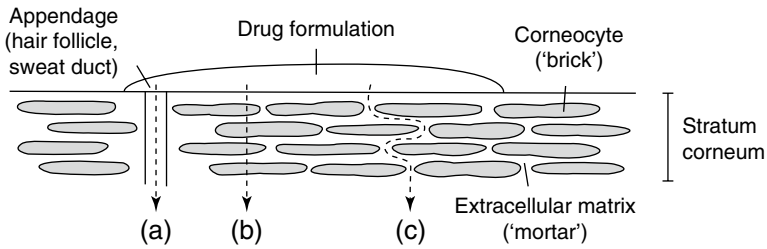

Fig. 1.3 Drug permeation pathways in the skin (stratum corneum shown): (*a*) the appendageal route, (*b*) the transcellular route, and (*c*) the tortuous extracellular route. The transcellular and intercellular routes constitute the transepidermal pathway

1.3.1.3 Relative Contributions of Permeation Pathways

It is widely accepted that the transepidermal pathway is *usually* the predominant pathway of skin permeation and that under sink conditions, diffusion across the stratum corneum constitutes the rate-limiting step that determines the overall flux of the permeant. The contribution of the appendageal pathway to percutaneous transport is generally considered secondary, since appendageal features typically account for only around 0.1 % of skin surface area (though this is higher at some body sites such as the forehead), and early studies suggested that the spatial density of appendages did not correlate with the flux of permeants across the skin (Scheuplein 1967). Nonetheless, the relative contribution of these pathways will vary depending on the physicochemical properties of the permeant and the formulation. Highly lipophilic drugs may be retained in the lipophilic stratum corneum and resist partitioning into the more hydrophilic viable epidermis. Thus, clearance from, rather than diffusion across, the stratum corneum may then become the rate-limiting step for highly lipophilic drugs. Similarly, the appendageal pathway may be more important for highly hydrophilic molecules such as caffeine (Trauer et al. 2010) and electrolytes, as well as large molecules with low diffusion coefficients which are thus effectively precluded from the transpidermal pathway. The relative importance of each pathway may also change with time – various studies have shown that the appendageal pathway rapidly but transiently predominates before being overtaken by the transepidermal pathway at steady state (Liu et al. 2011; Saar et al. 2011; Scheuplein 1967).

Conclusion

The skin owes its barrier properties primarily to the stratum corneum. The unique lipid composition and organisation within the stratum corneum are key determinants of skin permeability, which has important implications for drug permeation through the skin. Painstaking research in the last few decades has elucidated the mechanisms of drug transport through this highly effective barrier and generated structure-activity relationships that permit accurate prediction of drug permeation profiles. However, the challenge remains that the majority of drugs do not exhibit satisfactory skin permeation and innovative strategies are needed to enhance their uptake via the skin. Many such innovations are described in detail within the following specialised chapters of this book.

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Epidermal Lipids and the Intercellular Pathway

2

Philip W. Wertz

Contents

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2.1 Lipid Alteration with Keratinization

The pioneering work of G. Maurice Gray and Harold Yardley and their associates (Gray and Yardley 1975a, b; Gray et al. 1978a, b; Gray and White 1978; Yardley and Summerly 1981) done during the mid- to late 1970s demonstrated that lipids associated with epidermal keratinocytes increased in amount and altered dramatically in composition as a function of differentiation. Thus, the relatively undifferentiated basal keratinocytes contain about 7 pg of lipid per cell consisting of small amounts of cholesterol and phospholipids including sphingomyelin, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, and phosphatidyl serine. The more mature granular cells contain about 324 pg of lipid per cell, and this includes more cholesterol and phospholipids of the same types, but in addition, there are now also significant amounts of glucosylceramides, ceramides, and fatty acids. Finally, the terminally differentiated stratum corneum cells contain about 3,576 pg of lipid consisting mostly of ceramides, cholesterol, and fatty acids. There are also small proportions of cholesterol esters and cholesterol sulfate in the cornified layer, but the ceramides, cholesterol, and fatty acids are thought to be mainly responsible for the permeability barrier of the skin.

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DOI 10.1007/978-3-662-45013-0_2,

2.2 Lamellar Granules

Much of the lipid that accumulates with increasing differentiation in epidermis is packaged into small organelles known as keratinosomes (Wilgram 1965), cementsomes (Hashimoto 1971), Odland bodies (Oashi et al. 1973), membrane-coating granules (Hayward 1974), lamellar bodies (Elias and Friend 1975), or lamellar granules (Landmann 1986). Lamellar granules are round to ovoid in shape, are about 0.2 μm in diameter, and consist of a unit bounding membrane surrounding one, or sometimes several, stacks of internal lamellae (Elias and Friend 1975; Landmann 1986; Wertz 2000). Several lamellar granules are shown in Fig. 2.1. In the uppermost cells of the granular layer, the lamellar granules migrate to the apical end of the cell, where the bounding membrane of the lamellar granule fuses into the cell plasma membrane and the lamellar granule contents are extruded into the intercellular space. This not only delivers lipids, which are thought to be flattened vesicles, to the intercellular space, but also a battery of hydrolytic enzymes are delivered by this mechanism (Freinkel and Traczyk 1985; Grayson et al. 1985; Madison et al. 1998).

Since lamellar granules are lipid-rich, they have a low buoyant density. This unique property has been exploited to isolate lamellar granules (Freinkel and Traczyk 1985; Wertz et al. 1984; Grayson et al. 1985; Madison et al. 1998; Sando et al. 2003). Direct chemical analyses of isolated lamellar granules (Freinkel and Traczyk 1985; Wertz et al. 1984; Grayson et al. 1985) have shown that these organelles are rich in phosphoglycerides, sphingomyelin, glucosylceramides, and cholesterol. Fatty acids, cholesterol esters, and ceramides were minor lipid components. The most abundant of the glucosylceramides is a structurally unusual linoleate-containing acylglucosylceramide (Wertz et al. 1984; Madison et al. 1998). This consists of 30- through 34-carbon ω-hydroxyacids amide-linked to sphingosine (and dihydrosphingosine) with linoleate ester-linked to the ω-hydroxyl group (Wertz and Downing 1983; Abraham et al. 1985). Linoleate has long been known to be required for proper formation and maintenance of the permeability barrier of the skin, and it has been proposed that this unusual sphingolipid is directly involved in this requirement (Wertz and Downing 1982). Based on extensive electron microscopic studies, Landmann has proposed that the internal lamellae of the lamellar granules are stacks of flattened lipid vesicles (Landmann 1986). It was proposed that in assembly of these stacks of flattened vesicles, the long ω-hydroxyacyl chain of the acylglucosylceramide completely spans one region of bilayer, while the linoleate inserts into an adjacent region of bilayer, thus riveting the two sections of bilayer together at a molecular level. In support of this suggestion, it was shown that acylglucosylceramide does cause flattening and aggregation of synthetic lipid vesicles. Lamellar granules and acylglucosylceramides have been found in keratinizing oral epithelium, but neither the organelle nor the unusual sphingolipid are present in nonkeratinizing oral epithelium or other tissues. Likewise, lamellar granules and acylglucosylceramides have been

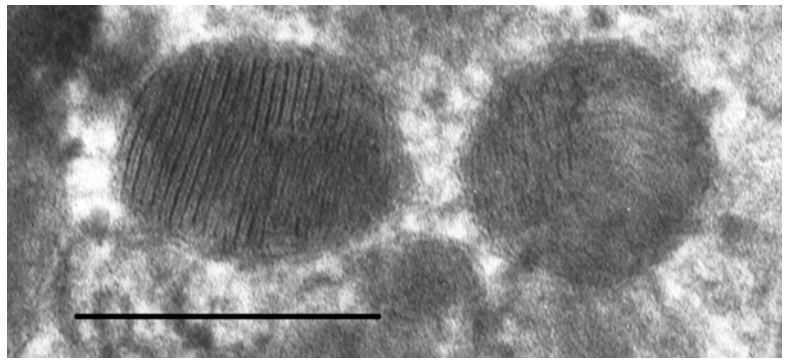

Fig. 2.1 Transmission electron micrograph of several epidermal lamellar granules. Bar = 200 nm

found in epidermis of a number of terrestrial mammals, birds, and reptiles, but both are absent from epidermis of fish and amphibians, where there is no stratum corneum.

The hydrolytic enzymes delivered to the intercellular space by lamellar granules, in general, have low pH optima like lysosomal hydrolases (Freinkel and Traczyk 1985; Wertz et al. 1984; Grayson et al. 1985; Madison et al. 1998). The enzymes detected include acid phosphatase, aryl sulfatase, galactosidase, galactosaminidase, glucosidase, glucosaminosidase, phospholipase A, sphingomyelinase, carboxypeptidase, cathepsin B, acid lipase, and ceramide glucosyltransferase. Of these enzymes, the specific activities in the isolated lamellar granules were lower than found in crude homogenates for arylsulfatase (both A and B) as well as sterol sulfatase (Grayson et al. 1985). The lipid-hydrolytic enzymes are important in converting the initially extruded lamellar granule lipids into the ceramide, cholesterol, and fatty acid mixture that is found in the intercellular spaces of the stratum corneum. It is this intercellular lipid that determines the permeability barrier of the skin, under normal circumstances (Wertz 2000).

2.3 Intercellular Lamellae

Among the enzymatic actions that occur at the boundary between the granular layer and the stratum corneum are the deglycosylation of glucosylceramides and the hydrolysis of sphingomyelin to produce ceramides. Representative structures of the ceramides found in porcine stratum corneum are presented in Fig. 2.2, along with the ceramide nomenclature system proposed by Motta and colleagues (Wertz and Downing 1983b; Motta et al. 1993). In the Motta nomenclature system, one letter is used to indicate the type of fatty acid (N for normal fatty acid, A for

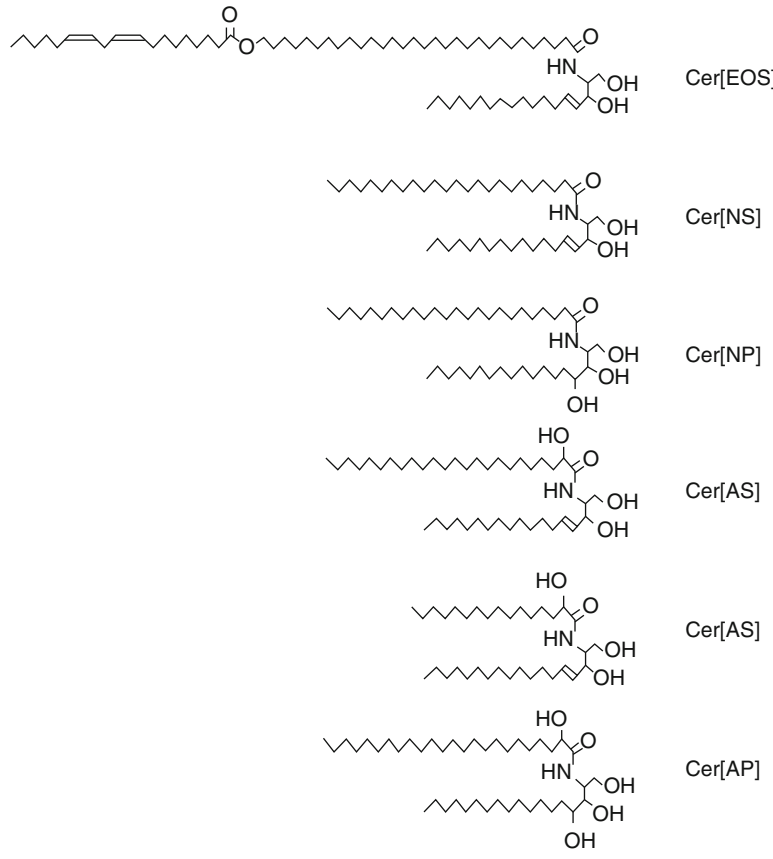

Fig. 2.2 Structures of stratum corneum ceramides and a proposed system for nomenclature (Wertz and Downing 1983; Motta et al. 1993)

α-hydroxyacid and O for ω-hydroxyacid), one letter is used to indicate the long-chain base (S for sphingosine, P for phytosphingosine, and H for 6-hydroxysphingosine), and the presence of an ester-linked fatty acid is indicated by a prefix E. Thus, the ceramide-containing saturated fatty acids amide-linked to sphingosine bases can be designated as Cer[NS]. The unusual ceramide at the top of Fig. 2.2 is an acylceramide or Cer[EOS]. Most of the ceramides are cylindrical in shape, which favors formation of highly ordered, and thereby impermeable, membrane domains. The unusual Cer[EOS] is thought to play a central role in formation of 13 nm trilaminar lipid structures (Madison et al. 1987; Kuempel et al. 1998; Groen et al. 2010). In these trilaminar structures, it has been proposed that the ω-hydroxyacyl portion of Cer[EOS] spans the outer layers, while the linoleates insert into the central lamella (Hill and Wertz 2003). With this arrangement, the outer two lamellae are highly saturated, while the central lamella contains all of the double bonds from the linoleate chains. This stabilizes the trilaminar structures into 13 nm units that have been seen using transmission electron microscopy with ruthenium tetroxide fixation (Madison et al. 1987; Kuempel et al. 1998) and with X-ray diffraction (Groen et al. 2010). One consequence of this arrangement is that the central lamella will reduce more ruthenium than the outer lamellae. This results in alternating broad-narrow-broad lucent bands in the electron micrographs, as can be seen in Fig. 2.3.

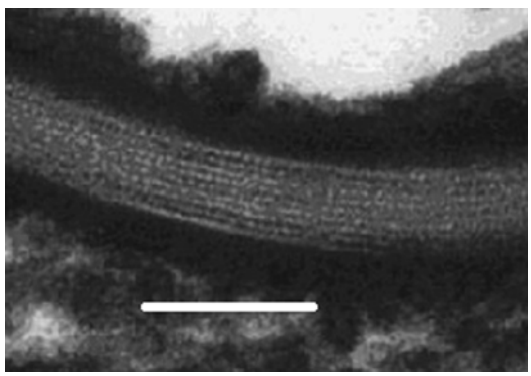

Fig. 2.3 Transmission electron micrograph of intercellular lamellae in the stratum corneum. Bar = 80 nm

The lateral packing of the lipids in normal human stratum corneum is predominantly orthorhombic (crystalline) with small amounts of hexagonal packing (gel) (Pilgram et al. 1998; de Jager et al. 2004). In some skin diseases in which lipid composition is altered and barrier function is diminished, such as atopic dermatitis and lamellar ichthyosis, the hexagonal phase increases relative to orthorhombic phase (Pilgram et al. 2001).

2.4 Covalently Bound ω-Hydroxyceramide

It is thought that approximately two-thirds of the acylglucosylceramide molecules associated with the lamellar granules are present in the bounding membrane with the glucosyl moiety at the inside surface of the granule (Wertz 2000). When the bounding membrane of the lamellar granule fuses into the cell plasma membrane, this inverts the orientation of the acylglucosylceramide. The glucose is then removed, and two stereoselective lipoxygenase attacks on the linoleate chain precede its removal and transfer of the resulting ω-hydroxyceramide to the outer surface of the cornified envelope (Zheng et al. 2011). The attachment of the ω-hydroxyceramide to the cornified envelope is through ester-linkages that may be produced through the action of a transglutaminase (Nemes et al. 1999). Thus, the stratum corneum becomes an array of flat keratin-filled cells bounded by a cornified envelope with a monolayer of covalently bound lipids on the outer surface embedded in a multilamellar array of free lipids.

2.5 Penetration Pathways

In a classic review, Scheuplein and Blank considered potential pathways through which small molecules could pass through the stratum corneum (Scheuplein and Blank 1971). These included a paracellular route through the intercellular spaces, a transcellular route, follicular penetration, and entry through the sweat ducts.

For human skin, they argued that entry through sweat pores would be insignificant due to the small fraction of the skin surface occupied by such openings. Although a similar argument pertained to follicular penetration, it was acknowledged that under some conditions follicular penetration could be significant. The follicular route has some advantages for drug delivery in that it can accommodate nanoparticles and microparticles with potential slow drug release (Knorr et al. 2009; Lademann et al. 2011). A major question was whether interfollicular stratum corneum was amenable to transcellular penetration, paracellular penetration, or some combination of the two. This remained a point of contention until 1980, when Nemanic and Elias (1980) visualized the intercellular pathway followed by N-butanol diffusing across stratum corneum by in situ precipitation combined with transmission electron microscopy. The penetration pathway for this molecule was exclusively via the paracellular route. Squier and Lesch (1988) subsequently demonstrated an exclusively paracellular route for both polar and nonpolar small molecules using microaudioradiography.

So for passive diffusion of drugs across the stratum corneum, the paracellular route is considered the predominant pathway. Chemical permeability enhancers are thought to act primarily by fluidizing the intercellular lamellae of the stratum corneum, thereby reducing diffusional resistance (Thong et al. 2007; Ahad et al. 2009). Some physical means of enhancing drug delivery may also alter the intercellular lamellae; however some physical enhancement methods do alter pathways. For example, microneedle arrays create direct channels across the stratum corneum (Coulman et al. 2006). Iontophoresis may effectively deliver drugs through sweat ducts (Dixit et al. 2007). Sonophoresis may both alter the physical state of the intercellular lamellae and create transcellular pathways (Rao and Nanda 2009). Electroporation increases the permeability of the stratum corneum by opening or creating aqueous channels, through which relatively large water soluble molecules can pass (Singh et al. 2012).

Conclusions

Epidermal keratinocytes differentiate to produce the stratum corneum which consists of flat, keratin-filled cells bounded by a complex cornified envelope and embedded in a matrix of lamellar lipid. The lipids in the intercellular spaces of the stratum corneum consist mainly of a structurally heterogenous group of ceramides, cholesterol, and long, saturated fatty acids. These lipids are organized into trilamellar structures that, under passive conditions, provide the primary permeability barrier of the skin. There are a number of physical methods that are able to bypass this barrier, which should prove useful in development of transdermal drug delivery.

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The Importance of Stratum Corneum Lipid Organization for Proper Barrier Function

3

Annett Schroeter, Adina Eichner, Josefin Mueller, and Reinhard H.H. Neubert

Contents

3.1 Introduction

Obstacles such as the complexity and chemical variability of the lipids present in the stratum corneum (SC), disturbing other material like proteins, ethical issues related to the use of excised human provided by biological material like excised skin, hinder elucidating the molecular morphology of the SC lipid matrix. These difficulties led to an increasing use of synthetic SC lipids in SC research. To allow for a systematic evaluation of the relevance of single ceramide (CER) species, multilamellar model membranes containing simplistic mixtures of synthetic SC lipids represent a suitable approach as shown in numerous previous works (Kessner et al. 2008a, b; Kiselev 2007: Kiselev et al. 2005; Wegener et al. 1997; Zbytovska et al. 2009). Such a systematic determination of the impact of particular CER subclasses is important for a detailed understanding of mechanisms in skin diseases.

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This knowledge supports the development of new therapeutic approaches. In addition, enhanced understanding of the function of different SC lipids in the process of barrier formation helps to develop new carrier systems being able to overcome the penetration barrier more efficiently.

3.1.1 Ceramides of the Stratum Corneum Lipid Matrix

It is generally known that the main constituents, the ceramides (CER), play a key role in the structuring and hence the maintenance of the barrier function of the skin (Coderch et al. 2003; Holleran et al. 1991). They are a group of structurally heterogeneous sphingolipids and consist of a long-chain fatty acid bound to the amino group of a long-chain di- or trihydroxy sphingoid base (sphingosine, phytosphingosine, and 6-hydroxysphingosine). The bound fatty acid of the CER consists predominantly of a very long almost entirely saturated alkyl chain (Wertz et al. 1987) and can be hydroxylated at the α-position to the carbonyl oxygen, at the end of the hydrocarbon chain (ω-position), or it contains no further hydroxyl group (Coderch et al. 2003). The first nomenclature used to label the different CER was based on their mobility in the thin-layer chromatography (Wertz and Downing 1983). As the number of the identified CER increased, this method of labeling was insufficient. Consequently, the nomenclature of the CER is based on their chemical structure and was developed by Motta and coworkers (Motta et al. 1993). In this system the ceramides are labeled with letters, whereby the last letter assigns the type of sphingoid base (*S*… sphingosine, *P*… phytosphingosine, *H*… 6-hydroxysphingosine). The long-chain fatty acid bound to the amino group can be differentiated due to their hydroxylation. This was taken up in the nomenclature as amid-bound fatty acids without a hydroxyl group were labeled with the letter N (=non-hydroxy), while ceramides with an omega- and alpha-hydroxylated fatty acid receive either the letter O or A, respectively. Furthermore, within the group of ceramides, the exceptionally long-chain ω-acyl ceramides exist, which are esterified with a long unsaturated fatty acid. In line with the way of labeling, these ceramides received the letter *E* (=esterified). Up to now, 12 major CER classes have been identified within the SC lipid matrix (Holleran et al. 2006; Masukawa et al. 2008) (see Fig. 3.1), but detailed information about their specific role for the barrier formation and function of the SC needs to be elucidated. Especially the long-chain CER[EOS], [EOP] and [EOH] are discussed to be of particular relevance because of their unique structure. As mentioned above these CER have in addition to the amidation either a phytosphingosine (P), sphingosine (S), or hydroxysphingosine (H) base, while the ω-hydroxylated fatty acid is esterified with a linoleic acid (EO) in ω-position (Coderch et al. 2003). Due to the esterified fatty acid, those CER have a chain length of 30–32 carbon atoms (Raith et al. 2004). However, in recent years also the short-chain CER such as CER[AP] and CER[NP] have been in the focus of various investigations, and it became evident that these CER seem to play a more important role in the structural organization of the uppermost skin barrier than previously assumed. But, it is most likely the broad distribution of alkyl chain lengths and the heterogeneity in the head groups which guarantee the integrity and functionality of the lipid lamellae of the SC (Norlen 2001).

3.2 Stratum Corneum Lipid Nanostructure Investigated With Neutron Diffraction

3.2.1 Basic Principles of Neutron Diffraction

The SC research comprises many biophysical approaches such as Fourier transform infrared spectroscopy, differential scanning calorimetry, atomic force microscopy or nuclear magnetic resonance spectroscopy. Among those the scattering techniques X-ray and especially neutron diffraction are very potent methods to investigate the structure of isolated SC (Bouwstra et al. 1991) as well as SC model membranes constructed from extracted and synthetically derived SC lipids (Bouwstra et al. 1991, 1996, 1998; Friberg and Osborne 1987; Kuempel et al. 1998; McIntosh

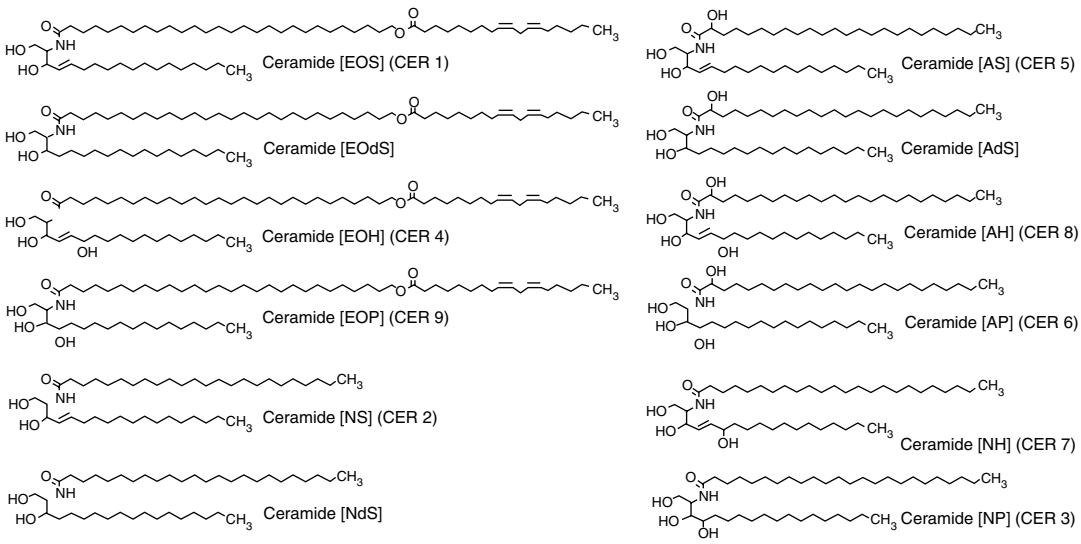

Fig. 3.1 Chemical structures of the ceramides found in the human stratum corneum. *S* Sphingosine, *P* Phytosphingosine, *H* 6-Hydroxysphingosine, *N* non-hydroxy fatty acid, *A* alpha-hydroxy fatty acid, *O* ω-hydroxy fatty acid, *E* esterified, *D* dihydro. In addition the old nomenclature according to the mobility of the CER in the thin layer chromatography was included for clarification

2003; McIntosh et al. 1996). Both neutron and X-ray diffraction are similar techniques, with the exception of the irradiation source. While X-rays primarily interact with the electrons of an atom, the interaction of neutrons with the atomic nucleus is short-ranged. To explain the several advantages of neutron over X-ray diffraction a brief explanation of these methods is necessary.

The technique of neutron diffraction is a versatile method to study the structure and dynamics, which specifically applies to biological samples. Due to their specific properties, neutrons may provide structural insights that are hardly obtained by other techniques, e.g., X-ray or light scattering. As non-charged particles, neutrons are enabled to penetrate matter deeply due to the small probability of interaction (Harroun et al. 2006). In contrast to X-rays, which are scattered by the electron cloud, neutrons interact with the atomic nucleus and are scattered isotropically (Dachs 1978). Hence, while the ability of elements to scatter X-rays increases with the atomic number throughout the periodic table of elements, such a correlation does not exist for neutrons (Cantor and Schimmel 1980). Particularly hydrogen, a light atom that is almost invisible for X-rays, is a strong scatterer for neutrons (see Fig. 3.2).

This makes neutron diffraction a particular valuable instrument to investigate structural and dynamic features especially in biological samples, which are rich in hydrogen. Moreover, neutrons show isotope sensitivity, i.e., even different isotopes of one element may have different scattering power for neutrons, for which hydrogen (1H) and deuterium (2H, D) are the most prominent examples (see Fig. 3.2) (Dachs 1978). The possibility to distinguish between components differing in their ability to scatter neutrons in one single sample, the so-called neutron contrast, is of great advantage for the study of biological systems like lipids and proteins (Büldt et al. 1978; Gutberlet et al. 2001; Tomita et al. 1999).

In a typical scattering experiment, a well-collimated neutron beam with a defined wavelength λ irradiates a sample, whereby the neutrons are scattered in all directions depending on the interactions between the sample material and the neutrons. The incoming neutrons interact with the sample and thereby experience a change in their momentum, which appears as a change of neutron direction and/or velocity. Consequently, monitoring the alterations of the neutron's momentum provides information regarding the

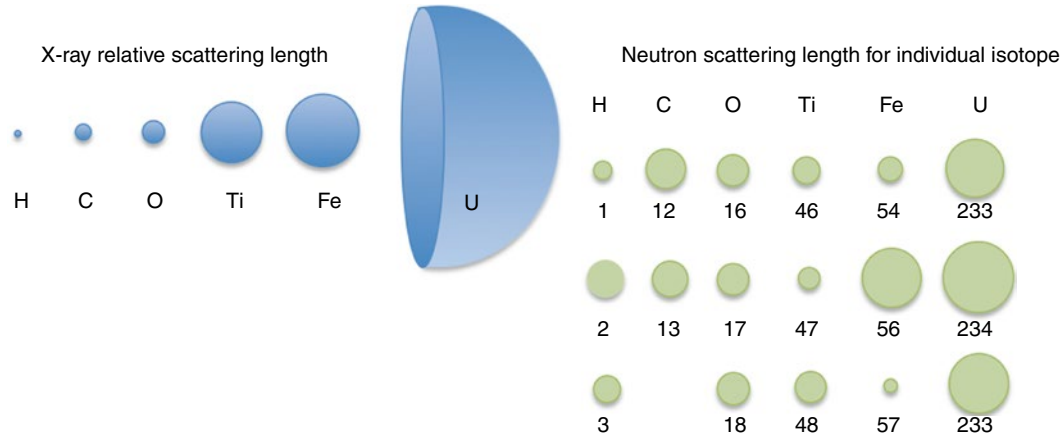

Fig. 3.2 Schematic comparison of the X-ray relative scattering lengths and neutron scattering lengths of different elements and their isotopes

structure and dynamics of the sample matter. To describe the change in momentum, the so-called momentum transfer vector, or *scattering vector* $\vec{Q}$, was introduced and is defined as the difference between incoming $\vec{k}_i$ and scattered $\vec{k}_s$ wave vectors $\vec{Q} = \vec{k}_i - \vec{k}_s$. In addition to a change in direction, the magnitude of $\vec{k}$ can also change as energy is transferred between incident neutrons and sample. When no energy is conveyed, the scattering process is considered to be totally elastic; therefore, $\vec{k}_i$ has to be equal to $\vec{k}_s$. Taking this in account, the scattering vector $\vec{Q}$ can be evaluated as $\vec{Q} = 2\vec{k}_i \cdot \sin\theta$, including the Bragg angle, which in case of crystalline and lamellar material appears at $\vec{Q}$ values equivalent to the reciprocal spacing of the lattice: $\left|\vec{Q}\right| = \frac{2\pi}{d}$, where by d denotes the characteristic spacing of a set of crystal planes. The complete Bragg formula $\lambda = 2d/\sin\theta$ can be received when the wave vector $\vec{k}_i$ is appropriately substituted with $\vec{k}_i = 2\pi / \lambda$.

Diffraction can be considered as a special type of scattering, whereby an organized structure such as a crystal or a lamellar arrangement is analyzed. According to Bragg's law, the incident beams are diffracted at a defined angle 2θ, and due to the interference between the waves, scattered from the parallel planes, diffraction occurs as depicted in Fig. 3.3.

The neutron scattering experiment now measures the scattering intensity I as a function of the scattering direction; the interpretation of the data offers information about the structure of the analyzed sample.

3.2.2 Investigation of Stratum Corneum Lipid Model Membranes with Neutron Diffraction

The initial biological materials, analyzed with neutron diffraction, were phospholipids due to the ability to form stable and highly organized multilamellar lipid bilayers necessary for neutron diffraction experiments. In recent years this technique has also been successfully introduced into the elucidation of the structural arrangement of the stratum corneum lipids. The application of neutron diffraction offers new possibilities to investigate the nanostructure of SC lipid systems and especially to gain information about the impact of the different CER subspecies as shown by Charalambopoulou and coworkers on fully hydrated human SC (Charalambopoulou et al. 2002) and by Kiselev et al. on well-defined SC lipid model membranes (Kiselev et al. 2005).

3.2.2.1 Evaluation of the Neutron Diffraction Data

As mentioned above, the scattering process is assumed as an elastic event with no energy transfer taking place. Consequently, the scattering

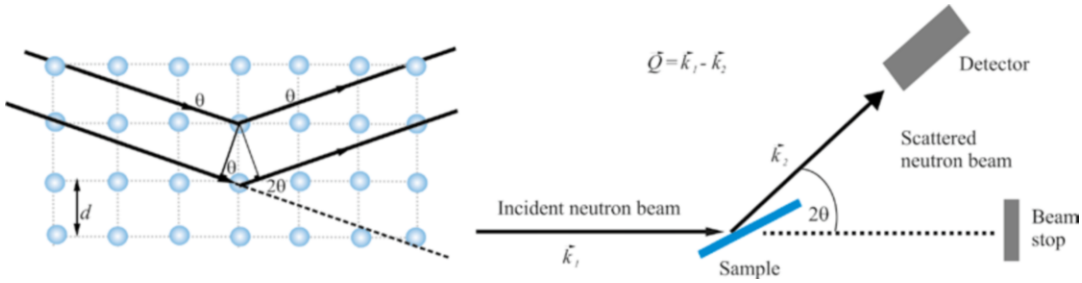

Fig. 3.3 Schematic drawing of the scattering process from ordered material. (*Left*) Neutrons strike an array of atoms (*spheres*) from the left side and are scattered to the right. The planes of atoms are separated by the interplanar distance *d*. The angle θ to the plans of atoms of the incident and the scattered beam are identical. The path length difference between the waves interfering constructively is equal to $2d \sin \theta$. (*Right*) A typical experimental setup of a neutron diffraction experiment, whereby $\vec{k}_i$ designates the incoming wave vector, while $\vec{k}_s$ represents the scattered neutron wave vector

vector $\vec{Q}$ can be correlated to the scattering angle 2θ. Furthermore, the intensity of the scattered neutron is measured as a function of the scattering angle 2θ. As the Bragg condition is complied, the integrated intensities can be calculated by using Gaussian fits to the received Bragg reflections. In order to gain deeper insight into the nanostructural arrangement of the SC lipids, it is necessary to compute the absolute value of the structure factors F_h from the integrated peak intensities: $|F_H| = A_h(\theta) \cdot \sqrt{h \cdot I_h}$ with Lorentz correction h and absorption factor A_h (θ) (Franks and Lieb 1979). The structure factor F_h serves as a mathematical description in which mode the incoming neutron wave is scattered by the investigated material (Franks and Lieb 1979; Nagle and Tristram-Nagle 2000a, b). The SC lipid multilamellar layers are composed of numerous bimolecular lipid membranes, which in other terms can be described as two equal monolayers facing each other. Such stacks of lipid layers are considered centrosymmetric for the neutron diffraction experiment, which allows for the construction of the neutron scattering length density (NSLD) profile $\rho_s(x)$ across the bilayer as Fourier transform

$$\rho_s(x) = \frac{2}{d} \sum_{h=1}^{h_{max}} F_h \cdot \left(\frac{2 \cdot \pi \cdot x}{d} \right)$$

(Nagle and Tristram-Nagle 2000b). In order to calculate the NSLD profile, it is essential to define the sign of the structure factor F_h. This can be easily done by variation of the D_2O/H_2O ratio in the surrounding atmosphere, the so-called contrast variation (Wiener and White 1991), assuming that water can penetrate between the bilayer sheets (Franks and Lieb 1979; Worcester 1976). It was shown for such symmetrical and hydrated bilayers that the phase problem of $F_h s$ simplifies to the determination of the sign of + or – (Franks and Lieb 1979) and can be derived from the slope of the correlation of F_h against the D_2O content in water vapor as shown in Fig. 3.4.

The NSLD profile offers detailed information about the nanostructure of the investigated lipid membrane and can also contribute to assign the position and orientation of the bilayer constituents. Furthermore, the evaluation of the NSLD profile allows for determining specific membrane regions such as the polar head groups, CH_3 groups, hydrocarbon chain region, and the region of cholesterol location (Kiselev et al. 2005). Next to the assignment of the position of these groups in the lipid bilayer membrane, the determination of parameters such as the region of polar head group or thickness of the intermembrane space further improves and intensifies the knowledge about such SC lipid organization.

3.2.3 Advantages and Disadvantages of Neutron Diffraction

As up to now a clear and detailed picture of the organization of the SC lipid matrix on a molecu-

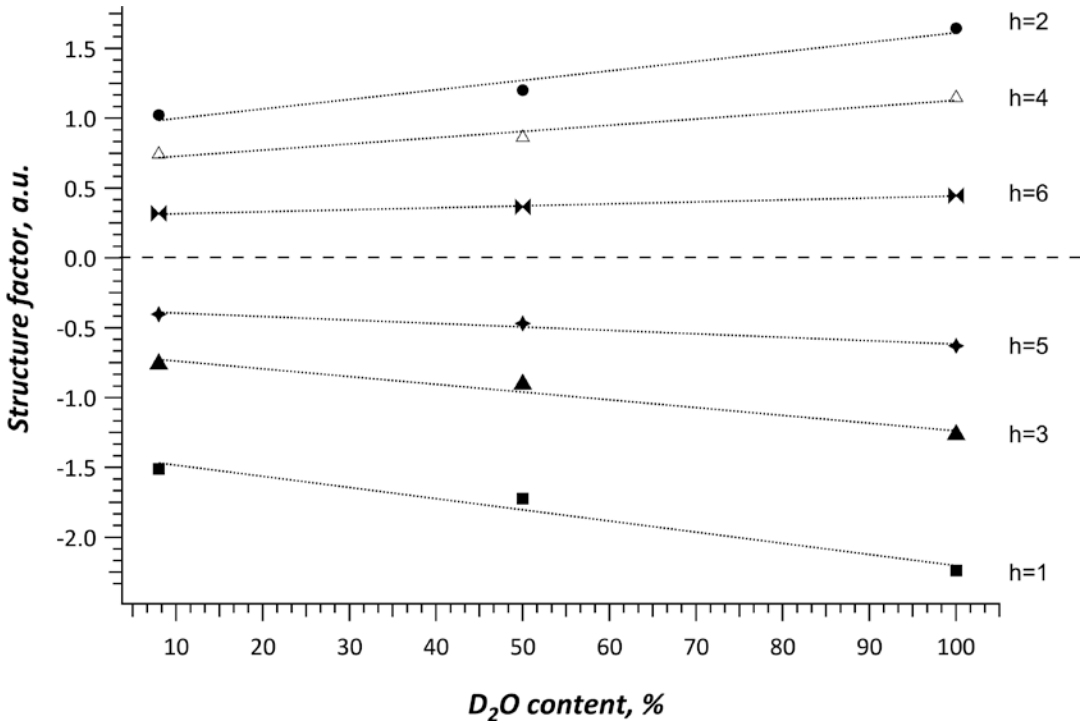

Fig. 3.4 Illustration of the dependency of the membrane structure factor F_h of the orders h = 1, 2, 3, 4 and 5 on the D_2O content on the water vapor a SC lipid model system

lar scale has not been elucidated, it is of supreme importance to comprehend the mode of action of the different SC lipid classes and particular the impact of the different ceramide subspecies. For the investigation of the driving forces and mechanisms that govern the self-assembling process of such lipid layers, the native SC lipid membranes are too complex objects to probe, especially with neutron diffraction. Consequently, for such an approach, model membranes will be the objects of choice. Moreover, issues due to the variability of the native lipids, for example, the variability in the head group architecture, can be overcome.

Neutrons as irradiation source are non-charged particles; they have only small interaction potential with matter, which enables a deep penetration into the studied material. This makes this method particularly suitable for biological issues such as the investigation of the structural arrangement of the SC lipids. In addition, as mentioned before the neutrons are scattered differently by different isotopes of the same element (see Fig. 3.2). This special feature renders the possibility of the contrast variation as described before. In the same line, there is another distinct advantage of the neutron diffraction technique, which is the possibility of specific deuteration, as the coherent scattering length b_{coh} (the scattering ability) of hydrogen (^1H) and its isotope deuterium (^2H) differs significantly ($b_{coh}(^1H) = -3.741$ fm, $b_{coh}(^2H) = 6.671$ fm). Accordingly, hydrogen atoms in a lipid molecule can be specifically substituted by deuterium, which does not alter the properties of the lipid molecule. When a partially deuterated lipid sample is compared to its protonated counterpart, it is possible to identify the exact position of the labeled group within the lipid membrane (see Fig. 3.5). This is a distinct advantage of neutron diffraction over X-ray diffraction, which does not allow for such localization. As the SC lipid molecules are rich in hydrogen atoms, there is a variety of substitution positions, which then permit to make distinction between different conformational states of the studied lipid species. This feature is of high inter-

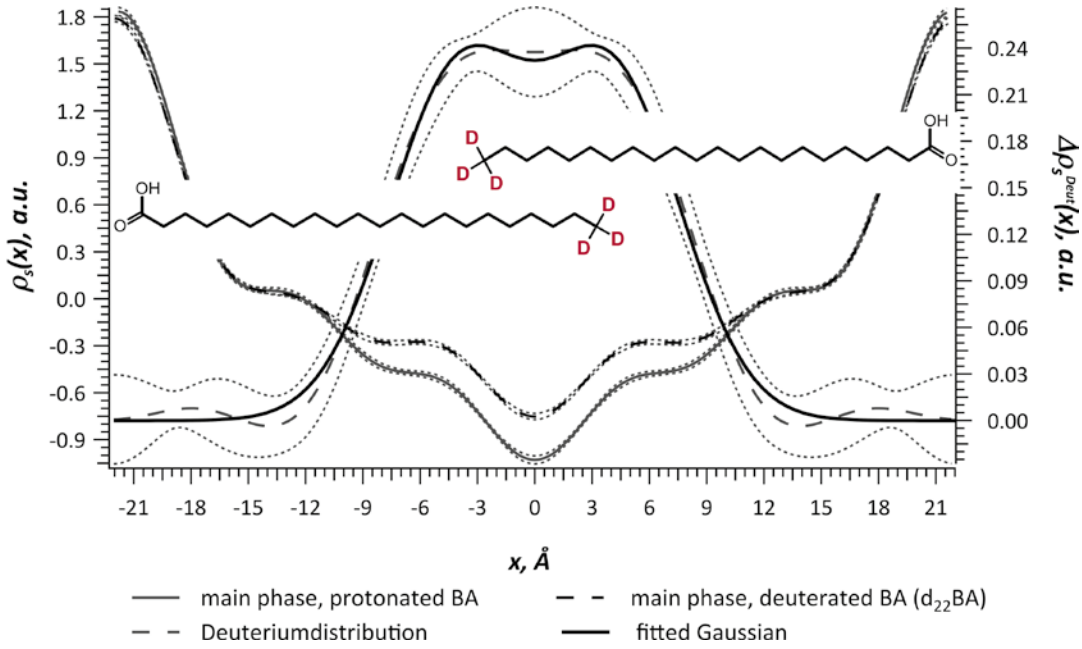

Fig. 3.5 Example of the localization of partially deuterated behenic acid (BA) molecules (d_{22}BA) in a SC lipid model membrane composed of ceramide [AP], cholesterol, d_{22}BA and cholesterol sulfate. The neutron scattering length density (NSLD) profiles display the comparison of the sample membrane containing either deuterated (*dashed line*) or protonated BA (*solid line*). *Dotted lines*: corresponding errors, *Long dash*: difference NSLD profile, *Fat solid line*: fit of the difference NSLD profile by two Gaussian functions (deuterium distribution). All measurements were carried out at 57 % relative humidity, at 8 % D_2O in water vapor and T = 20 °C

est, especially for the investigation of SC lipids, as it is known for CER to exhibit different conformational states (Dahlen and Pascher 1972, 1979; Raudenkolb et al. 2003a, b, 2005).

The disadvantages are the limiting factors for application of the neutron diffraction for exploration of the SC lipid matrix. When compared to X-rays the neutron fluxes are relatively small, which necessitates a much longer experimental timescale and a higher amount of lipid material to achieve a reasonable signal-to-noise ratio. Furthermore, when native SC is probed with neutron diffraction, this yields to only one or two diffraction orders, which are not sufficient for the analysis of the NSLD profile (Charalambopoulou et al. 2002). Consequently only SC lipid model systems can be studied.

Another drawback of the neutron diffraction technique is its availability, as there exist only a few neutron facilities at which such an experiment can be carried out (e.g., Helmholtz Centre Berlin for Material and Energy (HZB) and Institut Laue-Langevin (ILL), Grenoble, France).

3.2.4 X-Ray Diffraction for the Investigation of the Stratum Corneum Lipids

As mentioned before, X-ray diffraction has been widely used for the investigation of the structural arrangement of the SC lipids. So Hatta and coworkers could establish the impact of ethanol on the lipid membranes. They discovered by X-ray diffraction, that lipid compounds can be extracted and even recrystallized as well (Hatta et al. 2001). The investigations of Kessner et al. could establish by X-rays that the phase behavior of the long-chain CERs is effected by their long acyl rests (Kessner et al. 2010).

Compared to other scattering techniques, it has a variety of advantages. In contrast to the above-described neutron diffraction technique, X-rays are scattered by the electrons surrounding the atomic nuclei. This results in peaks of high intensity and high resolution. Furthermore, many X-ray sources for such experiments are accessible.

Nevertheless, as described above the major drawback of the application of X-ray in the field of SC research is the low capability to depict light atoms such as hydrogen, which is one of the main components of a biological relevant material. Furthermore, as the number of electrons between different isotopes of the same element does not change, X-rays cannot distinguish isotopes. Its application as an irradiation source does not allow for the evaluation of the sign of the structure factor, which is essential in order to be able to calculate the scattering length density profile to gain deeper insight into the arrangement of the lipid bilayer. When the lamellar thickness of a lipid membrane is changed by way of varying the thickness of the water layer, it is possible to evaluate the sign of the structure factor and consequently calculate the electron density profile. However, it is well known that the repeat distances of SC lipid mixtures prepared from ceramides (CER), cholesterol (CHOL), and free fatty acids (FFAs) are very insensitive to hydration and that especially for the short periodicity phase (SPP), only a limited number of diffraction orders are obtained with X-ray diffraction. Therefore, again it is difficult to acquire an electron density profile by X-ray diffraction analysis.

3.3 ω-acyl Chain Ceramides and Their Influence on the Nanostructure of the Stratum Corneum Lipid Matrix

The lipids of the SC and particularly the ceramides (CER) are responsible to uphold the proper barrier function as pointed out in the introduction. The CER are a very heterogeneous group of sphingolipids (see Fig. 3.1), which can roughly be divided into two groups: (1) short-chain CER such as CER[AP] or CER[NP] and (2) the exceptionally long-chain ω-acyl CER such as CER[EOS] or CER[EOP]. So far, there has been a general consensus that especially the long-chain ω-acyl CER seem to be of particular relevance because of their unique structure.

Besides the assumed importance of long-chain ω-acyl CER in forming the so-called long-periodicity phase (LPP), their presence plays a key role with regard to some skin diseases. For atopic dermatitis there was found a decrease especially in the CER[EOS] content as proposed by Yamamoto and coworkers (Yamamoto et al. 1991), whereas psoriatic skin among others is thought to be caused by an increase in the CER[EOS] amount (Motta et al. 1994).

3.3.1 Physicochemical Aspects

In addition to the amidation of the sphingosine backbone, the ω-hydroxylated fatty acid is at its ω-position mainly esterified with a linoleic acid (EO) (Coderch et al. 2003). Nevertheless, Hinder and coworkers identified for CER [EOS] different amid-bound fatty acids with chain lengths varying from 17 to 22 carbon atoms (Hinder et al. 2011). The same chain length ranging was found for the sphingosine part. Therefore, it was concluded that not only linoleic acid as fatty acid compound is esterified to the sphingosine backbone. Subsequently, the influence of these different chain lengths was studied by de Sousa Neto et al. with small-angle X-ray scattering and Fourier transform infrared spectroscopy (de Sousa Neto et al. 2011). Their results show an important influence of chain length on the lipid organization. Whereas linoleate- and oleate-linked fatty acid CER[EOS] showed both the formation of the LPP and SPP, the stearate-linked variety did not form the LPP. Hence, unsaturated amid-bound fatty acids seem to be crucial for the formation of the LPP.

Due to the high mobility of the exceptionally long-chain ω-acyl residue, these CER form less ordered structures than it is known for short-chain CER (Raudenkolb 2002). Additionally, the melting points being 86 °C for CER[EOS] and above 100 °C for CER[EOP], respectively, hardly differ from those found for short-chain CER as stated by Kessner and coworkers (Kessner et al. 2010). The discrepancy in melting points of both ω-acyl chain CER was argued to be due to the more polar head group structure of CER[EOP] (2 OH groups for CER[EOS] versus 3 OH groups for CER[EOP]), which enables this CER to create more hydrogen bonds. In contrast to their expectations, Kessner and coworkers could additionally ascertain that the ther-

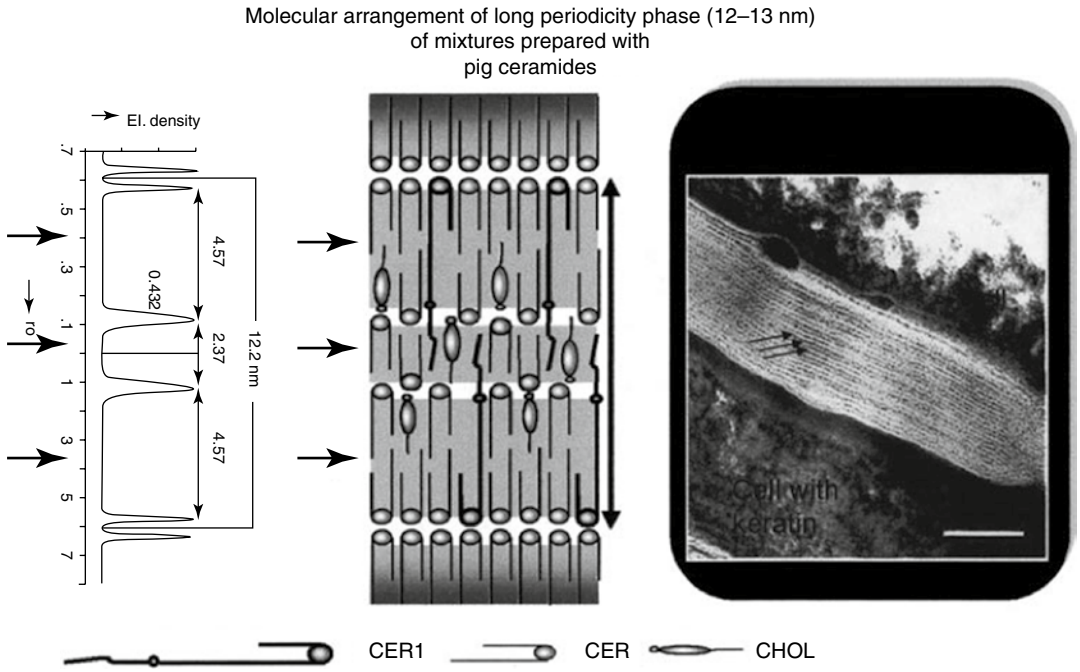

Fig. 3.6 Schematic presentation of the molecular arrangement of the long-periodicity phase (LPP). Reprinted from Bouwstra et al. (2001a) with permission from Karger Publisher

motropic phase behavior of these CER is reversible and the melting process does not induce major modifications of the lipids (Kessner et al. 2010). This is in line with other discoveries, which found the polar head group architecture to be responsible for polymorphism (Raudenkolb et al. 2003a, b, 2005).

3.3.2 Long-Chain ω-acyl Ceramides Studied Using Native Stratum Corneum

As there is a high demand to comprehend the structural arrangement of the different components of the SC lipid, especially, the long-chain CER were supposed to be of great influence. Consequently, the focus of many researchers was first placed on these exceptionally long-chain ω-acyl CER with their extraordinary characteristics concerning SC lipid organization. For example, Bouwstra et al. stated the importance of CER[EOS] not only for the formation of the LPP but also for the formation of a liquid phase which enables molecules to permeate along the lipid layer (Bouwstra et al. 2002). In this context the same group analyzed mixtures of CER, cholesterol (CHOL), and free fatty acids (FFAs) with regard to a diminishing content of CER[EOS] by X-ray and electron diffraction studies (Bouwstra et al. 2001a). According to their assumption, the fraction of lipids forming the LPP decreases by reducing the amount of CER[EOS]. Thus, they concluded that the presence of the long-chain ω-acyl CER is necessary for the formation of the LPP and subsequently for a proper barrier function of the SC lipid matrix. Resulting from different electron diffraction studies, the LPP is described as a trilamellar broad-narrow-broad arrangement of the SC lipids with a membrane thickness or repeat distance of 13 nm (Madison et al. 1987; White et al. 1988). Based on these insights Bouwstra et al. developed the sandwich model, depicted in Fig. 3.6. According to this model the liquid sublattice consists of CHOL and the linoleic acid residues of the long ω-acyl CER[EOS], [EOP], and [EOH], which are located in the center of this trilamellar structure and encompass nearly 3 nm of the total 13 nm thickness of the LPP. The adjacent crystalline phases with a broadness of 5 nm on either side are composed of long saturated hydro-

carbon chains of the CER and FFA (Bouwstra et al. 2001a). Except the research of McIntosh et al. (1996), in which isolated CER from native pig epidermis were employed, all studies mentioned above directly used native SC derived from a pig or mouse tissue for their investigations.

But as pig CER differ structurally from CER originated from human tissue, Bouwstra and coworkers compared their previous results of mixtures containing pig CER with those containing human CER (Bouwstra et al. 2001b). Similar to pig CER/CHOL mixtures, human CER/CHOL mixtures showed the formation of the LPP with the difference, that by addition of FFA the LPP disappeared and has been replaced by a short periodicity phase (SPP) (Bouwstra et al. 2001b). Contrary to these findings various work groups could not detect an LPP by cryoelectron microscopy (Al-Amoudi et al. 2005; Pfeiffer et al. 2000) and X-ray diffraction (Garson et al. 1991) in human SC. They explained the discovery of the LPP in former researches as an artifact due to the fixation with Ruthenium tetroxide. Ruthenium tetroxide is necessary for the electron diffraction and might yield to the misinterpretation of the received data. However, the fixation problem does not account for the X-ray diffraction results.

3.3.3 Synthetically Constructed Long-Chain ω-acyl Ceramides

There are several disadvantages when native SC lipids are used. For instance, the variability in chain length of either the FFA or the CER-bound fatty acids and differences in the CER head group architecture (see Fig. 3.1) can circumvent the assignment of different characteristics to individual lipids, especially the CER subclasses. In recent years synthetically constructed CER with defined chemical structures have been introduced into the SC lipid research, enabling a more reliable transduction of the physicochemical behavior to the structural characteristics of special CER.

This was taken into consideration by de Jager et al. (2004), who confirmed the existence of the LPP by investigating synthetic CER in mixtures with CHOL and FFA using small-angle X-ray diffraction. Additionally, they concluded that there is a close connection with the formation of the LPP and the presence of CER[EOS]. They stated that, while partial replacement of CER[EOS] by CER[EOP] does not influence phase behavior, complete substitution leads to a phase separation of CER[EOP] and a reduction of the LPP.

Another study performed by Kessner and coworkers also employed synthetically derived CER and applied both X-ray diffraction and Fourier transform Raman spectroscopy in order to ascertain these findings. Contrary to the previously described investigation, the physicochemical behavior of only the synthetic long ω-acyl CER[EOS] and [EOP] (Kessner et al. 2010) was studied without FFA or CHOL. These investigations revealed remarkable insights in this regard. While CER[EOS] in dry state only arranges in a SPP, CER[EOP] already forms the LPP. It was deduced that the differences in the head group architectures are responsible as this is the only dissimilarity between both CER species. They argued that the additional hydroxyl group in CER[EOP] is responsible for more hydrogen bonds and therefore enables the formation of a high-ordered package preventing the ω-acyl chains extending into the adjacent bilayer. Once both CER are hydrated, they are able to form the LPP with a repeat distance of 12 nm (Kessner et al. 2010).

To further examine the significance of these findings for the arrangement of the SC lipids, model membranes containing synthetic long-chain ω-acyl CER, CHOL, and FFA were analyzed. Using X-ray diffraction, mixtures of different CER, CHOL, and the FFA palmitic acid (PA) in an equimolar ratio were studied by de Jager et al. (2003). In mixtures containing CER[EOS]/CHOL/PA no LPP could be detected, while the same mixture comprising additionally CER[NP] showed a clear LPP with a repeat distance of 11.6 nm. The authors concluded that not only the presence of one single CER subclass can induce the formation of the LPP but a mixture of particular CER (de Jager et al. 2003).

Subsequently, Kessner and coworkers analyzed lipid mixtures containing CER[EOS], CER[AP], and CHOL. They detected a lamellar membrane with a thickness of two opposing CER[AP] molecules of approximately 45 Å indi-

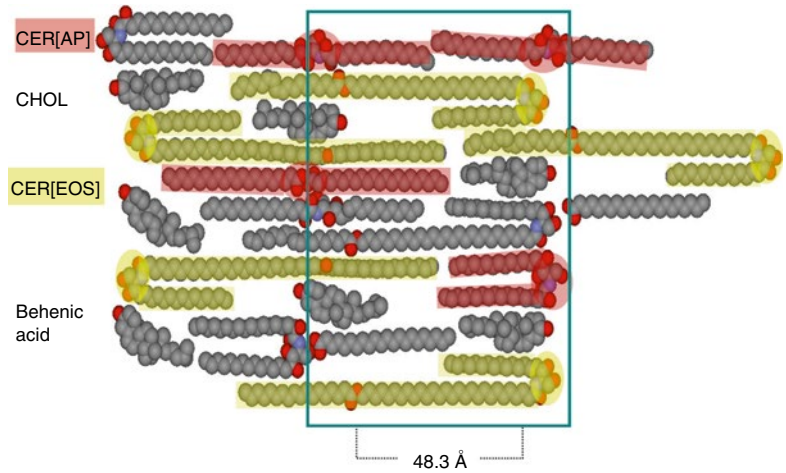

Fig. 3.7 Schematic presentation of the arrangement of CER[EOS] in the model matrix composed of CER[EOS]/CER[AP]/CHOL/BA (23/10/33/33, w/w) according to Schroeter et al. (2008)

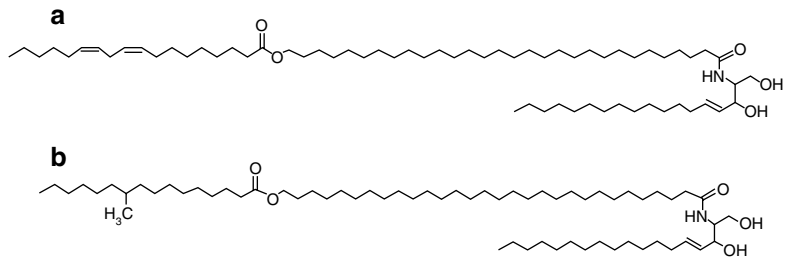

Fig. 3.8 Chemical structures of the native CER[EOS] specie (A) and the synthesized CER[EOS]_branched variety according to Engelbrecht et al. (2011)

cating that no LPP was formed (Kessner et al. 2008a). Even by adding of the FFA behenic acid (Schroeter et al. 2008), which was often reported to be required for the formation of the LPP (de Jager et al. 2003), only a slight increase of the repeat distance to 48 Å was perceived. Furthermore, from the neutron scattering length density profile, it was concluded that the long ω-acyl chain of CER[EOS] protrudes into the adjacent layer in order to fit into the membrane size created by CER[AP]. Consequently, CER[EOS] is positioned inside a phase with a short periodicity by spanning a bilayer and extending into adjacent layer (see Fig. 3.7).

It was concluded that the polar short-chain CER[AP] plays a key role in the formation of this lipid system. It dictates the arrangement of the other lipids within this mixture in a lamellar membrane, which covers the range of two opposing CER[AP] molecules. Consequently, the distinct head group polarity of CER[AP] exceeds the influence of the long ω-acyl chain of CER[EOS] (Kessner et al. 2008a; Schroeter et al. 2008). In another approach by Engelbrecht and coworkers, an artificial derivative of CER[EOS], the so-called CER[EOS]_branched with a methyl-branched and saturated ω-acyl chain, was synthesized and investigated in a model membrane applying neutron diffraction (Engelbrecht et al. 2011) (see Fig. 3.8). This molecule is less sensitive to oxidative stress and therefore more stable and easier to handle as the native specie.

To assure the comparability of the native CER[EOS] and the artificial CER[EOS]_branched derivative both Fourier transform Raman spectroscopy and differential scanning calorimetry were carried out, with the outcome that both species show a comparable phase and chain packing behavior (Engelbrecht et al. 2011). In order to elucidate the arrangement of this CER[EOS] derivative with respect to the previously described findings, Engelbrecht et al. additionally studied a model membrane system composed of CER[EOS]_branched, CER[AP], CHOL, and behenic acid with neutron diffrac-

tion (Engelbrecht et al. 2011). They found that the synthetically derived CER[EOS]_branched is able to serve as an appropriate substitute for the native CER[EOS] in terms of lipid arrangement and architecture. Even this more stable and saturated acyl chain of CER[EOS]_branched did not induce a formation of the LPP in the presence of CER[AP], as stated above for the non-branched species in such mixtures. Again, the protruding influence of the more polar CER[AP] induces the formation of the SPP. To further verify their results, molecular dynamic simulation was performed and confirmed the lamellar arrangement of this model membrane (see Fig. 3.9).

Contrary to these findings there are the results from X-ray diffraction and FT-IR studies conducted by Groen et al. (2010). For mixtures containing CER[EOS], CHOL, and different FFA in an equimolar ratio, they could detect a 14.7 nm lamellar phase. This very long repeat distance was discussed to result from two opposing CER[EOS] molecules with interdigitating linoleate residues. Accordingly they proposed a model arrangement in which the lamellae are divided into three different lipid layers referred to as (A) (containing the linoleate residues of CER[EOS]), (B) (composing CHOL and CER[EOS]-ω-bound fatty acids), and (C) (buildup of sphingosine residues and FFA) as depicted in Fig. 3.10. Although a long 14.7 nm lamellar phase could be observed, the LPP with a repeat distance of 13 nm was not detected.

Concluding, it has to be stated that the role of long-chain ω-acyl CER both in context with the formation of the LPP or in terms of skin diseases is not completely elucidated and still debated vigorously. Therefore, these CER are still a subject of great interest in SC lipid research.

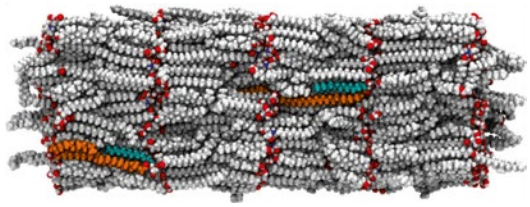

Fig. 3.9 Snapshot from molecular dynamic simulation of the lipid system consisting of CER[EOS]_branched/CER[AP]/behenic acid/CHOL (23/10/ 33/33 m/m) and H_2O. Color code: *orange*: CER[EOS]_branched, *blue* CER[AP]. Modified according to Engelbrecht et al. (2011)

3.4 Effect of Short-Chain Ceramides to the Structural Organization of Stratum Corneum Lipids

As outlined previously, the heterogeneous class of CER can be generally separated into two major subclasses: the very long-chain ω-acyl CER like

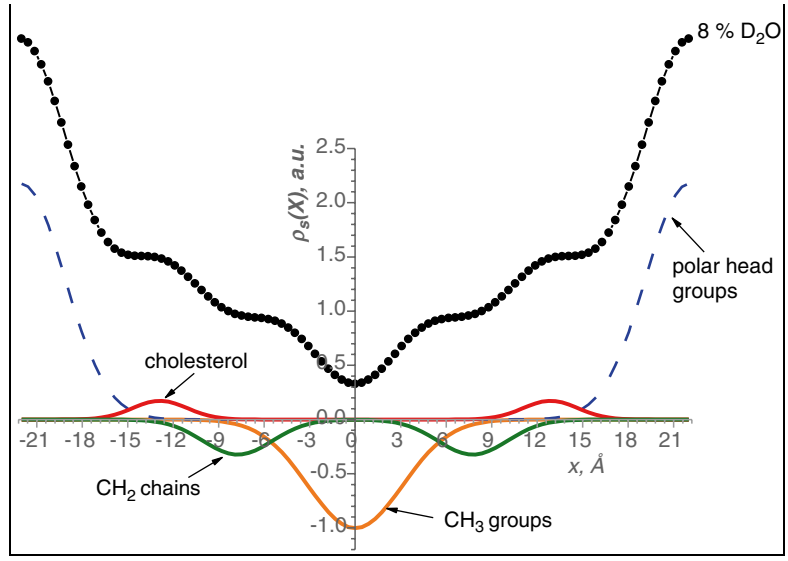

Fig. 3.10 Model calculations of the neutron SLD profile of a SC lipid system composed of CER[AP], CHOL, behenic acid (BA), and cholesterol sulfate modified according to Ruettinger et al. (2008). The fitted curves for each group are: polar head groups (*blue dash*), CH_2-groups (*green*), CH_3-groups (*orange*), cholesterol (*red*)

CER[EOS] or [EOP] and the short-chain CER, which can further be divided into the phytosphingosine type such as CER[AP] or CER[NP] and the sphingosine-type CER such as CER[AS] or CER[NS]. Furthermore, both subclasses are known to exhibit a broad distribution of their alkyl chain length, which is necessary for their proper functionality of the SC lipid matrix (Norlen 2001). During the last years, especially the short-chain CER of the phytosphingosine variety have gained in interest in the SC lipid research, as specific, their role for a proper barrier function has not been fully elucidated up to date. In order to evaluate the specific function of each CER subclass nowadays, well-defined model systems are investigated using different techniques.

The various experimental techniques such as X-ray diffraction, vibrational spectroscopy, or differential scanning calorimetry (DSC) to characterize the thermotropic and/or lyotropic properties of the ceramides as bulk substance and in different mixtures have been extensively reviewed before (Kessner et al. 2008c; Wartewig and Neubert 2007). In order to understand the impact of different CER species for the formation of the SC lipid matrix, the analysis of the CER as bulk material and then consequently in mixtures with other SC lipids is mandatory for the interpretation of the behavior in the complex multicomponent SC lipid membranes.

3.4.1 Elucidation of the Nanostructure of Stratum Corneum Lipid Models Based on CER[AP] or CER[NP]

As a first step to investigate the influences of different short-chain CER, Kiselev and coworkers prepared a SC lipid model membrane composed of CER[AP], cholesterol (CHOL), the free fatty acid (FFA) palmitic acid and cholesterol sulfate as oriented multilamellar membrane and investigated it with neutron diffraction (Kiselev et al. 2005). They showed by determining the internal membrane nanostructure and the water distribution across the bilayer, that such model membrane exhibits very low hydration, with a water layer thickness of about 1 Å at full hydration. From the neutron scattering length density (NSLD) profile, they further derived information about the position of the molecular groups of the lipids within the lipid bilayer. This was achieved by fitting the NSLD profile with Gaussian functions, which resemble the position of the polar head groups, the CH_3 group, the hydrocarbon chain region, and the region of cholesterol location, respectively (see Fig. 3.10). Furthermore, they established that a decrease in the amount of CHOL in the model system correlates with an increase in the membrane thickness (Kiselev et al. 2005).

In order to identify the exact position of the CHOL molecules in this model membrane based on CER[AP], Kessner et al. employed two partially deuterated CHOL derivatives (Kessner et al. 2008b). From the neutron scattering length density (NSLD) profiles, they concluded that the CHOL molecules are immersed in the hydrocarbon chain region of the membrane bilayer, with the isopropyl residue positioned in the center of the membrane as depicted in Fig. 3.11.

As the interaction of the different lipid species of the SC matrix is of high interest, the influence of the FFA chain length to the above-described model membrane based on CER[AP], CHOL, and FFA was investigated also applying neutron diffraction (Ruettinger et al. 2008). In this study, within SC lipid model membranes, only the FFA chain length (C18:0 stearic acid, C22:0 behenic acid, C24:0 lignoceric acid, C26:0 hexacosanoic acid) was varied and the results were compared to each other. The membrane thickness for all investigated model systems was found to be in the range of two opposing CER[AP] molecules. An increase of the FFA chain length did not cause an alteration of the internal nanostructure but led to a slight decrease in the membrane thickness, causing a partial interdigitation of the longer chained FFA. The reason for the unexpected result was placed on the presence of the polar short-chain CER[AP]. This molecule establishes a tight hydrogen bond network between the adjacent bilayers due to its four OH groups. Thus, the CER forces the long-chain FFA to incorporate into the unchanged spacing of the bilayer, thereby obligating the FFA to protrude partly through opposing leaflet as represented in Fig. 3.12.

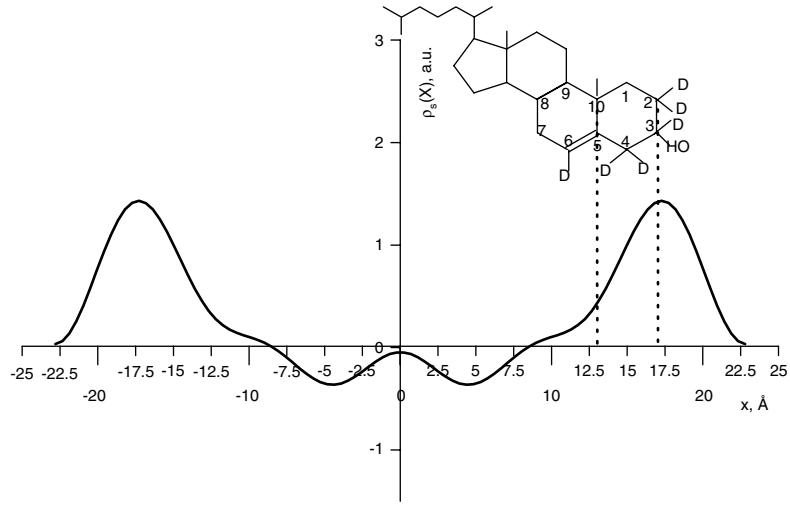

Fig. 3.11 Difference or deuterium distribution profile of the SC lipid model membrane derived from the difference between NSLD profile containing the deuterated CHOL derivative and the profile containing the protonated specie. Reprinted from Kessner et al. (2008b) with permission from Springer

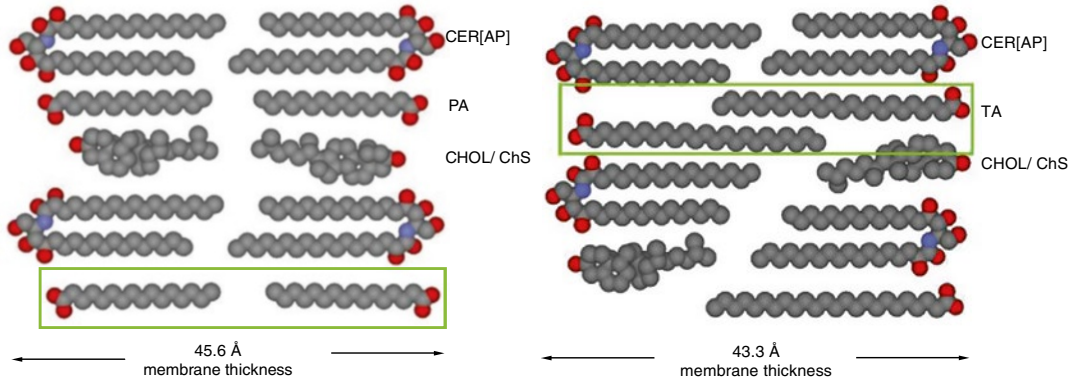

Fig. 3.12 Schematic presentation of the structural assembly of the SC model matrix based on CER[AP] according to Ruettinger et al. (2008). To demonstrate the influence of the longer chained FFA a model for the membrane containing palmitic acid (*left*) is compared to the matrix including tetracosanoic acid (*right*). *CER*[*AP*] ceramide [AP], *CHOL* cholesterol, *PA* palmitic acid, *TA* tetracosanoic acid

Consequently, CER[AP] creates a superstable structure, which is not influenced by the alteration of the FFA chain length. The resulting free space due to the interdigitation of the FFA can only be compensated by pulling the membrane together, hence, the slight decrease in the membrane repeat distance. Additionally, the experiments revealed that the longer-chained FFA tends to separate in an FFA-rich phase. It was reasoned that the elongation of the chain length of the FFA decreases the solubility of the FFA in the SC model membrane based on the short-chain CER[AP] (Ruettinger et al. 2008). To verify the interdigitation of the FFA in the CER[AP]-based SC lipid model membrane, the same group employed the partially deuterated FFA behenic-22,22,22-d3-acid and cerotic-12,12,13,13-d4-acid (Schroeter et al. 2009). The results from the neutron diffraction study provided the direct experimental evidence concerning the localization of the FFA in this SC lipid model system. Both the interdigitation and the presence of the FFA-rich phase could be proven by this method.

As mentioned earlier, such SC lipid membranes show very small head group hydration. Therefore, Ryabova and coworkers investigated the kinetics of the exchange of water in CER[AP]-based SC lipid model membranes with real-time neutron diffraction (Ryabova

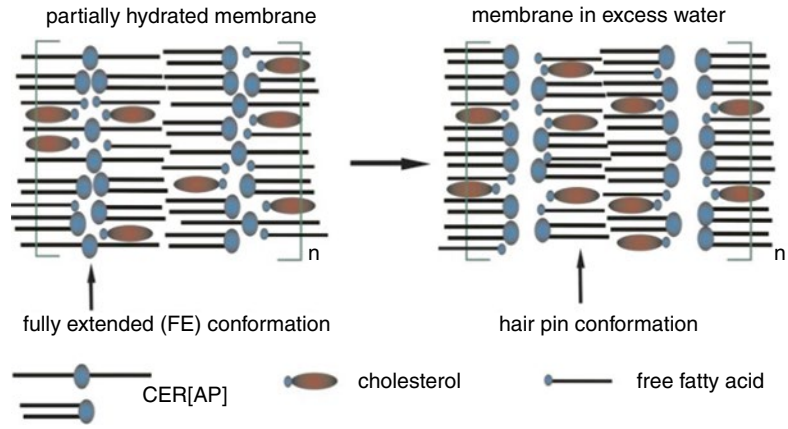

Fig. 3.13 Schematic presentation of the armature reinforcement model and transformation of SC lipid membrane from partly hydrated to fully hydrated state by the excess of water modified according to Kiselev (2007)

et al. 2009). The study revealed that the kinetic hydration comprises a fast initial segment, which is followed by two slow stages. By increasing the temperature to 57 °C, this process was significantly faster in its initial phase. Furthermore, they found that an irreversible phase separation at this temperature and low hydration level occurs, whereby they argued that the FFA separate. In a further investigation the same group studied the influence of both a realistic FFA mixture and cholesterol sulfate (ChS) to the structure of the above-described SC lipid model membrane based on CER[AP] (Ryabova et al. 2010). Again, as described by Ruettinger et al. (2008), not even a mixture of different FFA affects the nanostructure of this model system. Once more, the FFA needs to interdigitate in order to fit into the bilayer created by CER[AP]. Nevertheless, they found that their mixture containing six FFAs differing in chain length prevented the above-described FFA phase separation. Only the hydration behavior was altered due to the FFA mixture as Ryabova et al. discovered that the membrane swelling process was accelerated at low hydration levels, which was attributed to the less dense bilayer packing due to the interdigitation of the FFA (Ryabova et al. 2010). In the same study, no alteration of the rate of hydration was observed for the complete substitution of ChS by CHOL. However, the absence of ChS caused a phase separation, which was attributed to the missing sulfate group of ChS. As the sulfate group is negatively charged, it can increase the molecular area per lipid, which subsequently reduces the density of the lipid packing. This

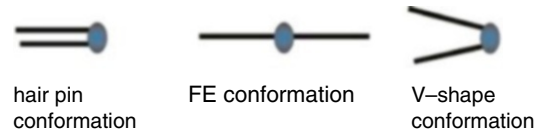

Fig. 3.14 Conformational states of the short-chain CER

density reduction increases the mobility of the lipids, which further promotes the miscibility of the lipids. When, on the other hand, the amount of ChS was increased at the expense of CHOL, the swelling of the membrane increased. Ryabova et al. argued that the higher ability to form hydrogen bonds of ChS is responsible for this effect (Ryabova et al. 2010).

As described in the preceding chapter, the presence of CER[AP] in an SC lipid membrane composed also of CER[EOS], CHOL, and FFA prevents the formation of the LPP, as it forces the long ω-acyl chain of CER[EOS] to protrude through the complete bilayer into the adjacent layer (Engelbrecht et al. 2011; Kessner et al. 2008a; Schroeter et al. 2008). These experimental findings contributed to or are in accordance with the so-called armature reinforcement model, a theoretical model describing the molecular arrangement of these lipids (Kiselev 2007) (see Fig. 3.13).

Here, the polymorphism of the short-chain CER (Pascher 1976; Pascher and Sundell 1992; Raudenkolb et al. 2003a, b, 2005) is taken into account, as the role of the fully extended conformation is discussed for the arrangement of the lipids (Fig. 3.14). This model conception assumes that CER[AP] in its fully extended

conformation adopts a sort of anchor function due to the strong intermembrane attractions, in which it is able to restrain the other lipids inside the membrane and, respectively, forces their arrangement within the membrane constraints. Upon hydration, CER[AP] is capable to perform a chain-flip transition to the one-sided or hairpin conformation, which accounts for the alterations in the structure observed in the hydrated state (Kiselev et al. 2005).

Next to neutron diffraction, there is also the very powerful technique of neutron small angle scattering (SANS), which allows for the investigation of the structure of vesicular lipids in excess of water. With SANS nanostructural parameters such as size of the vesicle, thickness of a lipid bilayer, thickness of hydrophobic and hydrophilic regions, and number of water molecules can be directly determined (Kiselev et al. 2006). Applying these methods Zemlyanaya and coworkers (Zemlyanaya et al. 2008) investigated CER[AP]-based quaternary unilamellar vesicles. In their investigation they detected a short-range interaction between the vesicles specimen, which leads to the formation of clustered structures. Furthermore, with this study they confirmed the chain-flip transition of the CER[AP] molecules described above in the *armature reinforcement model*.

As CER[NP] is one of the most abundant CER of the SC (Masukawa et al. 2009), the focus was also placed on this molecule. Compared to the above-described CER[AP], this CER also belongs to the phytosphingosine subclass, but does not have the α-hydroxy group present in CER[AP]. Accordingly, the lamellar nanostructure of a SC lipid model membrane based on CER[NP] was investigated in an interdisciplinary approach using both neutron diffraction and 2H-NMR spectroscopy and then compared to the above-described CER[AP]-based SC lipid model systems by Engelbrecht and coworkers (Engelbrecht et al. 2012). The authors indicated that in the presence of this CER subspecies a highly ordered lipid lamellae is formed and phase separation occurred, which was even at high temperature in a densely packed and stable bilayer. Here, intra- and intermolecular head group interactions of CER[NP] prevent the hydration of the head group region. From the neutron diffraction data, it was proposed that CER[NP] exhibits a V-shaped conformation in both lamellar phases, but with the distinction that one phase is phase-separated CER[NP] as portrayed in Fig. 3.15, which was further corroborated by ^{2}HNMR spectroscopy study.

Moreover, the model system based on CER[NP] a completely different diffraction pattern at higher temperature, when compared to the above-described CER[AP]-based lipid membranes. Thus, it was argued that the absence of just one OH group induces drastic structural alteration in the membrane arrangement.

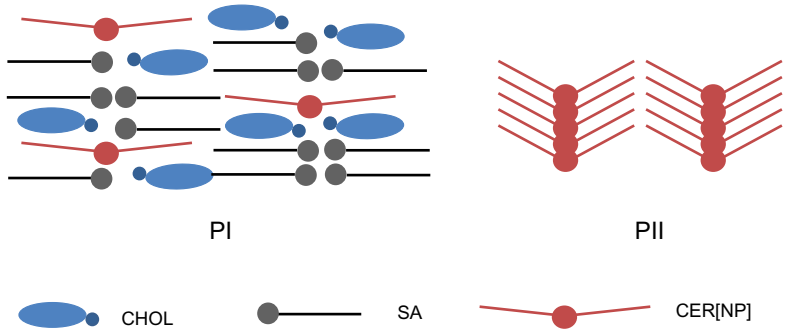

Fig. 3.15 Sketch of the assumed lamellar lipid assembly present in the phase-separated domains of PI and PII in the ternary SC lipid model membrane containing CER[NP], CHOL and SA at 80 °C and 99 % RH. While PII is constituted by crystalline CER[NP] showing a V-shaped conformation, PI is formed by CER[NP], SA and CHOL. Reproduced from Engelbrecht et al. (2012). with permission from The Royal Society of Chemistry

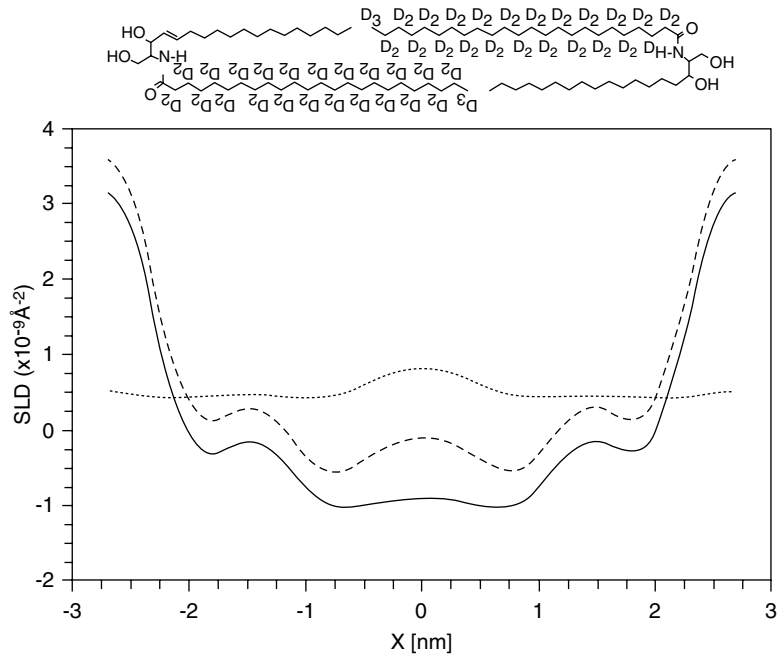

Fig. 3.16 Comparison of the Neutron scattering length density profile of the sample containing the protonated CER[NS] (*solid line*) and the deuterated variety (*dashed line*). Taken from Groen et al. (2011) with permission from Cell press

3.4.2 Investigating the Nanostructure of an Stratum Corneum Substitute

The above-portrayed investigations were mainly focused on the impact of *one* specific CER species in order to explicitly recognize the interaction occurring between the different lipids and identify the structure-function relationship of different CER subspecies. In another approach Groen and coworkers studied the nanostructure of SC lipid model membranes with neutron diffraction, which was composed of different CER species, CHOL, and FFA to closely mimic the SPP (Groen et al. 2011). In order to localize the CER, they applied a CER species (CER[NS]) with a perdeuterated acyl chain. In accordance with the finding described concerning the short-chain CER[AP], a bilayer arrangement with a membrane thickness in the range of two opposing CER molecules was detected. From the neutron scattering length density profile of the membrane containing the deuterated CER[NS] variety, they reasoned that the CER exhibits a symmetrical organization and excluded the asymmetric conformation of the CER inside the bilayer arrangement (Engelbrecht et al. 2012) as schematically displayed in Fig. 3.16.

However, as the acyl chain of CER[NS] is perdeuterated, only the presence of this chain can be deduced from the data. It is true that the chains need to be partially interdigitated in order to be in agreement with the neutron diffraction data. Nevertheless, another arrangement is possible whereby the CER molecules exhibit a fully extended conformation (see Fig. 3.14), the deuterated chains forming one leaflet, while the sphingosine backbone chain is pointed in the opposing direction.

Consequently, the drawback of the application of perdeuterated lipids in the neutron diffraction experiment is the fact that it does not yield to an unambiguous location of the labeled lipid species, and, furthermore, it does not give any information about the conformational state of the studied lipid.

3.5 Summary and Final Remarks

The intercellular lipid membranes of the SC are an excellent biological example for the relationship between the lipid composition, its physicochemical properties and biological function, as well as organization. To elucidate the special

assembly and the properties of the native SC lipid matrix is a very difficult task as the natural SC membranes are very complex. Therefore, in recent years the researches were primarily placed on the investigation of model systems in order to gain deeper insights into the driving forces of the lipid assembling process. Furthermore, not only model membranes are currently in the focus of different investigation but also rather simplistic, nonetheless realistic SC model membranes. As described, this offers the distinct benefit to analyze the different lipid species systematically. This approach is very important, as especially the impact of the various CER subclasses for the proper barrier function of the SC needs to be studied independently as shown for the very closely related CER[AP] and CER[NP].

So far a diversity of techniques have been introduced into this field, whereby neutron diffraction with its specific advantages seems to be the most promising one for the investigation of the nanostructure of the SC lipid matrix, as it enables the use of specifically labeled molecules. However, only a combination of different methods and approaches can provide a complete picture of the molecular arrangement of SC lipid matrix, as one technique alone can cover only a small field.

Up to date only a few CER subspecies have been investigated independently and are in parts debated controversially (see CER[EOS], for instance). Consequently, there is still a high demand to fully understand the impact of these lipids for a proper barrier function of the SC lipid matrix.

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Molecular Structure and Function of the Skin Barrier

4

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Contents

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4.1 Introduction

Terrestrial life was only made possible through the adaptive evolution of a waterproof barrier in the integument of organisms. In man, this barrier is constituted by a uniquely organized lipid material situated between the cells of the horny layer of the skin (Breathnach et al. 1973; Elias and Friend 1975). Recently, the lipid material's molecular organization was determined in situ with the aid of a novel experimental approach: high-resolution *c*ryo*e*lectron *m*icroscopy *o*f *v*itreous tissue *s*ection (CEMOVIS) defocus series combined with molecular modeling and electron microscopy simulation (Iwai et al. 2012). The lipid material is organized in an arrangement not previously described in a biological system – stacked bilayers of fully extended ceramides with cholesterol molecules associated with the ceramide sphingoid moiety (Iwai et al. 2012). This organization not only rationalizes the low permeability of the skin barrier but also its robustness. The new knowledge may serve as a molecular platform for in silico approaches to identify molecules for enhancing skin penetration for percutaneous drug delivery.

Below follows a brief account of the structure-function relationships of the human skin barrier.

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DOI 10.1007/978-3-662-45013-0_4, © Springer-Verlag Berlin Heidelberg 2015

4.2 Skin Lipid Composition and Phase State

The horny layer lipids consist of a heterogeneous mixture of saturated, long-chain ceramides, free fatty acids, and cholesterol in a roughly 1:1:1 molar ratio (Wertz and Norlén 2003). More than 300 different species have been identified in the ceramide fraction alone (Masukawa et al. 2009).

The most characteristic features of the horny layer lipid composition (Wertz and Norlén 2003) are (1) extensive compositional heterogeneity with broad, but invariable, chain length distributions (20–32 C; peaking at 24 C) in the ceramide fatty acid and free fatty acid fractions, (2) almost complete dominance of saturated very long hydrocarbon chains (C20:0–C32:0), and (3) large relative amounts of cholesterol (about 30 mol%).

These compositional features are typically those stabilizing lipid gel phases. It has therefore been proposed that the horny layer lipid structure exists as a single and coherent gel phase (Norlén 2001b). The viscous gel-like behavior of the lipid structure has recently been demonstrated by its remarkable malleability in situ (Iwai et al. 2012).

4.3 Skin Lipid Structure

CEMOVIS has recently shown that the extracellular lipid matrix of the horny layer is organized as a bilayer structure of fully extended (splayed chain) ceramides with the sphingoid moieties interfacing. Both cholesterol and the free fatty acids are distributed selectively: cholesterol at the ceramide sphingoid end and the free fatty acid at the ceramide fatty acid end (Iwai et al. 2012) (Figs. 4.1 and 4.2). A unique feature of the horny layer lipid organization is that the lipid molecules are arranged in the splayed chain conformation, with the two hydrocarbon tails pointing in opposite directions, contrary to conventional biological membranes where the lipids are arranged in the hairpin conformation with the two hydrocarbon tails pointing in the same direction. Further, the skin barrier organization differs from conventional fat crystals arranged in the splayed chain conformation, as the lipid layers in the skin are stacked in an alternate fashion as bilayers rather than as stacked monolayers.

4.4 Skin Lipid Formation

In order to appreciate the structure-function relationships of the skin barrier in vivo, it is of value to understand horny layer lipid formation, as the horny layer's lipid structure may represent a "frozen-in" or "immobilized" open biological system rather than a primary minimum energy order equilibrium system. Skin lipid formation is also central from a dermatological standpoint, since barrier malformation may be an etiological factor in barrier-deficient skin conditions such as eczema, psoriasis, and "dry skin."

It has recently been proposed that skin lipid formation proceeds via (1) membrane synthesis in the trans-Golgi of a membrane system with cubic-like symmetry, followed by (2) morphologically continuous (non-fusion-dependent) secretion of the cubic-like membrane system into the extracellular space, (3) phase transition from cubic-like to lamellar membrane morphology, (4) dehydration, (5) condensation, and (6) lipid chain rearrangement from a folded (hairpin) to an extended (splayed chain) stacked bilayer conformation (Norlén 2001a; Iwai et al. 2012). CEMOVIS

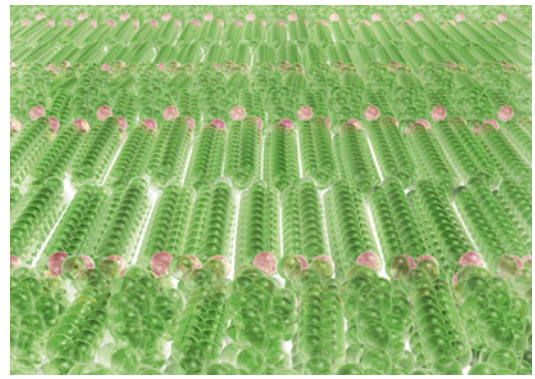

Fig. 4.1 Molecular organization of the skin barrier. The stratum corneum lipid layer is organized as stacked bilayers of fully extended ceramides with cholesterol molecules associated with the ceramide sphingoid moiety (Iwai et al. 2012). *Green spheres* represent hydrogen and carbon atoms in ceramides, cholesterol, and free fatty acids. *Red spheres* represent oxygen atoms

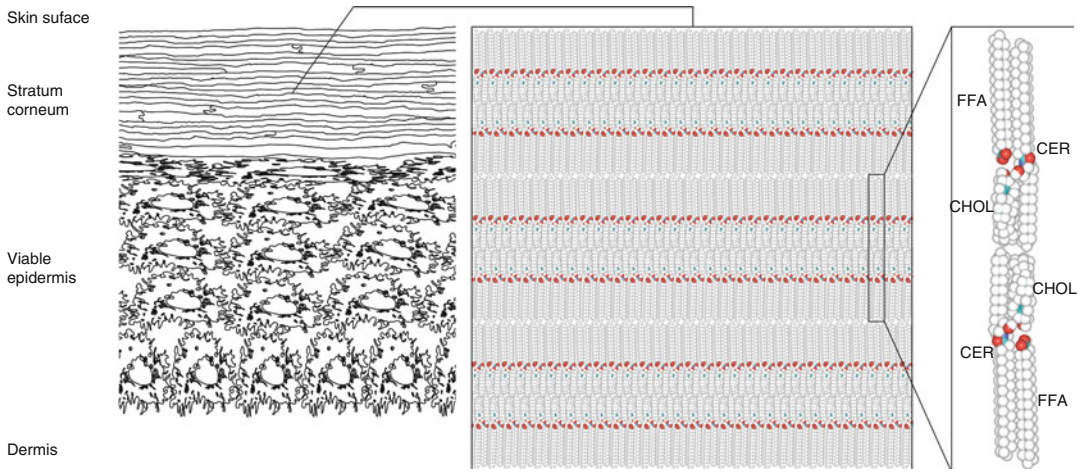

Fig. 4.2 Schematic drawing of the skin. *Left part* schematic cellular-scale drawing of epidermis. *Middle part* molecular-scale drawing of the lamellar lipid structure occupying the space between the cells of the stratum corneum. *Right part* atomic model of the lipid structure's repeating unit, composed of two mirrored subunits, each composed of one fully extended ceramide (*CER*), one cholesterol (*CHOL*), and one free fatty acid (*FFA*) molecule (Adapted from Norlén (2012), with permission)

supports the proposed continuity of the lipid secretion system as well as the proposed structural association of non-lamellar and lamellar lipid morphologies (Norlén et al. 2003; Al-Amoudi et al. 2005). However, structure determination of the intermediate stages of skin lipid formation may require access to native molecular resolution tomographic 3D data in situ (molecular tissue TOVIS (cf. Norlén et al. 2009)), a developing technology that may not yet have reached its full potential.

4.5 Skin Lipid Function

Current knowledge suggests that a stacked, fully extended (splayed chain) ceramide bilayer arrangement (Figs. 4.1 and 4.2) with a high cholesterol content and a heterogeneous, saturated, long-chain lipid composition represents an optimized barrier organization for skin. This is because it renders skin largely impermeable to water as well as to both hydrophilic and lipophilic substances due to its condensed chain packing and its alternating lipophilic (alkyl chain) and hydrophilic (headgroup) regions. Likewise, it is resistant to both hydration and dehydration because of its lack of exchangeable water between lipid leaflets. It is also resistant towards temperature and pressure changes because of its heterogeneous lipid composition and high cholesterol content, which stabilize gel-like chain packing and thereby prevent both lateral domain formation and induction of "pores" or non-lamellar morphologies. Further, this bilayer arrangement accounts for stratum corneum cell cohesion without advocating specialized intercellular adhesion structures such as desmosomes. The arrangement hence allows for sliding of stratum corneum cells to accommodate skin bending. Finally, as the interaction between the individual layers of the lipid structure involves only hydrocarbons, the layers may be relatively free to slide with respect to one another, making the lipid structure pliable. The fully extended ceramide bilayer arrangement with high cholesterol content and heterogeneous saturated long-chain lipid composition thus meets the barrier needs of the skin by being simultaneously impermeable and robust.

Conclusions

It was recently shown that the human skin barrier is organized as stacked bilayers of fully extended ceramides with cholesterol molecules associated with the ceramide sphingoid moiety.

The physical state of the skin's lipid structure has been proposed to be that of a single and coherent gel phase. Further, the lipid structure may be formed via a phase transition from cubic-like to stacked lamellar morphology followed by a flip of the constituent lipid components from a folded (hairpin) to an extended (splayed chain) ceramide bilayer conformation.

The skin's lipid structure is responsible for both the skin's low permeability towards water and hydrophilic and lipophilic substances and the barrier's robustness towards environmental stress, such as hydration and dehydration, temperature and pressure changes, stretching, compression, bending, and shearing.

The new molecular description of the skin barrier may serve as a molecular platform for in silico approaches such as molecular simulations, as well as for in vitro modeling, to underpin interactions of the lipid matrix with drugs and other chemicals. For example, it is foreseeable that this knowledge will now enable in silico screening to identify molecules for enhancing skin penetration for percutaneous drug delivery.

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The Increasing Importance of the Hair Follicle Route in Dermal and Transdermal Drug Delivery

5

Alexa Patzelt and Jürgen Lademann

Contents

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5.1 Introduction

For more than 200 years, constant attempts have been made to administer drugs via the skin and to enhance percutaneous penetration by various methods including mechanical, physical, and chemical manipulations to reduce the barrier function of the skin (Helmstadter 2011). In total, three potential penetration pathways have been identified. In addition to the well-described intercellular penetration pathway being mainly responsible for the percutaneous penetration effect, also the follicular route has been spotted to be of considerable interest as especially the upper portion of the hair follicle – the infundibulum – displays an area of additional absorption. However, in different skin sites, the size and number of hair follicles can differ tremendously (Otberg et al. 2004b); thus, also the influence of the follicular penetration route can vary. The transcellular penetration pathway, on the contrary, seems to be of inferior importance.

The aim of the present chapter is to describe and define the role of the hair follicle in the penetration process and to identify mechanisms which allow follicular penetration enhancement.

Due to the architectural structure of the hair follicle, follicular penetration is a complex process and has to be divided at least into two steps as illustrated in Fig. 5.1. It has to be distinguished between the penetration into the hair follicle and, in the second step, the transfollicular penetration

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into the living tissue surrounding the hair follicle, which cannot be observed for every applied substance, yet. Previous investigations could show that mainly the physicochemical properties of a topically administered substance determine its follicular penetration depth (Patzelt et al. 2011) and whether or not the substance is able to penetrate transfollicularly. Thus, modifications of the physicochemical properties of the substances such as size can be utilized as follicular penetration enhancers or inhibitors.

In comparison to the intercellular penetration process, topical drug delivery via the hair follicles provides additional features such as fast delivery into the systemic circulation if transfollicular penetration is applicable (Otberg et al. 2008) as well as long-term intrafollicular storage (Lademann et al. 2006) if transfollicular penetration cannot be realized. These and more aspects will be discussed in this chapter.

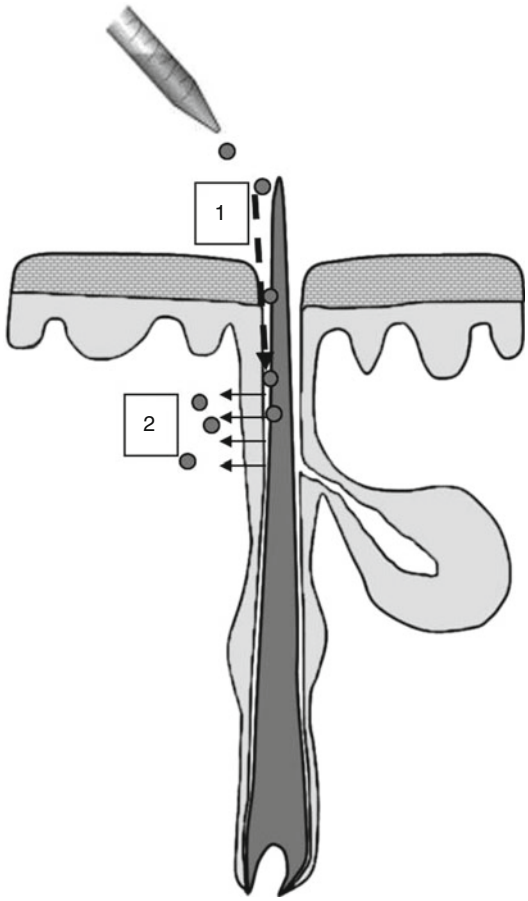

Fig. 5.1 Schematic illustration of the follicular penetration pathway which has to be divided into two steps: (*1*) intrafollicular penetration and (*2*) transfollicular penetration if allowed due to size reasons

5.2 Architectural and Physiological Features of the Hair Follicle with Regard to Follicular Penetration

Due to its complex and dynamic architectural structure, the hair follicle is predestined as a penetration and storaging organ, although the original functions of the hair follicle seem to be rather those of a sensory organ, sebum excretion and protection (Krause and Foitzik 2006). The infundibulum is the upper part of the hair follicle and consists of an upper and lower portion as depicted in Fig. 5.2. The epithelium of the upper infundibulum is continuous with the keratinized epidermis and covered by an intact stratum corneum, whereas the differentiation pattern of the lower infundibulum switches from epidermal to trichilemmal leading to an interrupted skin barrier with only few differentiated corneocytes

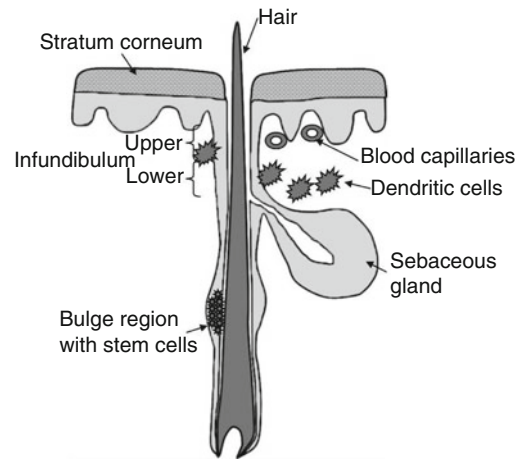

Fig. 5.2 Schematic overview of the follicular architecture especially depicting the structures relevant to the follicular penetration process such as the infundibulum surrounded by the blood capillaries and dendritic cells, the sebaceous gland, and the bulge region with stem cells

remaining (Blume-Peytavi and Vogt 2011) that are more prone to transfollicular penetration in this region. Moreover, the whole follicular infundibulum is supplied by a dense capillary network and surrounded by a high number of immune cells, on the one hand allowing the rapid systemic uptake of substances once penetrated transfollicularly and, on the other hand, rendering the hair follicle to be a promising target for immune therapy or topical vaccination (Patzelt et al. 2011; Vogt et al. 2008).

Additionally, as depicted in Fig. 5.2, also the sebaceous gland and the bulge region of the hair follicle which is hosting the stem cells are interesting structures within the hair follicle, at the same time being attractive targets for therapeutic interventions (Patzelt et al. 2011). The sebaceous gland is the organ of sebum excretion and additionally associated with a diversity of pathologies such as acne (Thiboutot 2004). It was suggested that by increasing the distribution of corresponding drugs in the sebaceous gland, the therapeutic effectivity could be significantly improved (Rolland et al. 1993). Respective efforts have already been made (Morgan et al. 1993; Ridolfi et al. 2012; Rolland et al. 1993), and some promising antiacne products are already on the market. The multipotent, highly proliferative, and easily accessible stem cells located in the bulge region (Ohyama 2007) are also to be integrated in the therapeutic concepts for cutaneous regenerative medicine or gene correction of congenital hair disorders or genetic skin diseases.

In total, more than 20 different cell types are involved in the structure of the hair follicle which underlies cyclical activity (Rogers 2004). Three different hair follicle types, namely, lanugo, vellus, and terminal hair follicles, have to be distinguished. Whereas the same hair follicle produces lanugo hairs in the fetal period and vellus hairs in the childhood, it produces terminal hairs in the adulthood (Blume-Peytavi and Vogt 2011). In total, each human individual displays an estimated number of five million hair follicles (Krause and Foitzik 2006) underlining its potential in the penetration process. The morphometry of vellus and terminal hair follicles has been well documented already (Vogt et al. 2007). A schematic overview of the follicular architecture is depicted in Fig. 5.2.

5.3 Mechanisms of Follicular Penetration and Transfollicular Penetration

5.3.1 Mechanisms of Follicular Penetration

The follicular penetration of topically applied substances represents a complex process which has not been clarified in detail until now. Whereas the retention of the particles in the follicular duct has been well documented, researchers are controversially discussing which substances are able to penetrate transfollicularly into the deeper skin layers, whereby the size next to other physicochemical properties seems to be the predominant parameter (Labouta and Schneider 2013). In the last years, predominantly particulates such as liposomes and micro- and nanoparticles have attracted attention as a result of their capability to improve penetration into the hair follicle. Here, a clear size dependency could be observed demonstrating that 320 nm sized polymer particles covalently labeled with a fluorescent dye penetrated significantly deeper into the hair follicles than the same fluorescent dye in nonparticulate form (Lademann et al. 2007). The optimum size for particles to penetrate deeply into the hair follicle was determined to be in the range of 400–700 nm, whereas larger and smaller particles reached significantly lower penetration depths (Patzelt et al. 2011) or remained even on the skin surface in the case of very large particles (Schaefer and Lademann 2001; Toll et al. 2004). As this effect was demonstrated for different solid particle preparations such as PLGA particles and silicium oxide particles, it was assumed that follicular particle penetration is a predominantly mechanical effect independently from the particle preparation (Patzelt et al. 2011). In Fig. 5.3, the dependency of the penetration depth on the particles' size is schematically represented. Lademann et al. (2009) hypothesized

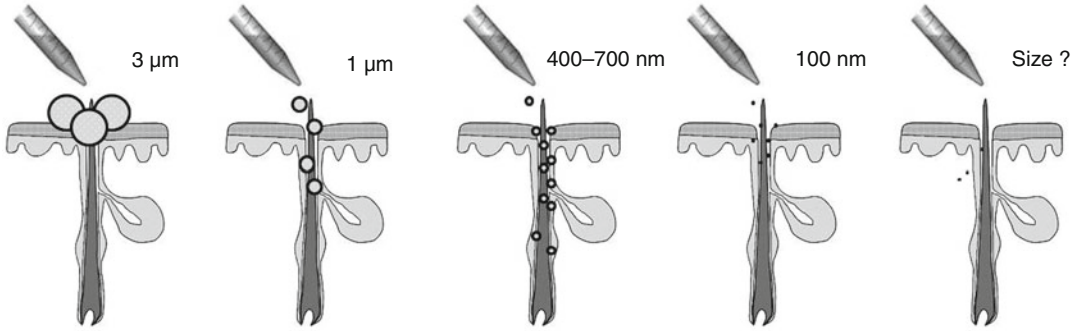

Fig. 5.3 Dependency of the penetration depth of the particles on the particles' size. Large particles (>3 µm) are only located on the skin surface and in the follicular orifice. Particles between 400 and 700 nm penetrate significantly deeper into the hair follicles than larger or smaller particles. A size threshold below which transfollicular penetration occurs has not been determined, yet

that the surface structure of the hair and the hair follicle, which is determined by the thickness of the keratin cells in the cuticula, being 530 nm in human hairs and 320 nm in porcine hairs, might act as a pumping system delivering the particles deeply into the hair follicle as demonstrated in Fig. 5.4. Whereas the movement of the hair occurs physiologically in vivo, it could be shown that this effect can be simulated in vitro by massage appliance (Lademann et al. 2007; Patzelt et al. 2011).

Once penetrated into the hair follicles, substances are stored over several days (Lademann et al. 2006) within this protected area if transfollicular penetration is not feasible. Whereas the reservoir of the stratum corneum is relatively unprotected and thus easily depleted by daily processes such as textile or water contact in combination with the physiological desquamation process removing one layer of corneocytes per day, the hair follicle represents a protected reservoir which can only be depleted by such slow outward-directed processes as sebum flow and hair growth. Previous investigations could show that a particle-containing formulation was still detectable within the hair follicle after 10 days, whereas the stratum corneum reservoir had already been almost completely depleted after 1 day (Lademann et al. 2006) as presented in Fig. 5.5. This long-term storage effect could be effectively utilized for therapeutic purposes as the application of drug-loaded particles with

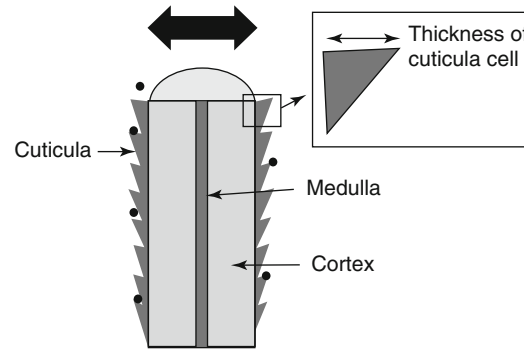

Fig. 5.4 Schematic cross section of a hair illustrating the specific structure of the cuticula cells. It has been hypothesized that this surface structure which is determined by the thickness of the cuticula cells might act as a pumping system delivering the particles deeply into the hair follicles when cuticula thickness and particle size are similar

retarded release could diminish the application frequency and thus increase the compliance of patients and the therapeutic outcome.

This therapeutic effectivity could even be enhanced when the correct skin site is chosen for the application. Otberg et al. (2004b) measured the follicular density, the volume of the follicular infundibula per square centimeter of skin, and the surface of the follicular infundibula per square centimeter of skin. The latter corresponds to the additional absorption area provided by the hair follicles. They could show that the forehead and the calf regions had the highest follicular volume per square centimeter of skin surface which was

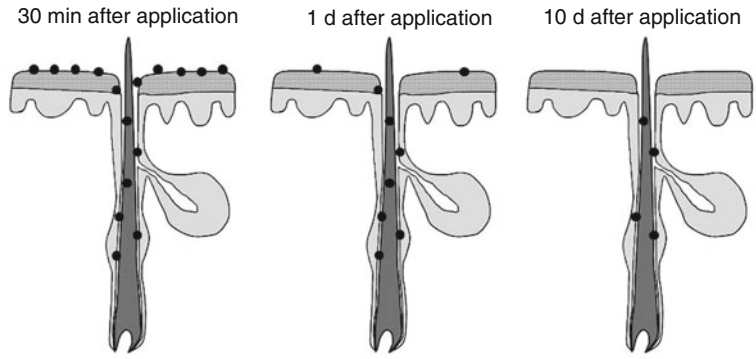

Fig. 5.5 The hair follicle is a long-term reservoir for topically applied substances. Directly after application, the applied particles are distributed on the skin surface and within the hair follicle. After 1 day, the stratum corneum reservoir is significantly depleted, whereas the particles are still available within the hair follicle. After 10 days, part of the particles are still detectable within the hair follicle

explained by the high follicle density on the forehead and the large hair follicles on the calf. It was estimated that the reservoir volumes of the hair follicles and of the stratum corneum were comparable in these body regions as demonstrated in Fig. 5.6, whereas the reservoir of the hair follicles in the region of the forearm was significantly lower by a factor of 20 in comparison to the stratum corneum reservoir.

The investigation of follicular penetration still represents a challenge as it requires spatial resolution. Nowadays, several methods are available to investigate follicular penetration reasonably. These methods have been recently summarized by Meidan et al. (2010) and include, inter alia, the selective artificial closing technique, where the hair follicles are selectively blocked with a varnish-wax mixture and thus excluded from the penetration process (Teichmann et al. 2006), the usage of a sandwich model (Barry 2002), where the top skin layer blocks the shunts in the bottom layer or the differential stripping method (Teichmann et al. 2005). Further novel optical devices are, e.g., autoradiography (Fabin and Touitou 1991), confocal laser scanning microscopy (Lademann et al. 2010) or combined confocal laser scanning microscopy with confocal Raman spectroscopy (Caspers et al. 2003).

Next to methodological challenges, also the selection of adequate model systems plays a superior role as could be demonstrated recently. Patzelt et al. (2008) could demonstrate that for

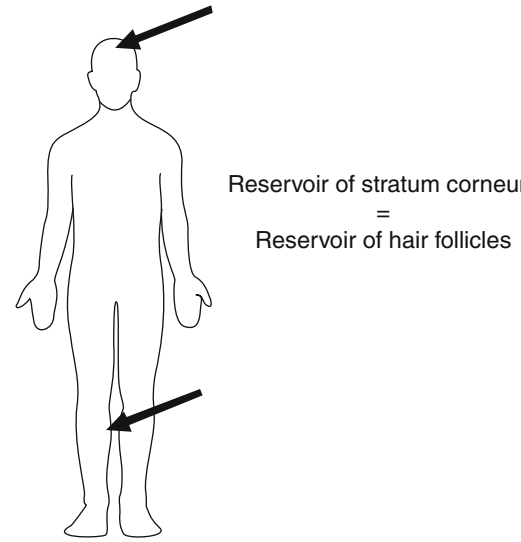

Fig. 5.6 The largest follicular reservoir can be found on the calf and the forehead. Here, the follicular reservoir is comparable to the stratum corneum reservoir

experiments performed on the same volunteers and each on the contralateral skin site, the in vitro follicular reservoir was only 10 % of the in vivo follicular reservoir. It was assumed that the elastic fibers surrounding the hair follicle contract during the excision. Whereas the removed skin sample can be re-stretched to its original size by expanding the interfollicular elastic fibers, those surrounding the hair follicle remain contracted and reduce the follicular reservoir significantly. Based on these observa-

tions, it can be stated that excised skin is not an appropriate model to investigate follicular penetration. However, in vivo investigations are not always feasible as substances or methods are mostly too injurious. As a result, the porcine ear model has been determined to be a suitable ex vivo model as the full skin can remain fixed on the underlying cartilage during the experiments, and the good and well-documented similarities of porcine and human skin architecture and structure allow a reasonable evaluation of the obtained data (Lademann et al. 2010).

In this context, it has to be emphasized that full skin samples including the subcutaneous tissue are very important for follicular penetration investigations. For diffusion cell experiments, however, mostly split skin or epidermal skin is utilized where at least the subcutaneous tissue is discarded meaning that the lower part of the hair follicle which reaches deeply into the subcutaneous tissue is cut off. This means that the inferior part of the hair follicle is open and the topically applied substances can diffuse directly into the receptor medium as demonstrated in Fig. 5.7. Similar concerns have already been raised by Senzui et al. (2010).

In addition to physicochemical properties of the applied substance and the pumping effect transporting the substances into the hair follicle by hair movement, also the activity status of the hair follicles seems to play an important role and decides whether or not follicular penetration occurs at all. As mentioned earlier, each hair follicle undergoes continuous cycling, which includes the complete remodeling of its nonpermanent portion and which influences the hair growth and sebum excretion activity of each hair follicle. A previous study could demonstrate that it can be distinguished between active hair follicles, which provide hair growth and/or sebum excretion and are open for penetration, and inactive hair follicles which provide neither hair growth nor sebum flow and are thus inaccessible for topically applied substances (Otberg et al. 2004a). For these inactive follicles a cover consisting of dried sebum and desquamated corneocytes was detected in the follicular orifices that prevents any penetration process. In the forearm region, one fourth of the hair follicles were shown to be unreceptive for penetration.

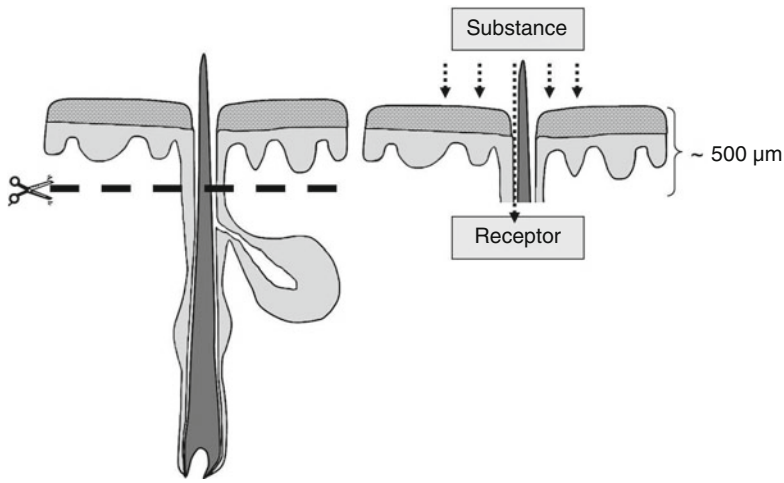

Fig. 5.7 Removing the subcutaneous tissue leads to a violation of the distal hair follicle. If, as usual, only the upper part of the skin is utilized for diffusion cell experiments, the topically applied substance can just diffuse into the receptor medium via the open inferior part of the hair follicle. Thus, the utilization of diffusion cell experiments is not an appropriate method to investigate follicular penetration

5.3.2 Mechanisms of Transfollicular Penetration

Transfollicular penetration of topically applied substances and mechanisms of transfollicular penetration have not been fully clarified, so far. It can be assumed, yet, that transfollicular penetration occurs mainly in the region of the infundibulum and specifically in the lower portion of the infundibulum where the barrier is interrupted due to the switch of a differentiation pattern as mentioned above and depicted in Figs. 5.1 and 5.3. For smaller non-particulates, i.e., substances such as caffeine or minoxidil, a rapid transfollicular penetration and systemic uptake has already been reported (Blume-Peytavi et al. 2010; Otberg et al. 2008). Interestingly, the systemic uptake was significantly faster (already after 5 min) when the hair follicles were accessible in comparison to skin areas where the hair follicles were selectively blocked previously. Here, the substances needed approximately 20 min to be detectable in the circulation. For particles the situation seems to be different, yet. There are clear indications that the transfollicular penetration process is predominantly determined by the size of the applied particles, although a clear size threshold for transfollicular penetration could not be defined so far. Recently, Labouta and Schneider (2013) reviewed the current literature focusing on skin penetration of inorganic particles. It could be shown that about half of the studies reported particle penetration or permeation. However, most studies' protocols involved either mechanical or chemical penetration enhancers (Dixit et al. 2007; Krishnan et al. 2010; Labouta et al. 2011; Mortensen et al. 2008; Paliwal et al. 2006; Upadhyay 2006; Zhang and Monteiro-Riviere 2008) or utilized excised either animal or human skin in in vitro diffusion cell experiments. With regard to risk assessment or mechanistic investigations concerning transfollicular penetration, the utilization of penetration enhancers or of in vitro diffusion cell experiments seems to be not reasonable as described above as overestimation of particle penetration can occur if penetration is either artificially enhanced or investigations are performed with dermatomed or split skin which always includes a violation of the distal hair follicle so that topically applied substances can just diffuse into the receptor medium. Whereas the contraction effect of the elastic fibers surrounding the hair follicle assumed by Patzelt et al. (2008) might be able to inhibit the diffusion of larger particles, it might explain that the detected diffusion of very small particles into the receptor medium is erroneously interpreted as penetration. This theory is supported by the fact that for all human in vivo studies reported by Labouta and Schneider (2013), no particle penetration or permeation could be detected. Only some studies were performed in vivo on animal skin, and within this group, only few studies reported a penetration into deeper skin layers of very small particles. The authors assumed that the gold nanoparticles utilized in their study might interact hydrophobically with the skin lipids leading to a disruption of the skin lipid layer structure, subsequent increased skin permeability and penetration into deeper skin layers (Huang et al. 2010). This assumption still needs further verification.

Summarizing the recent findings on transfollicular penetration, it can be stated that particles are well suitable to deliver active substances into the hair follicle, whereas transfollicular penetration is rather unlikely. For particles larger than 100 nm, intercellular or transfollicular permeation has not been observed in intact skin, yet; for smaller particles further research is necessary to define a clear threshold below which transfollicular and intercellular penetration can occur, which is also an important aspect with regard to risk assessment.

5.4 Enhancement of Follicular Penetration

As summarized above, particulate nanocarriers are excellent delivery systems for active substances into the hair follicle, which moreover, represents an interesting target site and permits fast access into the deeper viable skin layers by bypassing the complex intercellular penetration

pathways. The disadvantage – or advantage in terms of risk assessment – however is that particles have not been reliably demonstrated to penetrate intercellularly or transfollicularly.

Therefore, new approaches have to be developed to utilize the advantages of particulate delivery – such as deep follicular penetration, long-term follicular storaging, sustained release, and shielding from degradation – also for transfollicular transport of active substances.

One option is to utilize particles exclusively for delivery of substances into the hair follicle and its specific target sites. Recently, it was demonstrated that an antiseptic associated with approximately 300 nm sized carrier particles originating from a fat emulsion on the basis of medium- and long-chain triglycerides (Lipofundin® MCT/LCT, Braun, Germany) was able to achieve longer lasting antiseptic effects than the same substance in a non-particulate form (Ulmer et al. 2012). The study was based on the assumption that about 25 % of the resident bacteria colonizing the skin reside within the hair follicles (Lange-Asschenfeldt et al. 2011). Conventional non-particulate antiseptics, however, are not able to eradicate all bacteria from this follicular reservoir which leads to a fast recolonization of the skin. In contrast, it was shown that the particle-based antiseptic was able to penetrate deeply into the hair follicle. The recolonization was consequently retarded. Other examples for improved follicular penetration and optimized therapeutic effects include the application of encapsulated hair-growing substances or acne or rosacea therapeutics (Rolland et al. 1993; Shim et al. 2004; Tsujimoto et al. 2007). Some of the products are already commercially available (Papakostas et al. 2011).

If intrafollicular penetration of a therapeutic is not sufficient, strategies have to be developed to enhance the transfollicular permeation of the particles or of the active substance alone after particulate delivery into the hair follicle.

An easy, but invasive approach to translocate substances to the viable skin is to disturb the skin barrier prior to the application of the substance or particles, respectively. Here, several techniques are available such as cyanoacrylate skin surface biopsies, chemical enhancers, microneedles, electroporation, or ultrasound (Lawson et al. 2007; Vogt et al. 2008).

As a noninvasive alternative, the triggered release of substances from particle preparations has recently been introduced. In this approach, the particles exclusively serve as delivery systems to the desired penetration depth within the hair follicle which can be controlled by the particle size. Having reached the desired depth, the particles release their active agent by a specific triggering signal which can then translocate independently to the viable skin. The principle is illustrated in Fig. 5.8. By utilizing these stimuli-responsive controlled release systems, site-selective, controlled release patterns can be achieved leading to increased therapeutic efficacy and decreased side effects (Zhu et al. 2005).

At the moment, research especially focuses on the identification of appropriate

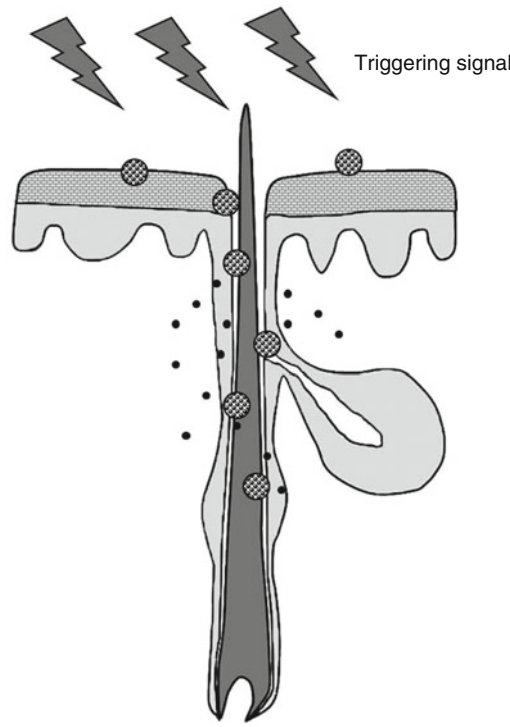

Fig. 5.8 Principle of stimuli-responsive release systems. The particles deliver the drug molecules to the desired depth within the hair follicle. After external or internal stimulus, the active drug is released from the particle and translocates independently to the deeper viable skin layers

stimuli-responsive release systems. Mak et al. (2011, 2012) recently introduced a release system based on the interaction of the particles with a protease. When the protease was likewise applied in particulate form, similar penetration depths were reached for the particles and the protease leading to a release of the active substance from the delivering particles also at significant depths of the hair follicle. Even an uptake of the model drug by the sebaceous gland could be detected.

Additional general approaches of controlled drug release include the application of high-frequency magnetic fields (Hu et al. 2008), ultrasound (Huang 2008), radiofrequency (Brazel 2009), light (Pissuwan et al. 2011), and pH drifts (Zhu et al. 2005). Controlled drug release could also be observed after utilizing CdS nanoparticles as caps for mesoporous channels and disulfide bond-reducing molecules physically blocking the drugs of certain sizes from leaching out (Lai et al. 2003). A new promising concept is also the application of gold nanoparticles in combination with near-infrared light. Gold nanorods have an absorption band in the near-infrared region and convert absorbed light energy into heat and can therefore act as a controller of a drug release system when combined with near-infrared light (Yamashita et al. 2011).

Most of these approaches have still to be verified in combination with particles in the follicular situation, which will certainly be a topic of future investigations.

Conclusion

The optimization of drug delivery to and via the hair follicle is getting more and more important as the hair follicle offers target sites of therapeutic interest and represents a rapid access to the deeper skin layers and the circulation if transfollicular penetration is feasible. Current aspects of optimized follicular drug delivery involve the adaptation of particulate carrier systems which have been demonstrated to deliver substances preferably deep into the hair follicle without allowing transfollicular penetration, yet. New approaches specifically aim at the development of controlled drug release systems where the particles only serve as transporters deep into the hair follicle. Here, the active drug is released by a specific triggering signal and can translocate independently to the deeper viable skin layers surrounding the hair follicles, subsequently. The controlled drug release represents a promising concept to utilize the advantageous delivering attributes of particles also for transfollicular penetration.

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The Correlation Between Transepidermal Water Loss and Percutaneous Absorption

6

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6.1 Introduction

6.1.1 What Is Transepidermal Water Loss?

Transepidermal water loss (TEWL) is the outward diffusion of water through the skin (Oestmann et al. 1993). An evaporimeter determines TEWL by measuring the pressure gradient of the boundary layer resulting from the water gradient between the skin surface and ambient air. TEWL measurements can reflect the general health of the skin via the assessment of skin barrier function and also assess treatment effectiveness or skin barrier repair by monitoring the change in TEWL over time (Nilsson. 1997; Pinnagoda et al. 1990). However, TEWL measurements cannot be simply compared across multiple experiments. TEWL measurements are subject intra-individual variation based on the anatomic site where the TEWL was measured and interindividual variation based on the extent of skin perspiration and skin surface temperature of the individual tested. In addition, TEWL measurements can be affected by experimental conditions such as the air convection, the ambient air temperature and air humidity of the room where the TEWL measurement was taken, and the method and type of instrument used to measure TEWL. Although TEWL can be influenced by many variables, experiments show

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that evaporimeter measurements generally are reproducible in vitro and in vivo (Pinnagoda et al. 1989, 1990; Elkeeb et al. 2010; Fluhr et al. 2006).

6.1.2 What Is Percutaneous Absorption?

Percutaneous absorption refers to the rate of absorption of a topically applied chemical through the skin. A compound's absorption rate is important for determining the effectiveness and/or potential toxicity of topically applied compounds. Since many topical formulations are used on diseased skin, where the integrity of the permeability barrier is in doubt, the dose absorbed into the body could vary greatly (Bronaugh and Stewart 1986). One rate-limiting step of a compound's absorption through the skin is the rate of diffusion through the stratum corneum (SC). The rate of absorption through the SC cannot be described by a zero- or first-order mathematical rate equation because the SC is a complex system variable in its penetration properties. Many factors contribute to the percutaneous absorption of a given chemical, such as methodology (including the effects of application time, method of measurement), physicochemical properties of the topical compound, interindividual variation (including the effects of skin condition, age of individual, and blood flow), and intra-individual variation (including the differences between anatomic sites) (Noonan and Gonzalez 1990; Wester 1993).

6.1.3 What Is the Significance of a Correlation Between TEWL and Percutaneous Absorption?

The extensive procedure required to measure percutaneous absorption versus TEWL enhances the desire to find a correlation between the two measurements in order to more easily assess skin barrier function and should aid in the understanding and development of penetration enhancers. In a review by Levin and Maibach in 2005, nine studies investigating the correlation between TEWL and percutaneous absorption were reviewed. Of the nine studies reviewed, a majority demonstrated a significant quantitative correlation, and a few found no quantitative correlation. At that time it was thought that the correlation between TEWL and percutaneous absorption may not hold for in vitro experimentation models, extremely lipophilic compounds, or possibly experiments performed on animal skin. Since then, several other studies have been published investigating the relationship between TEWL and percutaneous absorption using a very lipophilic compound (Hui et al. 2012), in vitro models (Elkeeb et al. 2010; Hui et al. 2012; Elmahjoubi et al. 2009), and animal skin (Elmahjoubi et al. 2009), and all studies have demonstrated a significant quantitative correlation.

In the next section, we review 12 studies investigating the correlation between TEWL and percutaneous absorption.

6.2 Pertinent Studies Investigating the Correlation Between TEWL and Percutaneous Absorption

Oestmann et al. (1993) investigated a correlation between TEWL and hexyl nicotinate (HN) penetration parameters in man. Penetration of HN was indirectly measured by means of laser Doppler flowmetry (LDF), which quantifies the increase in cutaneous blood blow (CBF) caused by penetration of HN, being a vasoactive substance. Lipophilic HN was chosen over hydrophilic methyl nicotinate because HN is a slower penetrant, hence, making it easier to distinguish an intact barrier from an impaired barrier.

LDF parameter initial response time (t_o) and the time to maximum response (t_{max}) were compared with corresponding TEWL values, and a weak quantitative negative correlation was found ($r=-0.31$, $r=-0.32$). This correlation suggests that when an individual's response time, t_o, was fast, the skin barrier was impaired. The weak negative correlation found that maybe LDF is not

as reproducible as other methods of measuring percutaneous absorption. Further research should investigate this weak correlation between TEWL and penetration of HN.

Lamaud et al. (1984) investigated whether TEWL correlated to the percutaneous absorption of the lipophilic compounds (hydrocortisone). Penetration of 1 % hydrocortisone and TEWL rates were recorded for the hairless rats in vivo before and after UV irradiation (660 J/cm^2). The results demonstrated a correlation between TEWL and the percutaneous absorption of hydrocortisone both before and after UV irradiation for application periods up to 1 h. In part two of the experiment, drug penetration was evaluated by urinary excretion 5 days after a single 24 h application of hydrocortisone on normal, stripped, or UV-irradiated skin of hairless rats. In this experiment the quantity of the drug eliminated correlated with the level of TEWL for up to 2 days for all skin conditions suggesting that TEWL can predict the changes of skin permeability to lipophilic drugs in normal and damaged skin.

Lavrijsen et al. (1993) characterized the SC barrier function in patients with various keratinization disorders using two noninvasive methods: measuring outward transport of water through skin by evaporimetry, i.e., TEWL, and the vascular response to HN penetration into the skin determined by LDF. Three of the five types of keratinization disorders studied, i.e., autosomal dominant ichthyosis vulgaris, X-linked recessive ichthyosis, and autosomal recessive congenital ichthyosis, have impaired barrier function and are a type of ichthyosis, while for the other two keratinization disorders studied, dyskeratosis follicularis and erythrokeratoderma variabilis, there were no prior information available on barrier impairment. In this experiment the two methods of barrier function assessment, TEWL and LDF, were correlated for all skin diseases and normal skin as a control.

TEWL measurements and the LDF parameter, $t_{o,}$ showed a high negative correlation in those with skin disease ($r=-0.64$) and a weaker negative correlation among the control healthy skin group ($r=-0.39$). Because TEWL reflects the steady state flux of a compound across SC and parameter, t_o is a function of the duration of the lag phase (not a steady state measurement), and this study suggested that these two methods, because they are measuring different things, should not be considered as exchangeable alternatives but rather as complementary tests to assess barrier function. On the basis of results of this chapter, however, it could be concluded that TEWL and HN penetration injunction are suitable methods to monitor skin barrier function in keratinization disorders.

Rougier et al. (1988) attempted to establish the relationship between the barrier properties of the horny layer using percutaneous absorption and TEWL measurements and discern the surface area of the corneocytes according to anatomic site, age, and sex in man.

The penetration of benzoic acid (BA) was measured in vivo at seven anatomic sites and compared to its TEWL value measured on the contralateral site. The amount of BA penetrated was measured through urinary extraction up to 24 h after application. It was discovered that irrespective of the anatomic site and gender, a linear relationship ($r=0.92$, $p<0.001$) existed between total penetration of BA and TEWL.

Comparing corneocyte surface area to permeability, Rougier et al. (1988) also found a general correlation of increasing permeability for both water and BA with decreasing corneocyte size. The smaller the volume of the corneocyte, the greater the intercellular space available to act as a reservoir for topically applied molecules, resulting in a higher absorption (Dupuis et al. 1984). This thinking is in accord with other studies who have shown that the smaller the capacity of the reservoir, the less the molecule is absorbed (Dupuis et al. 1984; Rougier et al. 1983, 1985, 1987a, b). In order to determine the influence of age on corneocyte size, Rougier et al. (1988) investigated the corneocyte size in the upper-outer arm for three groups of six to eight male volunteers: (1) 20–30, (2) 45–55, and (3) 65–80 years. No variation in corneocyte size up to 55 years was observed. The mean corneocyte size for the 20–30-year cohort was 980 ± 34 μm^2, and for the 45–55-year cohort, a value of 994 ± 56 μm^2 was recorded. The group aged 65–80 years did, however, show significantly larger corneocytes ($1{,}141 \pm 63$ μm^2)

relative to the other groups. Relatively small numbers of subjects were used by Rougier et al. (1988) which may explain the discrepancies when compared with data from other more recent studies (Leveque et al. 1984). Generally it is now understood that corneocytes generally increase in size with age (Machado et al. 2010) and TEWL and percutaneous absorption also increases with age (Roskos and Guy 1989); therefore, it seems that corneocyte size cannot explain the permeability changes in mature skin.

Rougier et al. (1988) used a detergent scrub technique to collect corneocytes at different anatomic sites from a group of six to eight male volunteers, aged 20–30 years. The rank order of the corneocyte surface area was forearm (ventral elbow) = forearm (ventral-mid) = arm (upper-outer) = abdomen > forearm (ventral-wrist) > postauricular > forehead. However when Rougier et al. (1988) investigated corneocyte size by anatomic site, he found that for certain anatomic sites where corneocyte size was similar (980–1,000 μm^2), there were large differences in permeability. Therefore, while percutaneous absorption and TEWL are quantitatively correlated, corneocyte size only partially explains the difference in permeability between the different anatomic sites and different age of the skin.

Lotte et al. (1987) examined the relationship between the percutaneous penetration of four chemicals (acetylsalicylic acid, benzoic acid, caffeine, and sodium salt of benzoic acid) and TEWL in man as a function of anatomic site. The amount of chemical penetrated was measured by urinary excretion for up to 24 h after application. For a given anatomic site, the permeability varied widely in relation to the nature of the molecule administered due to the physicochemical interactions which occur between the molecule, vehicle, and SC. For all anatomic sites investigated, irrespective of the physicochemical properties of the molecules administered, there was a linear relationship between TEWL and percutaneous absorption.

Aalto-Korte et al. (1993) attempted to find the precise relationship between TEWL and percutaneous absorption of hydrocortisone in patients with active dermatitis. Percutaneous absorption of hydrocortisone and TEWL was studied in three children and six adults with dermatitis. All the subjects had widespread dermatitis covering at least 60 % of the total skin area. Plasma cortisol concentrations were measured by radioimmunoassay before and 2 and 4 h after hydrocortisone application. TEWL was measured in six standard skin areas immediately before application of the hydrocortisone cream. Each individual TEWL value was calculated as a mean of these six measurements.

The concordance between the post application increment in plasma cortisol and the mean TEWL was highly significant resulting in a correlation coefficient of $r = 0.991$ ($p < 0.001$). In conclusion this study found a highly significant correlation between TEWL and percutaneous absorption of hydrocortisone.

Tsai et al. (2001) investigated the relationship between the permeability barrier disruption and the percutaneous absorption of various compounds with different lipophilicity. Acetone treatment was used in vivo on hairless mice to disrupt the normal permeability barrier, and in vivo TEWL measurements were used to gauge barrier disruption. The hairless mouse skin was then excised and placed in diffusion cells for the in vitro percutaneous absorption measurements of five model compounds: sucrose, caffeine, hydrocortisone, estradiol, and progesterone. The partition coefficient or lipophilicity of these compounds and compounds used in the subsequent studies are summarized in Table 6.1.

The permeability barrier disruption by acetone treatment and TEWL measurements significantly correlated with the percutaneous absorption of the hydrophilic and lipophilic drugs sucrose, caffeine, and hydrocortisone. However acetone treatment did not alter the percutaneous penetration of the highly lipophilic compounds estradiol and progesterone, hence, suggesting that there is no correlation between TEWL and the percutaneous absorption of highly lipophilic compounds. The results imply the need to use both TEWL and drug lipophilicity to predict alterations in skin permeability.

Chilcott et al. (2002) investigated the relationship between TEWL and skin permeability to tritiated water (^3H_2O) and the lipophilic sulfur mustard (^{35}SM) in vitro. No correlation was found

between basal TEWL rates and the permeability of human epidermal membrane to ^3H_2O ($p=0.72$) or sulfur mustard ($p=0.74$). Similarly, there was no correlation between TEWL rates and the ^3H_2O permeability on full-thickness pig skin ($p=0.68$). There was also no correlation between TEWL rates and ^3H_2O permeability following up to 15 tape strips ($p=0.64$) or up to four needle stick punctures ($p=0.13$). Taken together these results from this experiment indicate that under these experimental circumstances (i.e., in vitro human and pig skin) TEWL cannot be used as a measure of the skin's permeability to topically applied lipophilic or hydrophilic compounds.

Elkeeb et al. (2010) compared TEWL to the percutaneous absorption/flux rate of ^3H_2O in in vitro dermatomed clinically healthy human cadaver skin using three different evaporimeters to measure TEWL. Measurements were taken at baseline (i.e., at the start of the experiment) and then again at several time points over 24 h. The evaporimeters included an open chamber evaporimeter A (TEWameter® TM 210 (Courage and Khazaka, Cologne, Germany)) and two closed chamber evaporimeters B (VapoMeter™ (Delfin Technologies, Kuopio, Finland)) and C (AquaFlux AF200, Biox Systems, Ltd, London, UK). Open chamber evaporimeters are open to the ambient air, while closed chamber evaporimeters are closed systems that are not open to the environment. There has been controversy over the years as to whether open and closed chamber evaporimeters are equivalent in given accurate and precise TEWL measurements TEWL. Baseline TEWL measurements with evaporimeters A ($p=0.04$, $r^2=0.34$) and C ($p=0.00$, $r^2=0.50$) correlated with the percutaneous absorption or flux rate of tritiated H_2O, while evaporimeter B showed no statistically significant correlation ($p=0.07$, $r^2=0.31$). However, the pattern of changing TEWL values over 24 h was similar to that of the percutaneous absorption or tritiated water flux for all three evaporimeters A, B, and C ($p=0.04$, $r^2=0.34$,). The reason why evaporimeter B showed no significant correlation for baseline TEWL measurement remains unknown. Elkeeb et al. (2010) state that the results of this experiment imply the validity of using both open and closed chamber evaporimeters in the evaluation of skin barrier function.

Atrux-Tallau et al. (2007) demonstrated significant correlation between TEWL and the percutaneous absorption of caffeine (a hydrophilic compound) during an ex vivo experiment on heat separated epidermis and dermatomed human skin ($p<0.001$, $r^2=0.88$). Since caffeine is a hydrophilic compound and has a relatively small molecular weight of 194 Da, it was not surprising to the authors that the permeation behavior resembles that of tritiated water (22 Da).

Hui et al. (2012) investigated the correlation between TEWL and the percutaneous absorption of clonidine (a lipophilic compound) and ^3H_2O (a hydrophilic compound) in in vitro human cadaver skin. The partition coefficient of clonidine is reported in Table 6.1. TEWL measurements were made with a closed chamber TEWL meter (AquaFlux AF200). With the goal of discerning the potential differences in the correlation between TEWL and lipophilic clonidine, the correlation between TEWL and hydrophilic ^3H_2O percutaneous absorption and general differences in the percutaneous absorption of clonidine and ^3H_2O, the flux rate, skin distribution, and total amount of absorption for clonidine and tritiated water were recorded and compared. Statistical analysis indicated that the baseline TEWL values weakly correlated with the flux of [14C]-clonidine ($p<0.03$, $r^2=0.36$) and ^3H_2O ($r^2=0.34$, $p=0.04$). The correlation between fluxes of ^3H_2O and [14C]-clonidine was moderate (correlation coefficient = 0.675, $p<0.001$). In addition, TEWL and permeation data of ^3H_2O expressed as a percent dose of the amount in the receptor fluid correlated well throughout the experiment. However, the permeation curve of [14C]-clonidine as a percent dose in the receptor fluid differed from that of ^3H_2O and TEWL. The difference in the curves is likely secondary to differences in the hydrophilic/lipophilic properties of clonidine versus water. Therefore as Hui suggests, it may be necessary to combine the TEWL values with factors such as molecular weight and/or hydrophilicity/lipophilicity to gauge percutaneous absorption.

Elmahjoubi et al. (2009) investigated TEWL (using the AquaFlux evaporimeter) and the

Table 6.1 A summary of the compounds used in the correlation studies, their octanol-water partition coefficient, solubility classification, and whether or not their percutaneous absorption correlated with TEWL (Oestmann et al. 1993; Nilsson 1997; Elkeeb et al. 2010; Hui et al. 2012; Elmahjoubi et al. 2009; Lamaud et al. 1984; Lavrijsen et al. 1993; Rougier et al. 1988; Lotte et al. 1987; Aalto-Korte et al. 1987; Tsai et al. 2001; Chilcott et al. 2002; Atrux-Tallau et al. 2007)

Compound	Partition coefficient (log $P_{octanol/water}$)	Classification	Correlation
Sucrose	−3.7	Hydrophilic	Yes
Caffeine	−0.02	Hydrophilic	Yes
Water	1	Hydrophilic	Yes
Acetylsalicylic acid	1.13	Hydrophilic	Yes
Sulfur mustard	1.37	Lipophilic	No
Hydrocortisone	1.5	Lipophilic	Yes
Benzoic acid	1.87	Lipophilic	Yes
Sodium benzoate	1.87	Lipophilic	Yes
Estradiol	2.7	Highly lipophilic	No
Progesterone	3.9	Highly lipophilic	No
Hexyl nicotinate	4	Highly lipophilic	Yes (weak)
Clonidine	5.4	Highly lipophilic	Yes (weak)

percutaneous absorption/flux of ^3H_2O in full-thickness in vitro porcine skin both at baseline and after physical and chemical barrier disruption in multiple different experiments. The aim of these experiments was to further investigate the relationship between TEWL and ^3H_2O flux using the AquaFlux evaporimeter® (Bio Systems Ltd, USA) and to evaluate the use of porcine skin in vitro as a model to study the human skin barrier.

The first experiment investigated the relationship between basal TEWL rates and ^3H_2O flux in in vitro healthy full-thickness porcine skin. The results showed that basal TEWL values were linearly correlated with basal ^3H_2O flux values ($r^2=0.80$, $n=63$).

The second experiment examined the effect of physical barrier disruption with skin punctures on TEWL measurements. The results did not show a perfect correlation between skin punctures and TEWL measurements. TEWL increased significantly after the first skin puncture and then remained constant for punctures 2, 3, and 4. Another large increase in TEWL was seen with the fifth puncture. However no changes in TEWL values were seen with the sixth or seventh puncture suggesting that a threshold may have been reached after the fifth puncture.

The third and fourth experiments examined TEWL changes after chemical barrier disruption with surfactants. In the third experiment, anionic surfactants of differing alkyl chain lengths were applied to the full-thickness porcine skin in vitro to determine if measuring TEWL values could discern between mild and severe perturbations to the barrier function. TEWL was largely unaffected following cutaneous exposure to short and long alkyl chain surfactants and, however, was significantly elevated over control levels following exposure to those with intermediate chain lengths. Exposure to sodium lauryl sulfate (SLS), with an intermediate 12 carbon alkyl chain, produced the greatest increase in TEWL.

In the fourth experiment, the effect of varying SLS concentration, volume, and contact time on the TEWL in vitro in porcine skin was measured. The results showed a linear trend between TEWL and SLS concentration in the 0–1 % w/v concentration range. However, following treatment with 5 % w/v SLS, TEWL readings were only slightly higher than those following treatment with 1 % w/v surfactant. A linear correlation was also demonstrated between TEWL and surfactant solution volume ($r^2=0.87$), which was statistically significant ($p<0.01$). TEWL also increased as a function of increasing SLS treatment time, when concentration was fixed at 1 % w/v and volume fixed at 200 µl.

In conclusion, Elmahjoubi et al. (2009) found that baseline TEWL values correlated with the percutaneous absorption of ^3H_2O in vitro in healthy

porcine skin and the TEWL measurements linearly correlated with the exposure of porcine skin in vitro to increasing concentrations, time, and volumes of SLS. TEWL measurements did not demonstrate a linear correlation between skin punctures (i.e., skin damage) and TEWL. The authors feel that TEWL measurements in vitro in porcine skin may serve as a model for future studies in this area in contrast to the previous findings by Chilcott et al. (2002).

6.3 Discussion of the Assumptions Made in the Studies Investigating the Correlation Between TEWL and Percutaneous Absorption

Many of the experiments investigating TEWL and percutaneous absorption make large assumptions which could affect the results and hence be the source of controversy. For example, Tsai et al. (2001) and Chilcott et al. (2002) assume that in vitro measurements of TEWL and percutaneous absorption are equivalent to in vivo measurements, while Lamaud et al. (1984) assume that animal skin may serve as a permeability model for human skin. Great sources of error and variation can also be induced depending on the measurement device used to record TEWL rates and the choice of the compound and/or method used to measure percutaneous absorption rates. Because we do not completely understand the qualitative relationship between TEWL and percutaneous absorption, it is hard to determine which assumptions made during the experiment could be affecting the correlation results. This section investigates the probable causes that could influence the results of the correlation experiments. Provided in Table 6.2 is a summary of the major assumptions from 12 studies discussed in this chapter.

6.3.1 Using In Vitro Methods to Model In Vivo Experiments

Skin permeation can be measured in vivo or in vitro by using excised skin in diffusion cells. In theory, studies using in vitro or ex vivo are feasible models for in vivo experiments because passage through the skin is a passive diffusion process and the stratum corneum is nonliving tissue. Many studies comparing in vivo and in vitro TEWL and percutaneous absorption measurements have been conducted, and the results from those experiments support the contention that reliable measurements can be obtained from in vitro studies (Elkeeb et al. 2010; Noonan and Gonzalez 1990; Hui et al. 2012; Elmahjoubi et al. 2009; Nangia et al. 1993; Brounaugh et al. 1982b). While the consensus is that in vitro experiments are reasonable models for in vivo human experiments, some experiments note significant differences between these methods for measuring skin permeation. The most significant study by Bronaugh and Stewart (1985) found that the effects of UV irradiation could not be duplicated using an in vitro experimentation model, hence, suggesting that in vitro experiments examining the TEWL and percutaneous absorption after barrier damage may not be an acceptable model for correlation with in vivo studies. In vitro damage to the SC barrier may not be an accurate model to in vivo SC damage because in vivo exposure to skin irritants results in a cascade of reactions that do not occur in vitro in human cadaver skin (Nangia et al. 1993).

Chilcott et al. (2002) investigated the correlation between TEWL and percutaneous absorption in vitro after inducing different types of barrier damage. This was one of the rare studies which did not observe a correlation between TEWL and percutaneous absorption after barrier damage. It is possible that in vitro methodology in the experimental design may be responsible for the lack of correlation of TEWL to skin damage reported in this study. However, Fluhr et al. (2006) suggest that the conditions used in the study of Chilcott et al. (2002), i.e., the use of heat-split human epidermis and non-pigmented pig skin that had been stored for up to 14 days and penetration studies which extended over 96 h post-heat separation, likely contributed to their results. Fluhr (2006) states that the extracellular lipid matrix and corneocytes of the SC were potentially compromised from the heat separation. However, it is this author's opinion that even if the barrier was compromised by heat separation, these changes in barrier function should have been reflected both in the TEWL and percutaneous absorption

Table 6.2 A summary of the major assumptions made by the studies discussed in this chapter (Aalto-Korte et al. 1993; Atrux-Tallau et al. 2007; Chilcott et al. 2002; Elkeeb et al. 2010; Hui et al. 2012; Elmahjoubi et al. 2009; Lamaud et al. 1984; Lavrijsen et al. 1993; Lotte et al. 1987; Nilsson 1997; Oestmann et al. 1993; Rougier et al. 1988; Tsai et al. 2001)

Reference	In vivo vs in vitro (percutaneous absorption)[b]	Skin type	Percutaneous absorption measurement method	Compound[c]	Healthy skin vs damaged skin	Correlation results
Oestmann et al. (1993)	In vivo	Human	LDF	Lipophilic	Healthy	Yes
Lamaud et al. (1984)	In vivo	Animal	Urinary	Lipophilic	Both	Yes
Lavrijsen et al. (1993)	In vivo	Human	LDF	Lipophilic	Damaged	Yes
Rougier et al. (1988)	In vivo	Human	Urinary	Lipophilic	Healthy	Yes
Lotte et al. (1987)	In vivo	Human	Urinary	Hydrophilic and lipophilic	Healthy	Yes
Aalto-Korte et al. (1993)	In vivo	Human	Plasma cortisol level	Lipophilic	Damaged	Yes
Tsai et al. (2001a)[a]	In vitro	Animal	Diffusion cell	Hydrophilic and lipophilic	Damaged	Yes
Tsai et al. (2001b)[a]	In vitro	Animal	Diffusion cell	Highly lipophilic	Damaged	No
Chilcott et al. (2002)	In vitro	Both	Diffusion cell	Hydrophilic and lipophilic	Both	No
Elkeeb et al. (2010)	In vitro	Human	Diffusion cell	Hydrophilic	Healthy	Yes
Hui et al. (2012)	In vitro	Human	Diffusion cell	Hydrophilic and lipophilic	Healthy	Yes
Atrux-Tallau et al. (2007)	Ex vivo	Human	Diffusion cell	Hydrophilic	Healthy	Yes
Elmahjoubi et al. (2009)	In vitro	Animal	Diffusion cell	Hydrophilic	Both	Yes

[a]Reference Tsai et al. was divided into two experiments in this table since the study found a correlation between TEWL and percutaneous absorption with some compounds and no correlation with others

[b]TEWL in vivo and in vitro measurements are considered equivalent. We are only concerned with how percutaneous absorption measurements were performed

[c]Compounds were classified by their octanol-water partition coefficient, log $K_{octanol/water}$. See Table 6.1. Compounds possessing log $K_{octanol/water}$ values less than one are considered hydrophilic, while compounds with log $K_{octanol/water}$ higher than three were considered very lipophilic

and hence should have correlated if both measured variables truly reflect skin barrier function.

However since Chilcott et al.'s original publication in 2002, many studies demonstrating the correlation between TEWL and percutaneous absorption have been conducted in in vitro models (Elkeeb et al. 2010; Hui et al. 2012; Elmahjoubi et al. 2009), and it is more likely that the results of Chilcott et al. (2002) were an exception rather than the rule.

6.3.2 Using Animal Skin to Model Human Skin

Comparing the skin morphology and absorption of chemicals through human versus animal skin, it is clear that human skin is unique in both aspects and should be used for the most meaningful results (Bronaugh and Franz 1986). Yet an experiment by Bronaugh et al. (1982a) found that depending on the compound and the vehicle

used, permeability values obtained using animal skin can be well within an order of magnitude of the permeability values for human skin.

Independently, in vitro methods and animal skin models prove to be reliable models to predict percutaneous absorption in human skin in vivo. Therefore it seems logical to assume that the in vitro condition and the use of animal skin may be used in unison to accurately model in vivo absorption through human skin. However Rougier et al. (1987a, b) documented a distinct difference between animal studies performed in vivo versus animal studies performed in vitro when compared to the absorption of compounds through human skin in vivo. This experiment compared the permeability of human skin to the hairless rat (Walker et al. 1983) and the hairless mouse (Bronaugh and Stewart 1986) skin using molecules of widely different physicochemical properties. The results show that on in vivo animal or human skin, for whatever the molecule tested, the permeability ratios remained relatively constant, while in vitro they do not. Therefore, when application conditions are strictly identical in humans and in animals, it may be possible to predict percutaneous absorption in human skin in vivo by measuring in vivo absorption through animal skin, but not using in vitro animal absorption. The inaccurate results obtained when conducting experiments in vitro using animal skin may have affected the results studies by Tsai et al. (2001) and Chilcott et al. (2002) which were the only two studies using in vitro animal skin and showing no correlation between TEWL and percutaneous absorption.

However, Laumaud et al. (1984) conducted their study in porcine skin in vivo, and Elmahjoubi et al. (2009) conducted their study in porcine skin in vitro, and both found a correlation between TEWL and percutaneous absorption. This suggests that other factors than using animal in vitro model may have played a role in the lack of correlation found in studies by Tsai et al. (2001) and Chilcott et al. (2002). However, there is no doubt that there are distinct differences between animal skin and human skin when used as a model for human absorption, whether these differences are large enough to invalidate that the use of animal skin as a model for experimentation seems unlikely. However, further research may be warranted.

6.3.3 Differences in TEWL Measurement Methods

TEWL meters or evaporimeters can have an open or closed chamber system. Open chamber TEWL meters are open to the environment, and therefore their measurements are influenced by environmental factors such as room temperature or humidity. Closed chamber devices are closed systems that are not dependent on environmental variables. With adequate control of environmental variables, open chamber TEWL meters can provide reliable and reproducible measurements that are comparable to closed chamber TEWL meters (Pinnagoda et al. 1990, 1989; Elkeeb et al. 2010; Fluhr et al. 2006). Yet, only a limited number of comparisons between different types of TEWL meters have been described in the literature until the last few years, and TEWL meters are known to differ in their measurement range, speed, repeatability, and reproducibility (Hui et al. 2012).

Elkeeb et al. (2010) and Fluhr et al. (2006) performed studies which exemplify the general comparability of TEWL meters, but also exemplify their differences. As mentioned in the previous section, Elkeeb et al. (2010) compared TEWL to the percutaneous absorption/flux rate of $^{3}H_2O$ in in vitro human cadaver skin using three different evaporimeters: open chamber evaporimeter A (TEWameter® TM 210, Courage and Khazaka, Cologne, Germany; Acaderm Inc., Menlo Park, CA, USA) and two closed chamber evaporimeters B (VapoMeter™, Delfin Technologies, Kuopio, Finland) and C (AquaFlux AF200, Biox Systems, Ltd, London, UK). TEWL values correlated at baseline and over the 24 h experiment for evaporimeters A and C. However TEWL values of evaporimeter B only correlated with evaporimeters A and C during the experiment and did not correlate at baseline.

An experiment by Fluhr et al. (2006) compared many different TEWL meters in vivo in human and murine skin and ex vivo in

murine skin. TEWL rates obtained with two closed chamber systems (VapoMeter™ (Delfin Technologies, Kuopio, Finland) and H4300 (NIKKISO YSI CO., Ltd, Tokyo, Japan)) and one closed-loop system (MEECO; MEECO, Warrington, PA, USA) under different experimental in vivo conditions were compared with data from four open-loop instruments, i.e., TEWameter® TM 210, TEWameter® TM 300 (Courage and Khazaka, Cologne, Germany), DermaLab (Cortex Technology, Hadsund, Denmark), and EP 1 (ServoMED, Stockholm, Sweden). Through his experiments, Fluhr demonstrated the ability of most of TEWL meters to detect minor, moderate, and severe changes in barrier dysfunction; however, none of the devices could detect minor improvements in barrier function, and there were differences in the TEWL meters' ability to detect differences between severe and very severe barrier dysfunction. However, analysis of all the data collected demonstrated a weak correlation between a few TEWL meters, but an overall good correlation between all the TEWL meters.

An additional study by Farahmond et al. (2009) found similar results to Fluhr et al. (2006) when studying the differences between two closed chamber TEWL measurement instruments. These instruments were designed based on different measurement principles and demonstrated slight differences in their ability to detect changes in skin barrier function despite that the values of all three instruments correlated well with each other ($p < 0.001$).

These studies by Elkeeb et al. (2010), Fluhr et al. (2006), and Farahmond et al. (2009) reveal that there are potential limitations to TEWL meters in experimentation and the TEWL meter must be chosen carefully based on the proposed study design. In general, TEWL meters produced comparable and reliable results; however, in both Elkeeb et al.'s (2010) and Fluhr et al.'s (2006), experiments there were reported TEWL measurements that did not significantly correlate with other measurements. These variations in measurement have the potential to influence experimentation.

6.3.4 Influences of Percutaneous Absorption Measurement Methods

The major factor affecting percutaneous absorption measurements is the used methodology (Bronaugh and Maibach 1989; Wester and Maibach 1992). Methods used for percutaneous absorption measurements are not equal and hence can give different results. Table 6.2 column 3 summarizes the percutaneous absorption measurement methodology used in these correlation studies.

The most common method for determining percutaneous absorption in vivo is measuring the radioactivity of excreta following topical application of a labeled compound. Determination of percutaneous absorption from urinary radioactivity does not account for metabolism by the skin but has been proven to be a reliable method for absorption measurements and is widely accepted as the "gold standard" when available.

The most commonly used in vitro technique involves placing excised skin in a diffusion chamber, applying radioactive compound to one side of the skin and then assaying the radioactivity in the collection vessel on the other side of the skin (Bronaugh and Maibach 1991). The advantages of using this in vitro technique are that the method is easy to use and that the results are obtained quickly. The disadvantage is that the fluid in the collection bath which bathes the skin is saline, which may be appropriate for studying hydrophilic compounds, but is not suitable for hydrophobic compounds. If the parent compound is not adequately soluble in water, then determining in vitro permeation into a water receptor fluid will be self-limiting.

When conducting in vitro experiments, animal skin often substitutes human skin. Because animal skin has different permeability characteristics than human skin, one should be careful which type of animal skin is used (see section on animal vs human skin). In addition, proper care should be taken in skin preparation of excised skin to not damage the skin barrier integrity. Anatomic site is also important, since the skin from different sites shows different permeability as well as using many different donor skin samples.

The only two experiments which did not find a correlation between TEWL and percutaneous absorption, Tsai et al. (2001) and Chilcott et al. (2002), were experiments that measured percutaneous absorption in vitro. Perhaps using a diffusion cell to measure percutaneous absorption is the reason for not finding a correlation.

Oestmann et al. (1993) and Lavrijsen et al. (1993) used laser Doppler flowmeter (LDF) to measure HN penetration. LDF measures the increase in cutaneous blood flow (CBF) caused by the penetration of HN, a vasoactive substance. One problem with this method is that LDF measurements are not only dependent on the amount of HN absorbed but also on the individual's vasoreactivity, gender, and age. This may be the reason why Oestmann et al. (1993) and Lavrijsen et al. (1993) obtained only a weak correlation between TEWL and percutaneous absorption of HN. Another disadvantage of this method is that LDF measurements have many sources of variation which make it difficult to compare inter-laboratory results.

6.3.5 Influence of the Lipophilicity or Hydrophilicity of the Compound Studied

The percutaneous absorption rate and/or total absorption of a compound varies greatly depending on the compound and its lipophilicity. Yet, many of the papers reviewed did not consider how lipophilicity of the test compound would affect percutaneous absorption and hence affect the correlation between TEWL and percutaneous absorption. Feldmann and Maibach (1970) measured both the total absorption and maximal absorption rate for 20 different compounds of different lipophilicities. The range for total absorption for the 20 compounds tested demonstrated a difference greater than 250 times in total absorption amounts, while the 20 compounds that had a difference in maximum absorption rate were greater than 1,000-fold (Feldmann and Maibach 1970). Because of the extreme range of absorption for topically applied compounds, it seems reasonable to assume that the correlation between TEWL and percutaneous absorption may not be independent of the physicochemical properties of the compound applied. Namely, can TEWL measurements predict the skin barrier's permeability changes to both hydrophilic and very lipophilic compounds?

Correlation between TEWL and percutaneous absorption was found in many studies, such as Oestmann et al. (1993), Lamaud et al. (1984), Lavrijsen et al. (1993), Lotte et al. (1987), Aalto-Korte et al. (1993), Tsai et al. (2001a), Elkeeb et al. (2010), Elmahjoubi et al. (2009), Hui et al. (2012), and Atrux-Tallau et al. (2007), which suggest that TEWL can predict the changes in skin permeability to topically applied hydrophilic and lipophilic drugs. However, Tsai et al. (2001b) found that the percutaneous absorption of the highly lipophilic progesterone and estradiol did not correlate with TEWL.

The most common lipophilicity scale of molecules is defined by the octanol-water partition coefficient ($K_{oct/w} = \log(p_{oct/w})$). Presented in Table 6.1 are the compounds used in the aforementioned studies, their octanol-water partition coefficient, their solubility classification, and whether or not their percutaneous absorption correlated with TEWL.

Looking closely at Table 6.1, the highly lipophilic compounds were the compounds that demonstrated a weaker correlation or no evidence of a correlation between percutaneous absorption and TEWL, while the moderately lipophilic compounds such as hydrocortisone and benzoic acid and the hydrophilic compounds did show a correlation. This should be further investigated. As stated previously, it may be necessary to use both TEWL and drug lipophilicity to predict alterations in skin permeability.

Conclusion

In 2005, Levin and Maibach reviewed nine studies investigating the correlation between TEWL and percutaneous absorption of actives. At that time seven of the nine studies demonstrated a quantitative correlation, yet two studies did not. Those studies that did not confirm a quantitative correlation (Tsia et al. 2001b; Chilcott et al. 2002) or only observed a weak correlation (Oestmann et al. 1993; Hui

et al. 2012; Lavrijsen et al. 1993) used different experimental methods, such as an in vitro model, animal skin, or extremely lipophilic compounds compared with the studies which found a quantitative correlation. The conclusion at this time was that those assumptions and differences in experimental design were likely responsible for the lack of correlation. Since then, new studies have been published investigating the use of lipophilic compounds, in vitro models, and animal skin as models for in vivo human skin barrier study (Elkeeb et al. 2010; Hui et al. 2012; Elmahjoubi et al. 2009). These studies have demonstrated significant correlation between TEWL and percutaneous absorption in vitro in human and animal skin for both lipophilic and hydrophilic compounds (Elkeeb et al. 2010; Hui et al. 2012; Elmahjoubi et al. 2009). In this updated overview, 10 of the 12 studies discussed here found some degree of correlation between TEWL and percutaneous absorption. It is uncertain why these two studies found no correlation; however, it seems likely after looking at the compiled data in Table 6.1 that TEWL can serve as a prediction for percutaneous absorption in both in vivo and in vitro models and in human and animal skin and those studies which did not report a correlation between TEWL and percutaneous absorption were the exception rather than the rule. Furthermore, it may be that evaporimeter choice may play a more important role in experimental design than previously assumed.

Taken together, the weight of evidence confirms a relationship between TEWL and percutaneous penetration of actives, yet, much remains to be understood.

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7 Influence of Excipients on Two Elements of the *Stratum Corneum* Barrier: Intercellular Lipids and Epidermal Tight Junctions

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7.1 Introduction: Epidermal Barrier Structure and Function

The skin is one of the most important organs in the human body due to its crucial role as an interface between the internal and external environments. It controls the entry of ultraviolet radiation, chemicals, pathogenic agents, free radicals, etc., as well as the water exchanges. The permeability barrier homeostasis is sustained by a combination of several factors, biochemical and structural, in response to the influences of the environment.

The permeability barrier function is fulfilled by the epidermis and mainly its external layer – the *stratum corneum* (SC). SC is composed of dead cells, corneocytes, issued from the terminal differentiation of epidermal keratinocytes. The losses of corneocytes by superficial desquamation are compensated by cornification of keratinocytes from the living layers of the epidermis and by cell divisions in the germinative layer (*stratum basale*).

The efficiency of the SC barrier is based on the composition and the correct arrangement of

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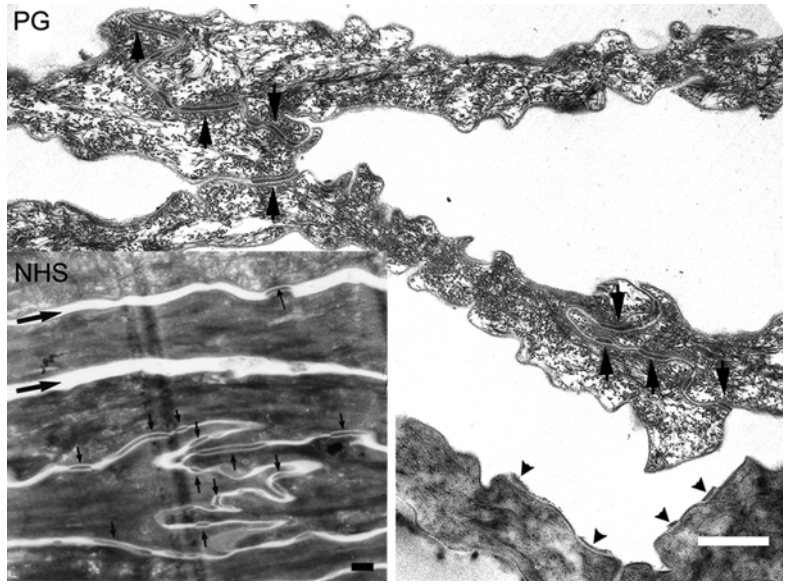

Fig. 7.1 In normal human skin (NHS), the SC may be subdivided into SC *compactum*, where the principal permeability barrier resides, and SC *disjunctum*, where the desquamation process takes place. Transition between the two regions is visualized in the inset. Several corneodesmosomes (*small arrows*) link SC *compactum* corneocytes both laterally and vertically. SC *disjunctum* is characterized by horizontal splitting between the successive corneocyte layers due to the loss of the interlayer corneodesmosomes. Exposure to propylene glycol (PG) induces basket-weave pattern in the entire SC. Interlayer corneodesmosomes become disrupted (*arrowheads*) because of a better accessibility of the catalytic enzymes, but the cells remain attached laterally (*big arrows*). This phenomenon is likely related to the presence of TJ-like structures sealing off the corneocyte periphery and therein located corneodesmosomes. Bars = 200 nm in NHS and 500 nm in PG

its principal elements: (1) corneocytes linked by corneodesmosomes (Fig. 7.1) and (2) intercellular lipids covalently bound to the cross-linked corneocyte envelopes and organized in a multilayered extracellular matrix (Wertz et al. 1989; Wertz 2000; Haftek et al. 2006). Molecular composition and structural arrangement of the hydrophobic lipid matrix are critically involved in the limitation of SC permeability (Bouwstra et al. 1991). Intercellular spaces of the SC contain also hydrophilic poaches or lacunae that may swell upon SC hydration and are compatible with the presence of catabolic enzymes and their natural inhibitors, essential for the regulation of epidermal desquamation (Menon and Elias 1997; Haftek et al. 1998). Most of the components of the SC intercellular spaces are produced in the last living layer of the epidermis, *stratum granulosum* (SG), and are delivered through exocytosis of the *trans*-Golgi-derived lamellar bodies (Ishida-Yamamoto et al. 2004). The oriented secretion of these latter organelles is also indispensable for the formation and function of the SC barrier. It has been proposed that epidermal tight junctions (TJ) may contribute to the polarization of the granular layer cells and constitute an additional permeability barrier (Brandner et al. 2010; Kirschner et al. 2010a, b).

In transmission electron microscopy, TJ appear as fusion points between external sheets of the plasma membranes of two neighboring cells. Such cell–cell membrane fusions become cross-linked during the keratinization process and persist in the SC in a form of TJ-like structures (Haftek et al. 2011). The resulting subdivision of the intercellular spaces of the SC into independent compartments appears to be important for appropriate regulation of the desquamation process and, thus, the barrier homeostasis.

At the molecular level, TJ are composed of transmembrane and cytoplasmic proteins connected to the actin cytoskeleton (Brandner et al.

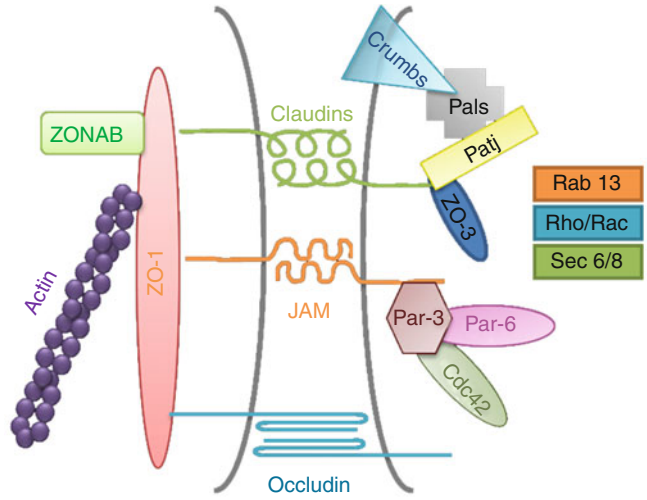

Fig. 7.2 Schematic representation of a tight junction (TJ) structure and of its cytoplasmic interacting proteins: transmembrane proteins, i.e., claudins and occludin, and junctional adhesion molecule (JAM) which interact in the intercellular space where linker proteins zonula occludens and zonula occludens-associated nucleic acid binding protein (ZO-1/3, ZONAB) attach TJ to the actin cytoskeleton. Regulatory proteins, e.g., *Sec 6/8* the secretion complex, *Pals* linking protein, *Patj* Pals-linking protein to tight junctions, *Par-3/6* partitioning defective 3 and 6, *Crumb, Cdc42* cell division control protein 42, *Rab, and Rho/Rac* small G-proteins are important for TJ formation and signaling

2002; Niessen 2007; Brandner et al. 2010). Several transmembrane TJ-specific proteins have been identified, e.g., occludin, several claudins, crumb, and JAM (junction adhesion molecules) (Fig. 7.2). Extracellular domains of these proteins interact to establish cell–cell contacts. The interaction between claudins engenders the formation of "pores" selective for various types and sizes of molecules (Tsukita and Furuse 2000; Furuse et al. 2002) (Fig. 7.3). The selectivity is dependent on the concentration and the type of claudins participating in the junction protein complex. Thus, the extracellular parts of TJ are not fully "tight." However, when distributed as a continuous band around a cell, TJ efficiently subdivides the cell surface into the apical and basal regions thereby contributing to creation of cell polarity.

Ken Hashimoto has observed TJ structures at the apical membrane of the SG keratinocytes as early as 1971 (Hashimoto 1971). However, this observation has been neglected for 30 years because lipids of the intercellular space of SC were considered the only efficient barrier to the water loss (Elias and Friend 1975). The beginning of the twenty-first century was marked by the revival of epidermal TJ studies. In 2002, Furuse et al. have demonstrated that biotin (557 Da) injected in a mouse dermis ascends to the epidermis via the intercellular spaces and stops in the SG at sites stained by antibody to occludin, thus indicating the presence of functional TJ at this level (Furuse et al. 2002). Moreover, in claudin-1 knockout mice that do not survive after birth due to the severe percutaneous water loss, the injected biotin crossed the level of SG, penetrating into the SC. This observation proved the presence of functional TJ in murine epidermis, capable of controlling the diffusion of molecules bigger than 557 Da.

In man, several authors have reported the presence of TJ-associated proteins in the epidermis and TJ-like structures in the SG of the fetal and, more recently, adult epidermis (Pummi et al. 2001; Brandner et al. 2002; Langbein et al. 2003; Schluter et al. 2004). These studies indicate that TJ provide a barrier against the permeability of solutes, also in human epidermis. However, doubt remains, concerning the exact role of TJ and their contribution to the barrier function, since the main permeability barrier at

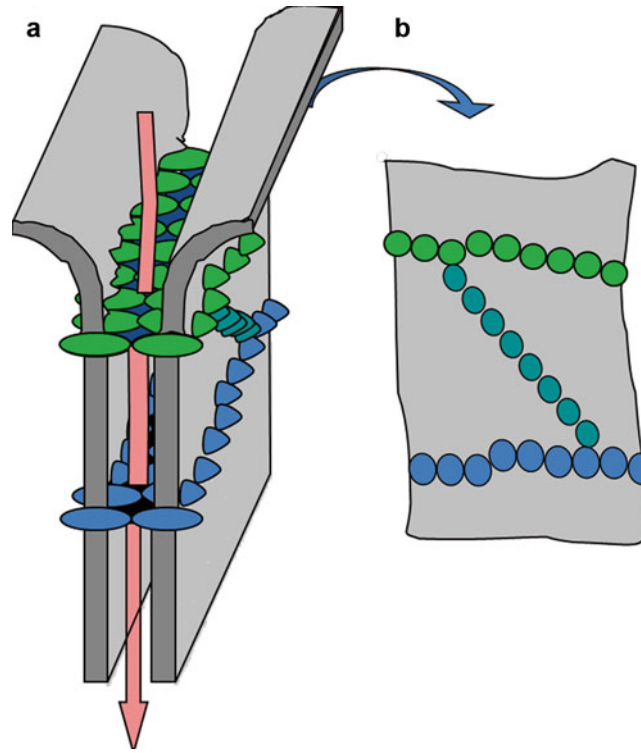

Fig. 7.3 Schematic drawing of molecule-selective "pores" engendered by tight junction (TJ) proteins in the intercellular spaces between the interacting cells. Pore-like structures are responsible for the control of paracellular diffusion of different molecules through the junctions. Claudins control the "tightness" and the selectivity of the pores within the TJ strands and, thus, TJ function relays largely on their claudin composition. A 3D representation of the arrangement of TJ proteins at the cell surface as well as the "pores" created after interaction of the extracellular domains of claudins and occludin are shown to the *left* (**a**). To the *right* (**b**) – distribution of the lined-up transmembrane proteins as it can be visualized at the cell surface with freeze-fracture

this anatomic site is apparently provided by the SC components.

One possibility is that TJ play an important role in case of rupture of the SC barrier and during the formation of this layer. In fact, TJ belts distributed around the granular layer keratinocytes could contribute to the apical extrusion of lamellar bodies. The oriented delivery of lipids to the interface between the viable epidermis and the SC is necessary for filling of the intercellular spaces with a hydrophobic material and thus for the correct barrier function (Brandner et al. 2010).

As cornified envelopes of corneocytes are impermeable to most diffusing substances, the main penetration pathway through the SC remains the intercellular one (Elias 1983). Lipids of the SC constitute the main component of the hydrophobic human skin barrier regulating water homoeostasis. Lipid composition determines the lipid organization in the SC and is therefore a key factor underlying the skin barrier function (De Jager et al. 2004). The lipid matrix is mainly composed of equimolar mixture of ceramides (CER, approx. 50 % by weight), free fatty acids (FFA; approx. 10 % by weight), and cholesterol (approx. 20 % by weight and its derivate cholesterol sulfate – approx. 5 %) (Wertz 2000; Weerheim and Ponec 2001; Feingold 2009). Triglycerides are also present in the SC barrier but, unlike viable cell membranes, the SC is almost completely deprived of phospholipids. Cholesterol plays an essential role in the maintenance of the membranes' fluidity, as it promotes intermixing of different lipid species, whereas cholesterol sulfate takes part in the modulation of desquamation because of its inhibitory action upon serine proteases. FFA consists predominantly of saturated long-chain species. Oleic acid (6 %) and linoleic acid (2 %) are the only unsaturated fatty acids detected unbound in the SC.

To date, in human SC, 12 ceramide subclasses with a wide distribution of the chain length have been identified (Masukawa et al. 2009; Van Smeden et al. 2011). All ceramides bear a polar head group and two long carbon chains: a sphingoid base and a fatty acid. They are necessary for

the formation of the covalently bound lipid envelope of corneocytes (Behne et al. 2000; Zheng et al. 2011) and play a key role in the functioning of the SC (Coderch et al. 2003). Ceramide-1 is particularly important for the intercellular lipid organization, because its long acyl chain is able to span more than one lipid bilayer and, therefore, it helps to rivet the multilayered matrix (Bouwstra et al. 2002). In general, a reduction in chain length of CERs has a stronger impact on the lamellar lipid organization and permeability than a change in the ratio between CER subclasses keeping the chain length approximately equal (De Jager et al. 2006; Groen et al. 2011; Neto et al. 2011). In atopic dermatitis patients, a reduction in overall ceramide chain length is observed and correlates with the impaired barrier function (Di Nardo et al. 1998; Janssens et al. 2012).

In normal human SC, lipids are arranged in two coexisting lamellar phases, a long periodicity phase (LPP) and a short periodicity phase (SPP) with repeat distances of around 13 and 6 nm, respectively (Madison et al. 1987; Bouwstra et al. 1991). Within the lipid lamellae, lipid head groups display an orthorhombic lateral organization (solid crystalline phase) at the skin temperature of around 30–32 °C. However, a subpopulation of lipids can also be less densely packed, showing a hexagonal pattern (gel crystalline phase) or, the most fluid, liquid crystalline phase (Bommannan et al. 1990; Ongpipattanakul et al. 1994; Pilgram et al. 2001a). Although the orthorhombic packing is predominant in the SC, the uppermost SC layers have the highest extent of the liquid crystalline phase, a consequence of the presence of sebum. As shown in *in vitro* experiments, cholesterol and ceramides are very important for the formation of the lamellar phases. After addition of FFA, the lipids are organized in an orthorhombic packing with a small proportion of lipids in a liquid crystalline phase. Deficiencies in any one of the three main lipid species result in barrier abnormalities as well as observable alterations in the ultrastructural features of the SC extracellular domains (Holleran et al. 2006). Lamellar lipid organization is considered to play an important role in the barrier function of the skin, especially the LPP (Bouwstra and Ponec 2006). Orthorhombic phase is considered as the least permeable structure, whereas the liquid crystalline phase is highly permeable to compounds (Groen et al. 2011). On the other hand, liquid phase seems to be necessary for formation of the LPP pattern (Bouwstra et al. 1999). Moreover, in dry skin, a pure liquid crystal system allows a rapid water loss through the bilayers with a moderate barrier action. The solid system could cause a water loss due to breaks in the solid crystalline phase (Fluhr et al. 2008). Maintaining the balance between the phases of different physical properties is probably required for optimal barrier function in preventing water loss (Thau 2002).

7.2 Excipients: Their Nature, Roles, and Modes of Action

According to the European Medicines Agency (EMA, earlier EMEA), excipients may be defined as the constituents of a pharmaceutical formulation which are not the active substance. Excipients include, e.g., coloring matters, antioxidants, preservatives, adjuvants, stabilizers, thickeners, emulsifiers, solubilizers, penetration enhancers, flavoring and aromatic substances, as well as constituents of the outer covering of the medicinal products.

Penetration enhancers are excipients which have the ability to modify the penetration of active substances through the skin and therefore could significantly influence the *in vivo* performance of a dermal and transdermal formulation. Development and control of action of these substances are essential for all dermal and especially transdermal formulations, where a constant and persistent release of active molecules over several hours or even days is mandatory for therapeutic efficacy.

7.2.1 Excipients' Action on the SC Lipids

Most of the skin penetration enhancers are designed to modify the intercellular SC domains in order to reduce the resistance of barrier lipid bilayers. They affect the strength of interactions

between the polar head groups, the conformational state, and/or the lateral packing of the lipids (Tfayli et al. 2012). Recent studies have also emphasized the effects of detergents on lipid synthesis, on lipid-metabolizing enzymes and, generally, on keratinocyte differentiation (Wei et al. 2006; Torma and Berne 2009). Several mechanisms of action are individualized and may intervene separately, depending on the nature of the excipient, although combined actions are most frequently encountered.

7.2.1.1 Extraction of Lipid Components

Ethanol at concentrations between 40 and 80 % extracts lipids from the skin and promotes the permeation of polar and nonpolar drugs through a hypothetical "pore" pathway (Manabe et al. 1996; Levang et al. 1999). Such lipid depletion or "degreasing" of the skin, i.e., the ability of solvents or detergents to solubilize and remove SC lipids, may also be involved in the damaging effect observed after prolonged or repetitive exposure to such substances.

7.2.1.2 Fluidization of the Intercellular Lipid Matrix

The enhanced permeation observed in the presence of ethanol was proposed to be associated with different mechanisms including the fluidization of SC lipids (Panchagnula et al. 2001). Fourier transform infrared (FTIR) experiment showed increase in SC lipid fluidity upon application of 20–60 % ethanol, which may enhance the permeation of lipophilic drugs, especially through the intercellular lipid pathway (Manabe et al. 1996).

7.2.1.3 Disorganization of the Lipid Lamellae

In normal SC, it is thought that the ratio of lipids in ordered and disordered (liquid crystalline) phases modulates the SC barrier function properties (Boncheva et al. 2008). The orthorhombic packing is the most tightly packed lipid barrier. It depends on the presence of long-chain fatty acids combined with ceramides in association with cholesterol (Bouwstra et al. 1998). Towards the skin surface, sebum lipids can induce a transition to a less tightly packed hexagonal phase (Pilgram et al. 2001b). Soaps associated with an extensive hydration, e.g., during bathing, can also promote a less organized state of the SC lipids (Rawlings et al. 1994). Moreover, some detergents induce horizontal splitting in the lipid layers leading to the loosening of the intercorneocyte spaces and thus facilitate the mobility of the extracellular enzymes implicated in the SC barrier modulation (Haftek et al. 1998) (Fig. 7.4; see also Fig. 7.1).

7.2.2 Excipients' Action on the Tight Junctions

TJ modulators interact with the extracellular loops or the membrane domain of TJ. Such modulators or excipients can belong to various families of molecules, i.e., lipids, surfactants, and proteins (Wong and Gumbiner 1997; Kondoh and Yagi 2007; Kondoh et al. 2008; Johnson et al. 2008). Wong et al. have proved that a 44-amino-acid-long peptide is able to increase the cellular turnover of occludin, one of the TJ complex proteins (Wong and Gumbiner 1997). This reversible effect results in an increase of the paracellular permeability of TJ.

Another example of a peptide capable of disintegrating the TJ structure by simple interaction with one of its proteins is the C-terminal fragment of *Clostridium perfringens* enterotoxin. This peptide interacts with claudin-4 and leads to the internalization of TJ. It could be used as a poison that provokes the complete loss of the intestinal TJ barrier (Morita et al. 1999; Sonoda et al. 1999).

7.3 Methods to Evaluate Influence of Excipients on the Epidermal Barrier

7.3.1 Methods to Follow the Structural Modifications

7.3.1.1 Microscopy Methods

Microscopy can be used to detect the influence of excipients on the extracellular lipid matrix as well as on the TJ. Light microscopy combined with immunodetection is usually employed to

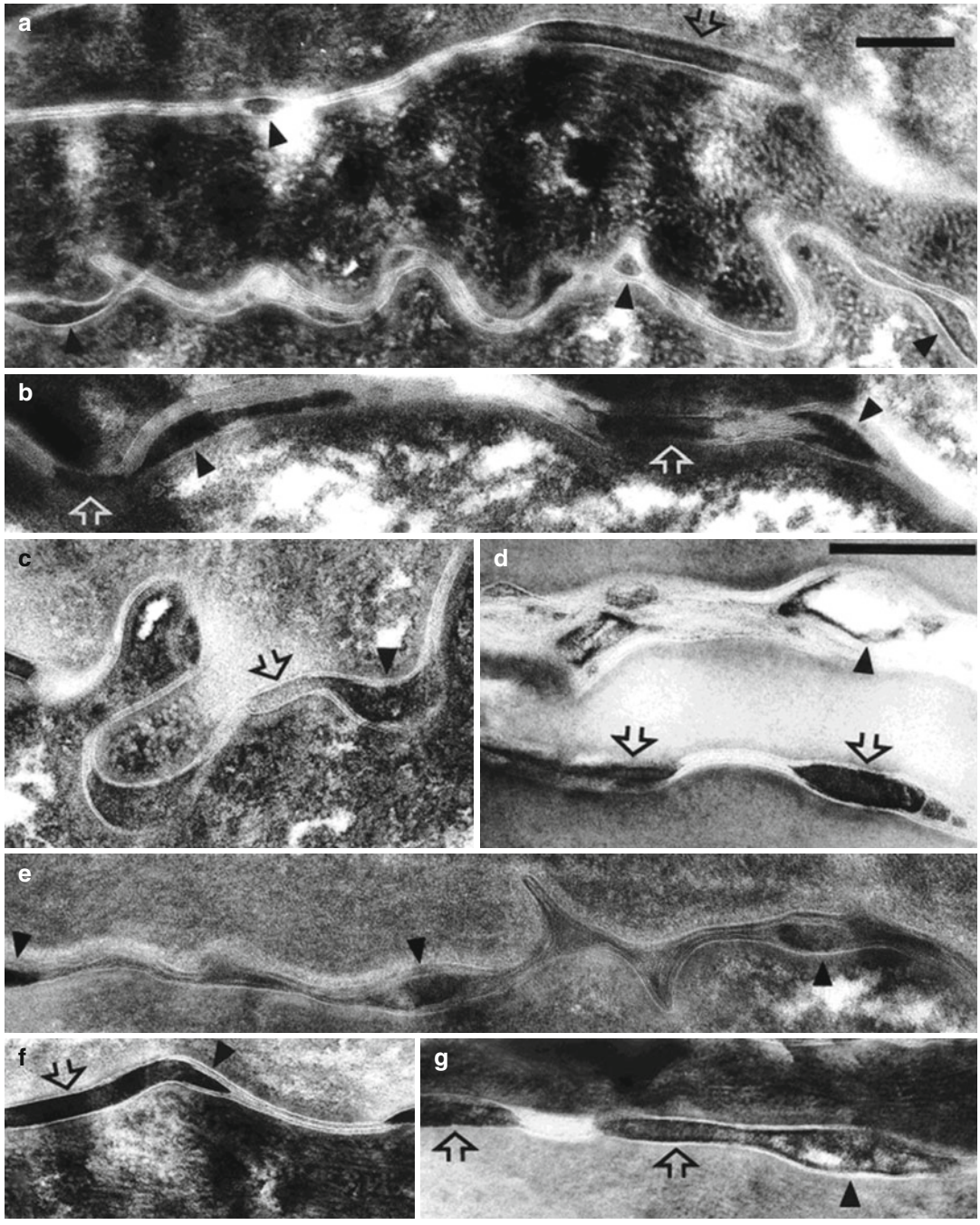

Fig. 7.4 Ultrastructure of human *stratum corneum compactum* post-fixed with ruthenium tetroxide (RuO4): (**a**) nonoccluded; (**b**, **c**) simple occlusion; (**d**–**g**) permeabilization with propylene glycol. Electron-dense lenticular dilatations (*arrowheads*) are localized between the intercellular lipid lamellae. In the occluded SC, these hydrophilic compartments are often found in contact with corneodesmosomes (*open arrows*). Propylene glycol promotes further expansion of the dark pools of intercellular substance and corneodesmosome degradation. The junctional plugs appear frequently fragmented and unevenly stained. At places where the corneodesmosome edges remain in direct contact with equally electron dense dilations, distinction between the two elements is difficult (**f**, **g**). Typical triple-band structures of the corneodesmosome cores are observable mostly with low levels of RuO4 staining, rendering lipid lamellae less visible (Reproduced with permission from Microscopy Research and Techniques, John Wiley and Sons; Haftek et al. (1998))

follow changes in expression and distribution of protein components such as TJ molecules. Immunofluorescent studies performed on frozen tissue sections allow the evaluation of "native" tissue, not fixed chemically and not exposed to solvents during the preparative methods. When combined with exploration in confocal laser scanning microscopy, where virtual optical sections are recorded within the immunolabeled tissue, this approach is very useful to evaluate TJ topography in 3D reconstructions (Brandner et al. 2002; Kubo et al. 2009). Immunostaining of dewaxed skin sections, following the standard histology protocol involving aldehyde fixation and paraffin embedding, should be interpreted with care when the SC is the compartment of interest. Indeed, solvents used during tissue dehydration and then during dewaxing efficiently remove intercorneocyte lipid matrix thus rendering impossible observation of excipient-induced changes in this localization. On the other hand, lipid removal may be beneficial for studies of TJ antigens persisting in the SC, as it improves accessibility of the latter to adequate antibodies.

Electron microscopy approach gives further insight into the structural composition of the SC barrier elements. Its "standard" transmission electron microscopy version, consisting of aldehyde fixation followed by osmium tetroxide post-fixation and resin embedding, is less suitable for studies of the SC barrier, essentially for the same reasons as the "standard" light microscopy. Although osmium tetroxide is able to partially preserve lipid bilayers from extraction during the subsequent dehydration procedures, visualization of the fine lamellar structures, within the extracellular spaces of SC, is not possible with this technique. Ruthenium tetroxide post-fixation solves this problem; however, incubation with this highly oxidizing reagent results in significant deterioration of the ultrastructure of living cells (Swartzendruber et al. 1995). Low-temperature embedding of samples in partially hydrophilic acrylate resins also preserves, to some extent, lipids from extraction by solvents used during the dehydration phase. However, it is not compatible with osmium or ruthenium tetroxide post-fixation because polymerization takes place under ultraviolet radiation which poorly penetrates tissue blocks turned black by the fixatives (Haftek et al. 1998). Acrylate-embedded samples are widely used for on-section immunolabeling with colloidal gold, including that of TJ (Haftek et al. 2011). Efficiency of ultrastructural immunolabeling can be further improved by the use of non-resin-embedded tissue ultra-cryosections (Igawa et al. 2011). Transmission electron microscopy is also used for observation of freeze-fracture samples, with or without immunolabeling (Corcuff et al. 2002). In this approach, the snap-frozen tissue is broken into pieces and the resulting fracture profile is covered with metal atoms. After removal of the underlying tissue, the metallic replica is examined with an electron microscope. Exposed fragments of the plasma membranes, where the split occurs preferentially, may show the presence of transmembrane molecules, such as TJ proteins (Kurasawa et al. 2009). The latter are arranged in a characteristic crisscrossed linear pattern and their TJ origin may be confirmed using immunogold labeling of the replicas with specific antibodies. Also, freeze-fracture studies efficiently help to investigate the lipid arrangement within the SC extracellular spaces (Van Hal et al. 1996).

Scanning electron microscopy methods appear to be less suited for SC barrier studies because of the magnification range, which is much lower than in the transmission variant, and due to accessibility limited to the surface of a sample. Nevertheless, recent technological improvements allowing examination in partial vacuum with signals obtained with secondary and retro-diffused electrons herald new applications using tape-stripped and immunolabeled SC [Haftek 2013, unpublished data].

7.3.1.2 Vibrational Spectroscopies

Vibrational spectroscopies are powerful nondestructive techniques that detect characteristic vibrational energy levels of a molecule. Infrared and Raman spectra contain detailed information about the structures and interactions of molecular classes of interest. Several studies have reported the potential of FTIR and Raman spectroscopies for the characterization of the molecular and supramolecular organization of lipids

and to follow changes in the lipid chain conformational order. More particularly, these techniques have already been used to characterize phase transitions of the SC lipids (Moore et al. 1997; Lawson et al. 1998; Ponec et al. 2000; Wartewig and Neubert 2007) of related model systems (Bouwstra et al. 2002) and to study polar and nonpolar lipid–lipid interactions (Corbe et al. 2007).

7.3.1.3 FTIR: Fourier Transform Infrared Spectroscopy

FTIR was used to obtain information on the lateral lipid organization and conformational ordering of the lipids (Janssens et al. 2012). FTIR spectroscopy can be used to investigate the biophysical alterations taking place in the lipid bilayer after treatment with penetration enhancers by studying the vibrational modes of its components. Different types of vibrations were monitored, the CH_2 symmetric stretching vibration and second derivatives of the scissoring bandwidth being the most informative for lateral organization (Boncheva et al. 2008; Damien and Boncheva 2010). For instance, the CH_2 symmetric and asymmetric stretching frequencies (υsym CH_2 and υasym CH_2) near 2,850 and 2,920 cm^{-1}, respectively, are primarily sensitive to lipid chain conformational order. A low (~2,848 cm^{-1}) wave number of the CH_2 symmetric stretching vibrations indicates the presence of a highly ordered lipid organization (either hexagonal or orthorhombic), while a high (2,853 cm^{-1}) wave number indicates the disordered liquid phase (Moore et al. 1997). However, a low average bandwidth of the scissoring vibrations represents a reduction of lipids in an orthorhombic organization and thus a less dense lipid organization (Janssens et al. 2012). Extraction of the SC lipids with solvents results in reduction of the CH_2 stretching absorbance. Indeed, the decrease in CH_2 stretching bandwidths accompanied by a decrease in CH_2 stretching band intensity suggests an overall extraction of SC lipids (Levang et al. 1999). The CH_2 rocking frequencies (710–735 cm^{-1}) are much more sensitive than the changes in CH_2 symmetric frequencies to the aforementioned hexagonal–orthorhombic phase transition. At temperatures below 40 °C, there are two distinct bands at approximately 720 and 729 cm^{-1}. The latter is a reliable marker of an orthorhombic phase in the SC lipids (Pensack et al. 2006).

7.3.1.4 Differential Scanning Calorimetry (DSC)

DSC is a thermoanalytical technique measuring heat capacity of a sample. It allows thermal analysis of an isolated SC and is used to evaluate the interaction of excipients with the skin.

Alterations of the characteristic temperatures of the endothermic peaks can be observed, relative to disorganization of the lamellar structure of lipids and to dehydration or denaturation of the proteins. DSC is less sensitive to phase transitions, presumably because of relatively low enthalpy changes resulting from the transition, whereas FTIR is able to detect them with relative ease.

7.3.1.5 Raman Spectroscopy

Raman spectroscopy reveals the spectral features specific to the modes of action of the penetration enhancers. These features can be simultaneously used as direct vibrational descriptors of the supramolecular organization, the polar interactions, and the conformational order of the CERs and as a descriptor of the penetration enhancer activity in drug delivery. Raman spectroscopy can be used to monitor not only the evolution of the lateral packing and the polar interactions due to penetration enhancer activity but also the intrachain conformational order and the chain-end conformers, such as resulting from the thermotropic behavior of ceramides (Tfayli et al. 2010). Such information cannot be obtained with FTIR spectroscopy.

7.3.1.6 X-ray Diffraction Studies

In this method, a primary X-ray beam, emitted by a source, is partially scattered by the structures present in a studied sample. When applied to SC, the obtained diffraction patterns give information about organization of the intercellular lipids. Position and intensity of the observed peaks are measured and interpreted, in order to evaluate the action of excipient on lipid organization (Bouwstra and Ponec 2006).

- Small-Angle X-ray Diffraction (SAXD)
  Diffraction pattern measured at low angle, typically between 0° and 5°, provides information about the larger structural units in the sample, such as the repeat distance of a lamellar phase (Janssens et al. 2012).
  At room temperature, two lamellar phases are present in human SC. One shows a periodicity of approximately 6.4 nm and the other of approximately 13.4 nm (Bouwstra et al. 1991). Since the 13 nm lamellar phase has always been present in all species studied so far, this phase is considered to be important for the skin barrier function.
- Wide-Angle X-ray Diffraction (WAXD)
  At wider angle, scattered X-rays contain information about smaller structural units in the sample, such as the lateral packing of molecules in a lamellar phase. In the liquid crystalline packing, the intermolecular distance is not very well defined. This results in a WAXD pattern that is characterized by a very broad peak at 0.46 nm. The diffraction pattern of the two-dimensional hexagonal lattice is characterized by a strong reflection at 0.41 nm. In the case of the orthorhombic organization, two strong reflections can be detected at a spacing of 0.41 and 0.37 nm. However, whether a hexagonal sublattice coexists with an orthorhombic one cannot be deduced from the diffraction pattern, since the 0.41 nm reflection, characteristic for the hexagonal lateral packing, is obscured by the 0.41 nm reflection attributed to the orthorhombic phase (Bouwstra and Ponec 2006).

7.3.2 Methods to Follow Biochemical Modifications

The presence of proteins of the TJ complex or enzymes implicated in the synthesis/degradation of lipids can be studied in tissue extracts. Similarly, quantitation of the corresponding messenger ribonucleic acid (mRNA) is also possible. As initial tissue localization of the obtained profiles is essential, these studies require precise sampling based on the structural data.

7.3.2.1 Molecular Biology

Molecular biology approach is required for the comprehension of viable cell responses. As such, it concerns exclusively studies of the living epidermal layers and not the SC. However, study of RNA gives an idea on the transcription rate of a specific gene and this, in turn, indicates possible repercussions on the subsequent SC formation and function. For example, quantification of mRNA coding for TJ proteins is possible using real-time polymerase chain reaction (RT PCR). Using this technique, one can monitor the presence of mRNA of various TJ proteins in keratinocyte cultures with different knockdown genes while testing permeability of the expressed TJ (Kirschner et al. 2013). Already in 2008, Yamamoto et al. have performed a posttranscriptional gene silencing of different TJ proteins to study the impact of these proteins on the formation of the SC barrier (Yamamoto et al. 2008). Keratinocytes were transfected with small interferent RNA (si-RNA) targeting either claudin-1 mRNA or occludin mRNA. It turns out that transfected cultures were not able to develop a functional barrier. Using molecular biology techniques, it would be possible to detect variations of the keratinocyte gene activity that excipients may engender when applied on the skin. Hybridization *in situ*, on tissue sections, is an approach combining structural information with mRNA detection. It is, thus, particularly well suited for studies of locally induced changes following topical treatments.

7.3.2.2 Biochemistry

Biochemistry assays are commonly used to analyze proteins as well as lipids extracted from a tissue.

Immunoblotting technique can detect the presence of a given protein. In fact, the presence of the mRNA in the cell cytoplasm is insufficient to predict the presence of the corresponding protein due to the complexity of the translation machinery. Western blot is a reliable qualitative and quantitative technique applicable to TJ protein studies (Kurasawa et al. 2009; Kirschner et al. 2013).

Lipid analysis may be performed on SC extracts using high performance thin layer

chromatography (HPTLC) (Rissmann et al. 2008; Popa et al. 2012). Combined with sequential tape-stripping, HPTLC approach allows following lipid composition of intercellular spaces between corneocytes in the SC according to the sample's depth (Popa et al. 2011).

7.3.3 Methods to Assess the Permeability Barrier Function

7.3.3.1 Transepidermal Water Loss (TEWL) Measurement

One of the key parameters to monitor the skin barrier function is the transepidermal water loss, i.e., the rate of water evaporation through the epidermis, independent of sweating. TEWL provides a noninvasive method for assessing changes in the barrier properties of the SC. It can be considered as a determinant indicative of the functional state of the epidermal barrier in normal, pathological, and experimental conditions (Janssens et al. 2012). Measurements are performed in standardized ambient conditions according to the well-defined consensus guidelines (Pinnagoda et al. 1990).

7.3.3.2 Diffusion Cells: *Ex Vivo* Permeation Studies

Diffusion cell is helpful to study the influence of excipients on drug permeation and penetration. Indirectly, information is gained on the functional state of the treated skin barrier. Skin permeation is evaluated by measuring the steady-state permeation flux of the tested drug. An enhancement ratio can be determined in the presence of the studied excipient. The diffusion cell consists of the upper, i.e., "donor," and the lower, i.e., "receptor," chambers, separated by a tested skin. The epidermis faces the donor chamber where the tested products are applied, and the dermis faces the receptor chamber filled with a receptor fluid, where the drug or another tracer will be measured after permeation. Temperature is controlled throughout the experiment and should be maintained at *in vivo* skin conditions (32 °C). Ensuring that the skin used for testing maintains its physical integrity is an essential factor to the successful performance of permeation experiments, as specified in the test guideline OECD 428 (Lévêque et al. 1993). The guidance document recommends the measurement of TEWL, transepithelial electrical resistance, or the use of tritiated water as permeation markers.

7.3.3.3 Transepithelial Electrical Resistance (TEER)

TEER measurement can be used to detect the TJ functionality status. Usually the functionality of TJ is evaluated *in vitro* on monolayer of cultured cells or on reconstructed human epidermis. Functional TJ significantly increase the tissue impedance towards a very weak electrical current generated by ohmmeters, by virtue of limiting ion flux through the intercellular space (Kurasawa et al. 2009; Abdayem et al. 2011). In normal human skin, SC by itself is able to prevent the passage of the testing current. Therefore, to detect any variation of resistance due to TJ modulation, the SC has to be partially or completely removed. This is usually done by tape stripping. In the case of *in vitro* reconstructed epidermis, the extracellular lipid matrix is not perfectly organized (Ponec et al. 2000) and the SC is fully hydrated. This results in readable TEER measurements, as the electrical current is able to cross this imperfect SC barrier (Abdayem et al. 2011). For the detection of the effects of different excipients on the TJ barrier, reconstructed epidermis is cultured in plastic inserts equipped with a permeable polycarbonate bottom. This experimental setting allows positioning of the ohmmeter electrodes on both sides of the cultured tissue. Tested excipients can be added at different doses either topically, i.e., directly on the SC surface, or "systemically," into the feeding medium penetrating the reconstructed epidermis from the bottom compartment. In case of "systemic" treatment, excipients are in direct interaction with the epidermal TJ and the SC remains intact. Topically applied agents have first to cross the SC barrier, before acting on TJ situated in the granular layer. Thus, TEER readings in the latter case reflect both TJ and SC permeability modulation.

7.3.3.4 Dye Penetration Test

Intact SC and functional epidermal TJ are able to block or to limit the passage of various molecules depending on their size and nature, e.g., lipophilicity, pH, and charge. Following the passage of a tracer applied at the surface of the skin or injected at the basal layers of the epidermis is another way to study the functional quality of the complementary SC and TJ barriers. For example, in the case of topical application of Lucifer yellow, detection of this hydrophilic fluorescent dye, with a confocal microscope, in the living layers of epidermis, may indicate the presence of poor SC barrier, either constitutive or induced (Kurasawa et al. 2009). Another widely used small molecular marker is biotin (433–666 Da) (Ding et al. 2011). After being injected to the dermis, the tracer diffuses through the intercellular spaces until it reaches the TJ barrier in the *stratum granulosum*. Immunohistochemical detection of the biotin on vertical skin sections reveals the sites where the diffusion has been stopped. These points are recognized as TJ because of their co-localization with anti-occludin staining (Furuse et al. 2002). Tracer penetration studies can also be performed at the ultrastructural level using electron-dense lanthanum salts. Ken Hashimoto was the first to describe TJ structures in human epidermis using, precisely, this approach (Hashimoto 1971).

7.4 Known Effects of Excipients on the Epidermal Barrier Structure and Function

Excipients influencing skin barrier demonstrate properties of penetration enhancers and are employed accordingly. The modes of action of skin penetration enhancers involve, in general, either the disruption of skin barrier properties or the increase of drug partitioning into the various layers of the skin, starting with the SC.

7.4.1 Propylene Glycol (PG)

PG is a small organic and hydrophilic molecule widely used as solvent and excipient. It is considered nontoxic and shows low relative irritancy *in vivo* (Lashmar et al. 1989; Barry 1991; Haftek et al. 1996). Haftek et al. (1998) have evaluated the ultrastructural spatial organization of the intercorneocyte spaces before and after application of PG. When applied *ex vivo* undiluted on the skin, PG was capable of inducing a pronounced loosening of the horny layer, observable in light and electron microscopy as the "basket-weave pattern." At the ultrastructural level, the SC dissociation could be linked to the expansion of water-containing domains within the intercorneocyte spaces associated with disorganization of the lamellar lipids and corneodesmosome degradation (Fig. 7.4). Thus, incorporation of PG into lipid layers does functionally change the properties of the intercorneocyte space and may increase mobility of the extracellular proteolytic enzymes (Haftek et al. 1996). This, in turn, promotes digestion of the intercorneocyte junctions and leads to desquamation. Interestingly, the PG-induced horizontal splitting occurring between the successive layers of corneocytes is not extended to the lateral cell–cell attachments (Fig. 7.1). Recent investigations on the TJ structures persisting in a cross-linked form between the SC corneocytes shed light on this curious phenomenon (Haftek et al. 2011). In fact, TJ-like structures located at the top of and within the lateral intercorneocyte contacts efficiently limit accessibility of the intercellular enzymes to the cell periphery and the corneodesmosomes located within it.

PG is also frequently employed to enhance drug release, from the dosage form (Panchagnula et al. 2001).

7.4.2 Fatty Acids: Oleic Acid (OA)

Percutaneous drug absorption is increased by a wide variety of long-chain fatty acids. Among the most frequently used is OA, as it moderately increases both drug diffusivity and drug partitioning parameters (Koyama et al. 1994). This long-chain monounsaturated fatty acid in cis configuration modifies the lipid domains of the SC. This effect appears to be due to lipid layers

fluidization and, predominantly, to phase separation (Naik et al. 1995). Indeed, after *in vivo* application, OA has been found to exist in a liquid phase at all levels of the spectroscopically examined SC. OA can be applied on the skin in mixture with ethanol and easily combined with other penetration enhancers, like propylene glycol or terpenes (Yamane et al. 1995; Larrucea et al. 2001).

7.4.3 Terpenes

Terpenes are organic compounds found in essential oils. They are used as co-enhancers of percutaneous penetration, and, thus, their influence is measured as the difference when compared to the results obtained with control mixtures that do not contain them. Koyama et al. evaluated the percutaneous absorption-enhancing effects of d-limonene in oleic acid using three drug models with different lipophilicities (Koyama et al. 1994). Pretreatment of the skin with limonene resulted in a large penetration enhancement for lipophilic and amphiphilic molecules but had little effect on the hydrophilic one. D-limonene increased mainly drug diffusivity in the nonpolar penetration route. Moreover, carvone, 1,8-cineole, and thymol were shown to enhance the percutaneous absorption of hydrophilic 5-fluorouracil in 50 % ethanol by either increasing the SC lipid fluidity, as revealed with FTIR, or perturbing the barrier integrity of the epidermis, as demonstrated with TEWL (Gao and Singh 1997).

Limonene, eugenol, and menthone were used in concentration of 5 % to investigate their input in percutaneous absorption of a moderately large molecule (tamoxifen) when applied in 50 % propylene glycol. As addition of any of these terpenes resulted in a significantly increased transcutaneous penetration, two mechanisms of action were suggested: lipid extraction deduced from FTIR spectra (Zhao and Singh 2000) and conformation changes of the lipid chains visualized with Raman spectroscopy (Tfayli et al. 2012). SAXD studies have also indicated that d-limonene and 1,8-cineole disrupt SC lipid bilayers. Similar conclusions were reached using FTIR and DSC for evaluation of the action of alcoholic terpenes, carvacrol, linalool, and alpha-Terpineol (Vaddi et al. 2002).

7.4.4 Azone®

Azone® (1-dodecylazacycloheptan-2-one or laurocapram) was the first molecule specifically designed as a skin penetration enhancer. Most probably, the bilayer arrangement of lipids is disrupted by the presence of this enhancer, capable of extracting cholesterol, as suggested by calorimetry results (Kang et al. 2006). However, its integration into the lipids is unlikely to be homogeneous, considering the variety of compositional and packing domains within SC lipid bilayers. Using electron microscopy, a "soup spoon" model for Azone®'s conformation within the SC lipids was observed. This model is compatible with a mechanism of action relaying on the creation of Azone® domains (Hoogstraate et al. 1991). Electron diffraction studies using lipids isolated from human SC provide good evidence that Azone® exists (or partially exists) in form of distinct phases within the SC lipids (Pilgram et al. 2001b).

7.4.5 Ethanol

Ethanol is commonly used as a "permeability enhancer" for transdermal drug delivery, as it is well known for its ability to increase skin penetration of drugs (Kwak et al. 2012). Ethanol-induced permeation of the SC depends on the alcohol content in the binary ethanol/H_2O system. It was found that for several different chemicals, the optimal range of ethanol concentration was between 40 and 80 % (v/v), while smaller and larger proportions of ethanol in water showed more limited penetration enhancing effect (Kuriharabergstrom et al. 1990; Chen et al. 1995; Panchagnula et al. 2001). The enhanced permeation observed in the presence of ethanol in diffusion cell studies was proposed to be associated with various mechanisms. The presence of alcohol increases the rotational

freedom of lipid acyl chains leading to an increase in fluidity of the intercellular lipid matrix (Panchagnula et al. 2001). Such a fluidization effect of lipid bilayers could be observed experimentally using FTIR (Chin and Goldstein 1977). Extraction of epidermal lipid components by this solvent may also lead to SC delipidization (Levang et al. 1999; Manabe et al. 1996; Kuriharabergstrom et al. 1990; Krishnaiah et al. 2004; Obata et al. 2006).

7.4.6 Dimethyl Sulfoxide (DMSO)

DMSO is one of the earliest but actually unused penetration enhancer. It exhibits a concentration-dependent effect (Williams and Barry 2012). Several theories have been proposed to explain its mechanism of action including extraction of skin lipids (Embery and Dugard 1971) and interaction with the head groups of lipid bilayers resulting in a change in the lateral packing and reduction of the compactness of lipid bilayers (Elfbaum and Laden 1968). In fact, DMSO affects interactions between the polar groups of lipid molecules mainly by reducing the strength of H-bonds (Tfayli et al. 2012).

7.4.7 Surfactants: Detergents

Surfactants are one of the chief ingredients in personal care products. However, skin barrier damage is observed after extensive exposure to detergents (Branco et al. 2005). Varying but overall small amounts of SC lipid are extracted by surfactants (Lévêque et al. 1993; Gloor et al. 2004). Nevertheless, disruption of lipid structural organization, observed using various physical techniques, such as X-ray diffraction, transmission electron microscopy, and DSC, has been demonstrated upon exposure of the SC to surfactants and was accompanied by an increase in TEWL (Ribaud et al. 1994; Warner et al. 1999; Jiang et al. 2003). Recent studies, using RT-PCR, have also emphasized the effects of detergents on lipid-metabolizing enzymes and on keratinocyte differentiation (Wei et al. 2006; Torma and Berne 2009). Moreover, *in vivo* studies combined with HPTLC analysis suggest surfactant-induced alterations in the SC lipid synthesis (Gloor et al. 2004; Heinemann et al. 2005).

Sodium lauryl sulfate (*SLS*) *or sodium dodecyl sulfate* (*SDS*) is one of the most used surfactants. SLS is likely to remove epidermal lipids through the solubilizing action. Froebe et al. (1990) examined this mechanism and demonstrated that ceramides were not substantially extracted by SLS. The total lipid removed after exposing SC to a 2 % solution of SLS was less than 4 % of the total lipid content in the SC. This result (and additional studies) has led to the assumption that there may be other mechanisms besides lipid depletion. Indeed, SLS treatment attenuates the peak of SPP at 6.5 nm in the X-ray diffraction pattern, thus indicating disruption of the intercellular lipid organization (Ribaud et al. 1994). Moreover, Saad et al. showed, by FTIR study, a loss of the orthorhombically packed lipids paralleled by an increase in lipids in the hexagonal phase (Saad et al. 2012). Other experiments on isolated SC examined with electron microscopy showed the disorganization of the intercellular lipid layers induced by SDS (Jiang et al. 2003). Lipid lamellae structures were obliterated and amorphous and/or flocculent material appeared in the dilated intercellular spaces. Interestingly, the damage to the lipid lamellar structure induced by exposure to water was similar to that induced by surfactants. In fact, hyperhydration of SC may lead to disorganization of the lipid lamellae as it increases the volume of the non-lipid fraction in intercellular spaces (Haftek et al. 1998).

Sodium caprate (*C10*) *and surfactants containing polyethylene glycol* (*PEG*) are excipients known as enhancers of the intestinal absorption by regulating jejunal TJ.

Kurasawa et al. (2009) have examined localization and expression of claudin-1 and of occludin in keratinocyte cultures after application of 10 mM sodium caprate (C10). Using immu-

nofluorescence microscopy, they have found that in intact cultures, both TJ proteins were present at the cell–cell contact regions, suggesting the occurrence of TJ. Sodium caprate application has resulted in the internalization of the proteins. This effect was reversible after discontinuation of the treatment. Examination of the cultures with freeze-fracture technique has confirmed the disappearance of TJ strands from the cell surface upon exposure to C10 and their reexpression after cessation of the treatment (Kurasawa et al. 2009).

Abdayem et al. (2011 and in preparation) have used *in vitro* reconstructed human epidermis and normal human skin *ex vivo* as examples of fully keratinized tissues and compared the effects of various excipients on the ultrastructure of epidermis and the functionality of epidermal TJ. The results indicated that after 24 h of topical application of 10 mM C10 and of PEG-8 caprylic/capric glycerides (Labrasol®, Gattefossé, France), the SC and the living layers of the reconstructed epidermis were altered. However, morphological modifications were clearly more pronounced after C10 application, showing extensive disorganization of the horny layer and vacuolization of the nucleated cells at all epidermal layers. Topical application of these excipients on intact human skin resulted in no remarkable modification of the tissue histology and only mild ultrastructural modifications could be detected in the SC.

When assayed with TEER measurements, the reconstructed epidermis exposed to either of the excipients demonstrated time- and dose-dependent loss in electrical resistance indicating permeabilization of the barrier. Such an effect was not observed *ex vivo*, suggesting that naturally formed SC barrier was much more resistant to the treatments than that of the epidermal equivalent. The effect of both excipients on TJ was verified by TEER measurement after exposure to the excipients added to the feeding medium of the reconstructed epidermis. Such a "systemic" treatment resulted in dose- and time-dependent response revealed with TEER. The response to 1 mM C10 was very rapid in every case. Treatment with Labrasol®, instead, led to the same final results in terms of the TEER loss, but the TJ permeation was more progressive and less destructive morphologically. Thus, although both C10 and Labrasol® are able to directly modulate epidermal TJ, the latter excipient appears to be better suited for *in vivo* applications aimed at percutaneous penetration enhancement.

7.4.8 Barrier-Restoring Molecules

7.4.8.1 Glycerin/Glycerol

Glycerin/glycerol, as a moisturizer, has been suggested to prevent crystallization of the skin lipids at low relative humidity (Froebe et al. 1990; Mattai et al. 1993). It has been hypothesized that glycerol can interact with polar head groups of the lipid bilayers rather than by penetrating the alkyl chains. Glycerol exists in the SC as a natural endogenous humectant and largely influences skin hydration, cutaneous elasticity, and epidermal barrier repair (Verdier-Sevrain and Bonté 2007; Fluhr et al. 2008). Upon topical application at low concentrations (1–10 %) in water, glycerol is able to restore water holding capacity of the skin barrier experimentally disrupted with the detergent (SLS). Although this effect is not immediately followed by the barrier repair, as measured with TEWL, it may constitute an important step favoring physiological process to reestablish water barrier function of the impaired skin (Atrux-Tallau et al. 2010).

Some moisturizing agents, including glycerol, also promote desquamation (Rawlings et al. 1995), probably by additionally increasing the volume of the non-lipid, hydrophilic fraction of the SC intercellular compartment and, thus, improving local conditions for the activity of endogenous catabolic enzymes. SC hyperhydration may be easily achieved through skin occlusion; however, hydrophilic moisturizers increase water levels in the SC without the formation of a water-impenetrable layer on the surface of

the SC (Lodén 2003). An interesting observation is that nonocclusive lipophilic moisturizers penetrate into the SC and, by changing its lipid organization to LPP and orthorhombic, actually increase the barrier function (Caussin et al. 2007). Generally speaking, moisturizers may have favorable or deleterious influence on the SC barrier function, depending on the treated skin condition and the nature of the employed agent. One of the obvious clinical situations is that of atopic dermatitis. Moisturizers with barrier-improving properties may delay relapse of the disease but treatment with moisturizing creams could also enhance transcutaneous penetration of potential allergens and thus increase the risks of dermatitis and asthma (Buraczewska et al. 2007; Lodén 2012).

7.4.8.2 Urea

Urea, a small polar molecule, is frequently used in dermatologic formulations as a "moisturizer." It does not seem to significantly affect the lipid matrix of the SC. In fact, it is able to replace water while keeping the physical properties unchanged. Urea was shown to protect living cells against osmotic stress by retaining the liquid crystalline phase of the lipid membrane even at low humidity, down to 64 % (Costa-Balogh et al. 2006). Consequently, maintaining the necessary degree of fluidity of the extracellular lipids may be the mechanism by which urea improves skin conditions in dry climates.

7.4.8.3 Petrolatum

Petrolatum also can help to restore the SC barrier by penetrating into its upper layers. It remains the gold standard for barrier repair ingredients because it is the nonphysiologic substance closest to the natural intercellular lipids and it can intercalate into the intercellular spaces. It does not penetrate to the living layers and, contrary to the physiologic lipids, i.e., ceramides, free fatty acids, and cholesterol, petrolatum is unable to stimulate the endogenous production of the SC intercellular matrix (Grubauer et al. 1987; Ghadially et al. 1992; Mao-Qiang et al. 1995).

7.4.8.4 Physiologic Lipid Mixtures (Mixtures of Ceramides, Fatty Acids, and Cholesterol)

Physiologic lipid mixtures (mixtures of ceramides, fatty acids, and cholesterol) have been observed to penetrate into the skin deeper than SC and to improve the skin barrier function (Mao-Qiang et al. 1995, 1996; Lodén and Bárány 2000; Chamlin et al. 2002; Na et al. 2010; Popa et al. 2012). The immediate effect of physiologic lipid mixtures is attributable to unspecific replenishment of the SC intercellular spaces, but, contrary to petrolatum, physiologic lipids in adequate proportions may be assimilated by keratinocytes from the living layers and used for the barrier repair in the longer term. Accordingly, mixtures of ceramides, fatty acids, and cholesterol, in equimolar proportions, allowed normal barrier recovery in acetone-treated murine skin, whereas two-component mixtures, e.g., fatty acids plus ceramides, cholesterol plus fatty acids, or cholesterol plus ceramides, delayed barrier recovery (Mao-Qiang et al. 1993, 1995). Cholesterol, applied as the dominant lipid in physiologic mixtures, accelerated barrier recovery in aged human skin, presumably because of a reduction in endogenous cholesterol synthesis underlying chronological skin aging (Zettersten et al. 1997). No acceleration of the barrier recovery in SLS-damaged human skin was detected after treatment with ceramide 3 alone in different emulsions (De Paepe et al. 2000). Neither did a "moisturizer" consisting of ceramide 3, cholesterol, and fatty acids (so-called skin-identical lipids) in a petrolatum-rich emulsion show superiority to pure petrolatum in human skin damaged by SLS and tape-stripping (Lodén and Bárány 2000). The absorption of ceramides and the superiority of some lipid mixtures to other lipids thus remain to be proven in randomized and controlled studies on humans, and a good guess is that these formulations should be tailored with respect to the epidermal abnormality to be treated (De Boer and Hillier 2001; Lodén 2003; Popa et al. 2012).

Conclusion

Excipients play an important role in the formulation of dermatological products. They may enhance transdermal penetration of molecules of interest through modulation of the composition, organization, and function of the principal skin barrier elements, i.e., the SC intercellular lipids and epidermal TJ. On the other hand, several excipients, useful in skin protection and restoration of the damaged barrier function, are also available. Their appropriate use is at the heart of the galenic art applied to the skin.

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Part II

Penetration Enhancement Techniques in Skin Delivery

8 Targets in Dermal and Transdermal Delivery and Classification of Penetration Enhancement Methods

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Contents

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8.1 Introduction

In the past three decades, the skin has gradually become recognized as an important drug delivery route. Being the most accessible organ in the body, the skin can be reached directly and so drug delivery to this tissue is assumed to be relatively easy. There is considerable interest in the skin as a site of drug application for both local (topical) and systemic (transdermal) effect, the first used in the treatment of different skin diseases and the latter as an alternative route for systemic drug administration. Advantages offered by this kind of drug delivery are numerous compared to other conventional routes (Parikh et al. 1984; Guy et al. 1987; Schreier and Bouwstra 1994; Paudel et al. 2010):

1. Transdermal drug delivery systems (TDDS) avoid hepatic first pass which allows for lower doses of drugs to be administered and that means these methods are safer for patients with liver diseases.
2. TDDS avoid the gastrointestinal tract and so bypass problems like drastic pH changes, the deleterious presence of food enzymes, variable transit times and rapidly fluctuating drug plasma concentrations.
3. TDDS are an acceptable, pain-free, non-invasive form of self-administration for patients which ensures easy patient compliance and quick ending of the therapy if necessary.
4. TDDS act as a "depot" controlling the rate of drug input over a prolonged period of time

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and ensuring constant plasma levels even for drugs with short half-times.

In the case of drugs with a narrow therapeutic margin, when applied onto the skin in TDDS, their undesirable side effects are reduced, particularly the effects associated with pulsed peak plasma levels. Furthermore, the dose interval can be reduced.

5. Topical drug delivery systems allow the drug to be directly applied to the skin and delivered to the site of disease in the skin.
6. Topical drug delivery systems like TDDS are non-invasive, avoid hepatic first pass as well as the gastrointestinal tract and problems associated with this drug application route, increase patient compliance and can be self-administered.
7. Specially designed topical delivery systems (like liposomes) may also form drug "depots" in the skin with sustained drug release.

The problems that topical/transdermal drug delivery systems encounter are the low permeability of the stratum corneum which limits the number of drugs available as transdermal products and the potential interaction of drugs with the skin causing irritation and sensitization.

8.2 Therapeutic Target Sites in Topical and Transdermal Drug Delivery

During topical and transdermal drug delivery, drugs are applied to the skin after which they should follow a route to one of the following target sites (Fig. 8.1): (1) the local tissues immediately beneath the application site, (2) deep regions in the vicinity of (but still somewhat remote from) the application site and (3) the systemic circulation (Flynn and Weiner 1991). Therefore, it is important to develop an adequate formulation which delivers the drug to the desired target in the skin or below the skin, i.e. to differ between topical, regional and transdermal drug delivery, since each application has its specific requirements.

Topical delivery can be defined as the application of a drug-containing formulation to the skin to directly treat cutaneous disorders or the cutaneous manifestations of a general disease. Topically delivered drugs should have their pharmacological or other effects confined to the surface of the skin or within the skin (Flynn and Weiner 1991). Formulations designed to target the skin surface include sunscreens, barrier products, cosmetics and insect repellents (Benson and Watkinson 2012). In addition to these, topical formulations can target appendages (hair follicles and sweat pores) and include antiacne products, antiperspirants, hair growth promoters and anti-infectives.

Regional delivery involves the application of a drug to the skin in order to treat diseases or alleviate disease symptoms in tissues that lie deeper, beneath the application site. Pharmacological targets of this type of drug delivery are within the musculature, vasculature, joints and tissues beneath and around the site of application. When targeting regional sites, drug formulations aim to have a regionally selective effect. Regional drug concentrations upon this route of drug administration are higher than the ones achieved by systemic administration (Flynn and Weiner 1991). For both topical and regional drug delivery, systemic absorption is unwanted but unavoidable.

In transdermal delivery drugs are applied to the skin with the aim of reaching the systemic circulation. The purpose of this type of drug delivery is to achieve a therapeutically relevant drug level in order to treat a systemic disease. Hence, the percutaneous absorption of the drug is essential, while the local deposition of the drug is unwanted, but unavoidable (Flynn and Weiner 1991). The use of transdermal delivery is limited to only a small pool of drugs (see Table 8.1) due to the selective barrier properties of the skin. The small number of candidates for this delivery route is a result of the fact that only a few drug molecules have skin permeability coefficients sufficiently high to achieve clinically active plasma levels. Currently, the market for transdermal patches comprises patches with a few low molecular weight drugs: scopolamine for motion sickness, clonidine and nitroglycerin for cardiovascular disease, fentanyl for chronic pain, nicotine to aid smoking cessation, oestradiol (alone or in combination with levonorgestrel or norethisterone) for hormone replacement and testosterone for hypogonadism (Benson 2005).

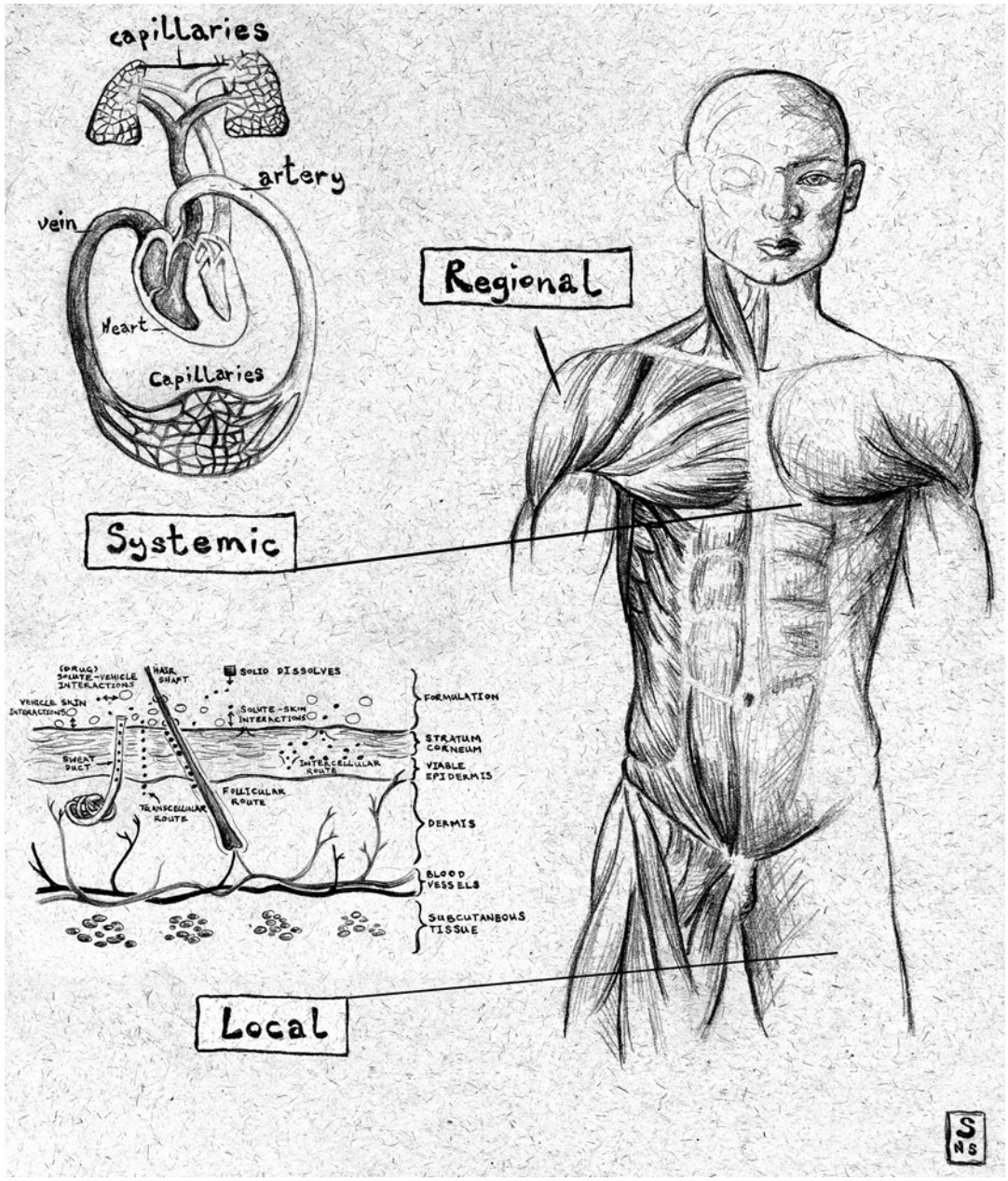

Fig. 8.1 Targets in dermal and transdermal drug delivery

Since percutaneous absorption is pivotal to the effectiveness of both topical and transdermal systems, significant efforts have been devoted to developing strategies to overcome the impermeability of the intact human skin. There are many ways for circumventing the stratum corneum, which provides the main barrier for drug penetration (Barry 2001).

8.3 The Skin

The skin is the largest organ in humans covering approximately 2 m^2 in an average-sized adult. Its main role is to prevent water loss and protect our body from undesired outside influences. This implies that the skin acts as a barrier for diffusion of substances into the

Table 8.1 List of marketed transdermal products

Generic drug	Indication	Product	Manufacturer
1. Scopolamine	Motion sickness	Transderm Scop®	Novartis
2. Nitroglycerin	Angina pectoris	Minitran®, Nitrol®, Transderm-Nitro®, Nitro-Dur®	3 M, Rorer, Novartis, Key Pharms
3. Clonidine	Hypertension	Catapres-TTS®	Boehringer Ingelheim
4. Estradiol	Postmenopausal related symptoms	Estraderm®, Climara®	Novartis, Bayer HealthCare
5. Nicotine	Smoking cessation	Nicoderm CQ®, Habitrol®	Sanofi-Aventis, Novartis
6. Testosteron	Hypogonadism	Androderm®, Testoderm®	Watson Labs, Alza
7. Fentanyl	Analgesia	Duragesic®	Janssen Pharmaceuticals
8. Estradiol and levonorgestrel	Postmenopausal related symptoms	Climara Pro™	Bayer Healthcare
9. Estradiol and norethindrone	Postmenopausal related symptoms	Combipatch®	Novartis
10. Ethinyl estradiol and norelgestromin	Contraception	Ortho Evra®	Janssen Pharmaceuticals
11. Buprenorphine	Analgesia	Bu Trans®	Purdue Pharma L.P.
12. Rivastigmine	Dementia associated with Alzheimer's and Parkinson's disease	Exelon®	Novartis
13. Oxybutynin	Overactive bladder	Oxytrol®, Kentera®	Watson Labs, Orion Pharma
14. Oxybutynin chloride	Overactive bladder	Gelnique®	Watson Labs,
15. Rotigotine	Parkinson's disease	Neupro®	UCB Inc
16. Granisetron	Nausea, vomiting	Sancuso®	ProStrakan Inc
17. Methylphenidate	*Attention deficithyperactivity disorder*	Daytrana	Noven Pharms Inc
18. Selegiline	Depression	Emsam®	Somerset
19. Lidocaine	Postherpetic neuralgia pain relief	Lidoderm®	Teikoku Phar
20. Lidocaine and tetracaine	Local dermal analgesia	Synera®	Zars Pharma
21. Capsaicin	Postherpetic neuralgia pain relief	Qutenza®	NeurogesX
22. Diclofenac epolamine	Topical pain relief	Flector®	Inst Biochem
23. Diclofenac sodium	Topical pain relief in osteoarthritis	Voltaren®	Novartis

underlying tissue (Schaefer 1996; Bouwstra et al. 2003). The main problem in the dermal/transdermal administration of drugs is overcoming this natural barrier (Barry 2001; Bouwstra et al. 2003).

The skin is composed of two anatomically distinct layers: the epidermis and the dermis. Beneath the dermis is the fatty subcutaneous layer hypodermis (See Fig. 8.1). The epidermis is composed of the stratum corneum (10–20 μm thick) and the underlying viable epidermis (50–100 μm), which consists of stratum granulosum, stratum spinosum and stratum basale. The viable epidermis is responsible for the generation of the stratum corneum (Schaefer 1996).

The stratum corneum (horny layer, SC) is the final product of keratinocyte differentiation (cornification). It is made of layers of metabolically inactive cells, embedded in an extracellular matrix of lamellar lipid bilayers. Corneocytes provide the physical and chemical stability of the SC, while the extracellular matrix gives it the

rigid structure, impermeable barrier for water and water-soluble compounds. The SC can be considered as a wall consisting of polyhedric squeeze-protein "bricks" and water-depleted stiff lipid sheets as "mortar" (Ghyczy 2002). The protection of the skin is provided primarily by the SC, which due to its specific structure provides the primary barrier to percutaneous absorption of compounds as well as to water loss (Lindberg and Forslind 2000; Bouwstra et al. 2003). In addition to the stratum corneum, recent findings showed that the viable epidermis is also a rate-limiting barrier to drug penetration (Andrews et al. 2012).

Corneocytes represent cell remnants of terminally differentiated keratinocytes of the viable epidermis. It is the composition of the corneocytes that gives the SC its strong barrier properties. Corneocyte layers are made up of cross-linked proteins and covalently bound lipids. The proteins resist chemical and physical denaturation and the lipids resist solubilization (Schaefer 1996; Downing and Stewart 2000; Williams 2003). In addition to these there is the corneocyte protein envelope which is added during the cornification process (Downing and Stewart 2000). The insoluble cornified envelope is stabilized through core proteins (90 % of its dry mass) cross-linked to the envelope and through covalently bound lipids (10 % of its dry mass) (Schaefer 1996). Therefore, the two layers of the envelope are the layer adjacent to the cytoplasm which is thick and composed of structural proteins and the layer on the exterior of the protein layer which is composed of lipids. The lipid layer serves as an anchor to the keratinocytes and links the proteinaceous domains to the intercellular lipid domains.

Intercellular lipids are arranged in a crystalline sublattice, with only a small portion of lipids in a liquid phase. The crystalline lipid sublattice is far less permeable to water than the liquid lipid phase. The low permeability of the SC is due not only to the unique lipid composition but also to the unique structural organization of the lipid phase (Downing and Stewart 2000; Lindberg and Forslind 2000; Bouwstra et al. 2003; Feingold et al. 1990).

The dermis (or corium) is typically 3–5 mm thick and is the major component of human skin forming the bulk of the skin. It is made of a network of connective tissue, and elastic tissue embedded in a mucopolysaccharide gel (Wilkes et al. 1973). The collagen fibres in the connective tissue give the dermis support and the elastic tissue provides flexibility. The following structures are embedded in the dermis: blood and lymphatic vessels, nerve endings, pilosebaceous units (hair follicles and sebaceous glands) and sweat glands (eccrine and apocrine). Fibroblasts, endothelilal cells and mast cells are present in the dermis, and during inflammation or wound healing, macrophages, lymphocytes and leukocytes may infiltrate (Schaefer 1996). Blood carries the molecules away from near the dermo-epidermal layer, making dermal concentrations of most permeants low. The formed concentration gradient provides the driving force for drug permeation. In addition to blood, the lymphatic system may also remove permeated molecules from the dermis, maintaining a driving force for permeation.

In terms of transdermal drug delivery, the dermis provides a minimal barrier to the delivery of most polar drugs, but may significantly limit the penetration of highly lipophilic molecules (Williams 2003).

8.4 Drug Transport Routes Through the Skin

A molecule placed on the skin surface could reach the viable tissue: (1) via the appendages, (2) across the transcellular route and (3) across the intercellular route (Fig. 8.1).

The transappendageal transport (shunt route transport) involves the transport through the pilosebaceous unit (hair follicles with sebaceous glands) and through sweat ducts. Hair follicles are the most important appendages in terms of surface area (Schaefer 1996). It is generally assumed that this pathway contributes marginally to the steady-state drug flux (Redelmeier and Kitson 1999; Agarwal et al. 2000; Barry 2006). The reason for this is that the skin area covered with the appendages is proportionally smaller than the total skin surface area (Scheuplein 1967).

However, appendages may function as shunts, which may be important at short times prior to steady-state diffusion (Barry 2006). Appendages can contribute to transdermal drug delivery to a varied degree. Some results show that their (appendageal) contribution is small (Siddiqui et al. 1989), whilst others showed that these shunts are indeed important in skin permeation for a range of drugs (Illel et al. 1991). The same author (1997) also suggested that hair follicles and sebaceous glands can be privileged pathways for some molecules or formulations, which enter faster into these shunts than they do through the SC. Changing certain parameters in a formulation (such as pH, solvent, penetration enhancers) can influence follicular drug permeation (Frum et al. 2008). In addition to this some methods used for transdermal permeation enhancement, such as liposomes and iontophoresis, increase the flux of drugs through hair follicles (Li and Hoffman 1997; Hoffman 1998; Ciotti and Weiner 2002). Lauer (1999) reviewed in detail the follicular delivery.

The transcellular route leads directly across the SC, involving the drug transport through keratinocytes and intercellular lipid lamellae. The consecutive partitioning of the drug between hydrophilic (keratine) and hydrophobic (lipids) parts of the SC makes this a difficult pathway. The nature of the permeant and the partitioning coefficient will influence the importance of this route. Hydrophilic molecules may prefer the transcellular route at a pseudo-steady state. However, lipid bilayers are the rate-limiting barrier for permeation via this route (Williams 2003).

The intercellular route is through the lipid bilayers, which comprise around 1 % of the SC diffusional area, yet provide the only continuous phase within the membrane. It is generally accepted that, except for some specialized cases (e.g. highly hydrophilic substances), the intercellular lipid route is the principal pathway by which most small, uncharged molecules traverse the SC (Loth 1992; Abraham et al. 1995; Roberts et al. 1996; van Kuijk-Meuwissen et al. 1998) and many methods for enhancing the drug penetration disrupt or bypass the intercellular lipid bilayers of SC (Barry 2006). According to the domain mosaic model of the skin barrier (Forslind 1994), the structural organization of the lipids of the SC has two phases: (1) lipids in crystalline/gel state surrounded by (2) lipids that form more fluid (liquid) crystalline domains. This second, more fluid lipid domains provide the pathway by which permeants traverse the SC. A method combining ultradeformable lipid vesicles (Transfersomes®) with confocal laser scanning microscopy (CLSM) showed the existence of two different hydrophilic pathways in the SC: an "intercluster" pathway and an intercorneocyte pathway (Schätzlein and Cevc 1998). The intercluster route runs between clusters of 3–10 neighbouring corneocyte "columns". This pathway has low resistance to molecule penetration and it comprises ≤1 % of the total skin surface or ≤20 % of the pathway area in the skin. The intercorneocyte pathway runs between all the corneocytes in a cluster and is therefore very tortuous. This transdermal permeation route resists penetration better and is more abundant (≥3 % of the skin or ≥80 % of the pathway area). Van Kuijk-Meuwissen et al. (1998) showed by CLSM that the liposomally entrapped fluorescent label travelled across SC mainly via the intercellular route.

8.4.1 Factors Affecting Drug Permeation Rate Through the Skin

Factors affecting the drug permeation rate through SC can be considered using the equation (Eq. 8.1) for steady-state flux (Barry 1983):

$$\frac{\mathrm{d}m}{\mathrm{d}t} = \frac{DC_0K}{h} \tag{8.1}$$

where $\mathrm{d}m/\mathrm{d}t$ is the steady-state flux, presenting the cumulative mass of the diffusant, m, passing per unit area through the membrane; 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; and h is the membrane thickness. From Eq. 8.1, the ideal properties for a molecule in order to penetrate SC well would be the following (Barry 2001; Benson 2005):

- Low molecular mass, preferably less than 600 Da, when D tends to be high.
- Adequate solubility in oil and water in order to achieve a high membrane concentration gradient, which is the driving force for diffusion (C_0 is large).
- High, but balanced (optimal) K, since a too high coefficient may inhibit clearance from viable tissues. This parameter is very important in establishing a high initial penetrant concentration in the first layer of the SC. Molecules showing intermediate partition coefficients (log K octanol/water of 1–3) have adequate solubility within lipid domains of the SC (to permit diffusion through this domain) whilst still having a sufficiently hydrophilic nature to allow partitioning into the viable epidermis.
- Low melting point, which correlates with good solubility as predicted by the ideal solubility theory.

When a drug possesses ideal physicochemical properties (as in the case of nicotine and nitroglycerin), transdermal delivery is feasible. If the drug does not match these ideal characteristics, manipulation of the drug or vehicle to enhance diffusion is necessary and/or penetration enhancement techniques are used.

8.5 Penetration Enhancement Technique Classification

Lots of techniques reported in literature (Barry 2001; Benson 2005; Rizwan et al. 2009) are successful in enhancing the drug delivery into/through the skin. These methods can be grouped initially into chemical and physical methods (Table 8.2). The most extensively studied chemical methods include chemical penetration enhancers (Williams and Barry 2004; Ahad et al. 2009), vesicles (El Maghraby and Williams 2009) and prodrugs (Kasting et al. 1992). Iontophoresis (Costello and Jeske 1995), electroporation (Wang et al. 1998), ultrasound (Cancel et al. 2004) and most recently microneedles (Sivamani et al. 2009) are the most studied physical methods.

Prausnitz and Langer (2008) proposed categorizing TDDS into three generations of development. Drugs in the first generation of TDDS have low molecular weight (Mw), are lipophilic, achieve efficacy at low doses and generally do not require penetration enhancement. The second generation of TDDS utilize enhancement, such as chemical enhancers, iontophoresis and ultrasound but have been limited to the delivery of small Mw molecules. The third generation of TDDS delivers macromolecules to the SC with the help of novel chemical enhancers, electroporation, cavitational ultrasound, microneedles, thermal ablation and microdermabrasion.

8.5.1 Chemical Methods for Penetration Enhancement

Chemical penetration enhancers are defined as agents that partition into and interact with the SC constituents to induce a temporary, reversible increase in skin permeability. These substances temporarily reduce skin resistance and thereby enhance drug flux (Barry 2001). Different groups of structurally related chemical compounds are used as penetration enhancers (see Volume 3, Part 2): water, surfactants, essential oils, terpenes and their derivatives, fatty acids, esters, ethers, Azone and its derivatives, transkarbams, amides, pyrrolidones, sulphoxides and their analogues, etc. (Buyuktimkin et al. 1997; Williams and Barry 2004; Babu and Pandit 2005; Bugaj et al. 2006; Puglia and Bonina 2008; Karande and Mitragotri 2009; Mittal et al. 2009; Brychtova et al. 2010; Ibrahim and Li 2010; Karakatsani et al. 2010; Salerno et al. 2010). Chemical penetration enhancers represent the most studied penetration enhancement method as they have been shown to enhance the topical as well as transdermal delivery of a broad range of drugs both lipophilic and hydrophilic. As an example pyrrolidones enhance permeation of hydrophilic (e.g. mannitol, 5-fluorouracil and sulphaguanidine) and lipophilic drugs (betamethasone-17-benzoate, hydrocortisone and progesterone) (Williams and Barry 2004), as well as terpenes, showing enhanced skin permeation of lipophilic

Table 8.2 Methods used in transdermal penetration enhancement

	Mode of action	Reference
Chemical enhancement methods		
Skin hydration	Increased drug solubility and/or disruption of the SC	Barry (2001)
Chemical penetration enhancers	Increased drug partitioning and/or diffusion in the SC	Williams and Barry (2004)
Vesicles	Drugs are encapsulated into vesicles which interact with the skin	Honeywell-Nguyen et al. (2004)
Prodrugs	Chemical modification of the drug	Qandil et al. (2008)
Ion pairs	Permeation is increased by neutralizing the drug charge with an ion of the opposite charge	Ren et al. (2008)
Salt formation	Drug is changed into a suitable salt form to increase its solubility	Cheong and Choi (2003)
Supersaturated solutions	Thermodynamic activity of the drug solution is shifted, thus increasing penetration rate	Dias et al. (2003)
Eutectic systems	The mixture of drug and another substance lowers the melting point and increases solubility	Ehrenstrom and Reiz (1982)
Physical enhancement methods		
Sonophoresis	Creation of microscopic holes for the transport of drugs	Tezel and Mitragotri (2003)
Iontophoresis	Cavitational ultrasound generates shock waves that disrupt the SC lipid structure	Costello and Jeske (1995)
Electroporation	Electrically driven transport of charged drug molecules	Denet and Preat (2003), Zewert et al. (1999)
Jet injections	Pore formation with short electrical pulses	Bremseth and Pass (2001)
Microneedles	High pressure acceleration of drug particles across the SC	Gill and Prausnitz (2007)
Dermabrasion	Selective removal of the SC by applying high pressure microparticles	Andrews et al. (2011)
Thermal ablation	Short intervals of localized skin heating that creates micropores	Park et al. (2008)
Laser	Thermal ablation of SC creating pores	Gomez et al. (2008)
Waves (radiofrequency, photomechanical, microwaves, photoacoustic)	Disruption of the structure of SC	Levin et al. (2005), Lee et al. (1999), Moghimi et al. (2010), Sa et al. (2013)
Magnetophoresis	Magnetic field is driving drug movement across SC and alters the SC structure	Benson and Watkinson 2012
Combination of techniques		
Chemical enhancers and microneedles, sonophoresis and electroporation		Mutalik et al. (2009), Mitragotri et al. (2000)
Iontophoresis and other physical methods (electroporation, sonophoresis or microneedles)		Hikima et al. (2009), Banga et al. (1999)
Sonophoresis and other physical methods		Mitragotri et al. (2000)
Electroporation and microneedles		Yan et al. (2010)

Table 8.2 (continued)

	Mode of action	Reference
Iontophoresis and chemical penetration enhancers		Wang et al. (2005)
Other methods		
Moxibustion	Increase in skin temperature and skin permeation	Cao et al. (2011)
Submicron injectors	Submicron injection system isolated from sea anemone accelerates the drug across the SC	Shaoul et al. (2012)
Mechanical methods (tape stripping, skin flexing, skin stretching, massage)	Different modes of action: removal of SC layer or reversible formation of micropathways	Rouse et al. (2007), Abdulmajed and Heard (2008), Benson and Watkinson (2012)

drugs, such as ketoprofen (Wu et al. 2001), ibuprofen (Brain et al. 2006), estradiol (Monti et al. 2002), tamoxifen (El-Kattan et al. 2001), zidovudine (Narishetty and Panchagnula 2004), hydrocortisone (El-Kattan et al. 2000) and hydrophilic drugs, e.g. propranolol hydrochloride (Zhao and Singh 1999), bupranolol (Babu and Pandit 2005), nicardipine hydrochloride (Krishnaiah et al. 2002, 2003) and others. Azone and its analogues have been used to enhance a wide range of drugs, too (Afouna et al. 2003; Jampilek and Brychtova 2012). Oleic acid is also widely studied and is one of the leading penetration enhancers used for transdermal applications (Prausnitz et al. 2004).

The limitations of using chemical enhancers are that they are not suitable for enhancing the skin penetration of high Mw drugs and that they often irritate the skin when used at concentrations necessary for achieving useful levels of penetration enhancement (i.e. they have low efficacy at low doses) (Prausnitz et al. 2004). In attempts to solve these problems, researchers have tried synthesizing novel chemical penetration enhancers (Akimoto and Nagase 2003), with optimal enhancer features such as laurocapram (Azone), which safely achieves therapeutic transport enhancement and its analogues (Jampilek and Brychtova 2012), or using two or more penetration enhancers together, because of their synergistic effect in augmenting the penetration of drugs into/through skin (Furuishi et al. 2010).

Barry and co-workers (Barry 1991; Goodman and Barry 1988; Williams and Barry 1991) devised the lipid-protein-partitioning (LPP) theory to categorize chemical penetration enhancers and to describe the mechanism by which they affect skin permeability. According to this theory, enhancers act by one or more of the three modes of action: (1) disruption of the intercellular bilayer lipid structure (lipid modification), (2) interaction with the intracellular proteins of the SC (protein modification) and (3) improvement of partitioning of a drug, coenhancer or cosolvent into the SC (partitioning promotion).

The aforementioned mechanisms of action of enhancers are direct effects of enhancers on the skin. Chemical enhancers can also act indirectly by modifying the formulation. These mechanisms include modification of thermodynamic activity of the vehicle, "drag effect" where the solvent permeating the skin carries the permeant with it and solubilizing the permeant in the donor (Williams and Barry 2004). For more details see Vol. 3 describing a vast range of different chemical penetration enhancers.

Vesicles are colloidal particles, made of water and amphiphilic molecules. The latter form one or more bimolecular layers enclosing an equal number of aqueous compartments. Vesicles can encapsulate hydrophilic drugs within the aqueous regions and lipophilic molecules within the lipid bilayers (Bangham et al. 1965; Williams 2003). There is a large body of research that use different types of vesicles for dermal and transdermal drug delivery (see Volume 2): liposomes, transferosomes, invasomes, ethosomes, niosomes, vesosomes, etc. (Schreier and Bouwstra 1994; Touitou et al. 2000; Cevc et al. 2008; Dragicevic-Curic

et al. 2008, 2009; El Maghraby et al. 2009). The results obtained from these studies are still not consistent and further investigations are needed to fully understand the nature of vesicle transport into/across the skin.

Salt formation is a drug manipulation process where the drug compound is changed into a suitable salt form (Cheong and Choi 2003) with a higher solubility and therefore increased permeation through the skin.

In *ion pair* strategy charged drug molecules penetrate the SC more easily, because the charge on the drug is neutralized by the molecule with the opposite charge, i.e. they form an ion pair (see for details Vol. 1, Chapter 13). In the epidermis the ion pairs dissociate and the drug then diffuses further (Megwa et al. 2000; Ren et al. 2008).

Eutectic systems are a form of penetration enhancement method that uses eutectic mixtures which are drug formulations that combine two substances in an adequate ratio, so that the mixture of substances has a lower melting point than each substance alone (see for details Vol. 1, Chapter 12). The lower melting point of a drug is a parameter that determines the solubility of the drug (see Eq. 8.1) and therefore influences the skin penetration. An example of an eutectic mixture for penetration enhancement is the EMLA® cream (AstraZeneca), being an eutectic mixture of lignocaine and prilocaine (1:1) used as a topical local anaesthetic, which significantly reduced pain associated with venous cannulation in children compared to placebo (Ehrenström-Reiz and Reiz 1982). It was also shown that terpenes form binary eutectic mixtures with ibuprofen and that the resultant melting point depression of the delivery system is correlated with a significant increase in transdermal permeation (Stott et al. 1998). Further, the itraconazole-phenol eutectic formulation enabled, despite the high molecular weight and hydrophobicity of itraconazole, the drug to permeate the skin (Park et al. 2012).

The use of *supersaturated solutions* for enhanced skin delivery of drugs is based on the fact that the maximum, passive flux of a drug across the skin is achieved when it is present in the applied formulation at its saturation concentration, while the drug delivery can further be increased by the creation of a transient, metastable or supersaturated state, whereby the drug's thermodynamic activity is increased above unity (Leichtnam et al. 2006). This approach has the advantage of providing improvement in permeation (proportional to the degree of saturation (DS)) without inducing skin irritation and it is an inexpensive enhancement method. The limitation of the supersaturation approach is its inherent problem of stabilization, and the need to find a way to maintain the metastable state for a period sufficiently long so that an impact on drug transport is apparent (Leichtnam et al. 2007). This period is frequently so short that no impact on transport is observed; however, there are examples of modest to significant effect, like modest increase of transdermal delivery of testosterone (Leichtnam et al. 2006) or significant transdermal delivery of ketotifen (Inoue and Sugibayashi 2012). For more details see Vol. 1, Chapter 11).

Hydration (see for details Vol. 3, Chapter 1) of the SC can enhance the permeation of a large number of drugs used in transdermal delivery, both hydrophilic and hydrophobic (Benson 2005). The mechanisms by which SC hydration increases drug penetration could be by expanding the solubility of the drug and/or by disrupting the structure of the SC due to swelling. The evidence for these mechanisms is not conclusive. In addition to this, hydration does not consistently enhance the penetration of drug molecules and extended occlusion could trigger skin injuries (Bucks and Maibach 1999).

Prodrugs (see for details Vol. 1, Chapter 10) are chemically modified drugs that can cross the skin barrier more easily than the original drug (Sloan et al. 2006). Once the prodrug crosses the SC, an enzymatic and/or chemical transformation will release the active parent drug, which can then exert the desired pharmacological effect. The goal when designing a transdermal prodrug is to alter the physicochemical properties of the drug in such a way as to increase their lipid and aqueous solubility and therefore facilitate the transfer of the drug across the skin. Challenges of the prodrug approach are an increase in size of the modified drug and gaining Food and Drug administration (FDA) approval. In addition to this, many

transdermal prodrug reports still use penetration enhancement techniques (Juluri et al. 2013; Liu et al. 2011; Milewski et al. 2010) showing that this approach is not completely straightforward.

8.5.2 Physical Methods for Penetration Enhancement

Iontophoresis (see for details Volume 4, Part 2) is an electrically assisted delivery to administer therapeutic amounts of the drug across the skin, which enables a significant increase in drug transport across the skin compared to passive drug permeation (even 184-fold; Kalaria et al. 2013). Iontophoresis helps both charged and uncharged drug molecules to migrate across the skin (Costello and Jeske 1995). The penetration enhancing properties of this method are in the electric driving force and not in changing skin permeability. A drug-filled electrode is placed on the skin and a low-voltage current is applied. The mechanisms that move charged and uncharged molecules are electrophoresis and electro-osmosis, respectively (Banga 1998). An advantage of this method is that the drug delivery can be controlled and regulated and application is relatively painless. Limitations of iontophoresis are its relatively high cost, narrow/fixed/restricted drug delivery rates determined by the maximum current applied (Prausnitz and Langer 2008) and molecular size restriction (up to 10–15 kDa) (Kalluri and Banga 2011). Iontophoresis is often used synergistically with other penetration enhancers: chemical penetration enhancers (Wang et al. 2005), ultrasound (Mitragotri et al. 2000) and electroporation (Banga et al. 1999; Alexander et al. 2012). See Volume 4, Part 2 for a detailed explanation of iontophoresis.

Electroporation (see Volume 4, Part 3) is another electrically assisted penetration enhancement method where short pulses of high voltage current are applied to the skin. Skin becomes temporarily permeabilized (the structure of the SC is disrupted) which facilitates the transport of drugs mainly by diffusion and electrophoresis (Denet et al. 2004). Studies show effective penetration enhancement of both small and large molecules by electroporation (Blagus et al. 2013; Denet and Preat 2003; Zewert et al. 1999), but they are at the moment confined to animal models and in vitro studies.

Sonophoresis (Volume 4, Part 1) is the method which uses ultrasound to aid topical and transdermal drug delivery at high (MHz) and low (kHz) frequencies (respectively). Its mode of action is via disturbing the lipid structure of the SC. The mechanisms associated with high- and low-frequency sonophoresis are different; especially, the location of cavitation and the extent to which each process can increase skin permeability are quite dissimilar (Polat et al. 2011). The major effect of low-frequency ultrasound is cavitation, the formation and collapse of air/gas bubbles in the liquid medium at the skin surface (Tezel and Mitragotri 2003). These bubbles oscillate and collapse forming shock waves which induce transient structural changes in the nearby tissue, lipid bilayers of the SC. Cavitational ultrasound can markedly increase drug flux across the skin (Mitragotri et al. 2000) and is non-invasive.

Microneedles are micron-sized needles which can create channels in the skin that penetrate the SC, but do not stimulate the nerves in deeper tissues. In this way drug delivery is increased, pain is avoided and skin invasion is minimal. Microneedles can be solid or hollow and can be used in different ways: piercing of the skin followed by drug-loaded patch; inserting solid microneedles coated with the drug; the drug can be encapsulated in a biodegradable microneedle; or infusion of drug formulation via hollow microneedles into the tissue. Small drugs as well as high Mw drugs, such as peptides, proteins and oligonucleotides, can be transported with this technology (Gill and Prausnitz 2007; Liu et al. 2013). See Volume 4, Chapters 21, 22, and 23 for further reading.

Laser ablation applies laser beams to thermally erode the SC making micropathways in the epidermis (Gomez et al. 2008; Paudel et al. 2010).

During *thermal ablation* skin permeability can be increased when the skin is heated to hundreds of degrees for a very short time, to avoid damage to the surrounding tissues (Park et al. 2008).

The authors suggest that skin changes its permeability upon heating because of the lipid and keratin disruption in the SC, resulting in the removal of SC and formation of microchannels through which a wide range of drug molecules can pass into the deeper layers of the skin. See Volume 4, Chapter 17 for further reading on ablation methods.

Radiofrequency waves can also cause ablation of the SC. Again, microchannels are formed as a result of localized heating (Sintov et al. 2003; Levin et al. 2005). See Volume 4, Chapter 10 for more details.

Microdermabrasion often used in dermatology and cosmetic treatments can also serve as a method for the enhancement of drug penetration into the skin. Removal of the SC is achieved by applying a stream of small crystals. Andrews et al. (2011) showed that microdermabrasion can increase the subcutaneous delivery of insulin in diabetic rats more if the epidermis is removed in addition to the removal of SC. See Volume 4, Chapter 17.

Jet injections are needle-free injections where liquid droplets or solid particles containing a drug are directed towards the skin in a pressurized manner. This high-velocity penetration of particles into or across the skin can be relevant in vaccine administration (Mohammed et al. 2010; Kim and Prausnitz 2011). Insulin can be delivered clinically by jet injection (Engwerda et al. 2011, 2013). See Volume 4, Chapter 15 for more details.

Magnetophoresis (Volume 4, Chapter 13) is a method that uses static magnetic fields to enhance drug penetration (Murthy et al. 2010). Pulsatile electromagnetic fields were used to enhance the penetration of naltrexone in human skin in vitro in a process termed dermaportation (Krishnan et al. 2010; Benson and Watkinson 2012). Dermaportation offers also a potential new delivery method for skin delivery of peptides for a range of dermatological and cosmetic applications (Namjoshi et al. 2008).

The above-mentioned physical enhancement methods can be used in combination; examples are ultrasound and iontophoresis (Le et al. 2000), electroporation and ultrasound (Kost et al. 1996), electroporation and iontophoresis (Banga et al. 1999) and microneedle and iontophoresis (Chen et al. 2009). For a detailed explanation see Volume 4, Chapters 23, 24, and 25.

Conclusion

In the last few decades, the research focus of transdermal drug delivery has been on improving skin permeability through the development of many new methods. Chemical enhancement methods, physical enhancement methods and combination of methods have all contributed to the transdermal industry to a varied degree. A further aim of transdermal systems is to extend the list of products on the market, both in terms of diversity of products and range of indications (diseases) treated. One way forward would be including more macromolecular drug formulations, and the recent method developments are promising to push these boundaries.

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Formulation Effects in Percutaneous Absorption

9

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9.1 Introduction

Skin is increasingly exploited as a site of application for drug delivery. The ultimate goal is to deliver the active ingredient to the site of action in therapeutic amounts over the intended duration. The outermost layer of the skin, the stratum corneum, is generally accepted as the major barrier for such delivery (Scheuplein and Blank 1971; Thong et al. 2007). Over the past decades, various techniques have been investigated and developed to promote active ingredient permeation across the skin (Benson 2005). This includes formulation optimisation, as an approach to promoting percutaneous absorption (Barry 2001; Benson 2005). An appropriate choice of ingredients and their concentrations are formulated together to promote penetration, through solute-ingredient or solute-ingredient-skin interactions mechanisms. Formulation optimisation gives us an opportunity to maximise, minimise or control the skin penetration of a solute using various ingredients mixtures. This is a complex process, given that ingredients are used not only for enhancing skin delivery but also for ensuring the physicochemical stability of the product, as well as its acceptance. It is very common that "real" formulations in the market are complex systems, such as microemulsions, liposomes and lipid nanoparticles. The more complex the ingredients used, the more complex the interactions might exist. While the rate of skin delivery from a simple formulation such as a single or binary mixture can be estimated, its prediction from more complex formulations remains a challenge.

In this chapter, we will review formulation optimisation for skin delivery. Our focus will be on the current understanding of the formulation approach to optimise delivery into or through the skin, known as dermal and transdermal drug delivery, respectively. We begin with a brief overview of skin structure and formulation in skin delivery. We then discuss formulation design, starting from theoretical considerations in skin delivery and its application in comparing various complex formulations. An alternative approach is proposed and current techniques to enhance percutaneous absorption are discussed based on their potential mechanisms related to this paradigm. In addition, formulation-based strategies for optimised delivery as well as importance of other ingredients commonly added in formulation for ensuring physicochemically stable formulations and better product acceptance are discussed. Finally, we give an overview of the current understanding of formulation effects in percutaneous absorption from a simple formulation followed by more complex drug delivery systems.

9.2 Skin Structure and Rate-Determining Pathways

A diagrammatic representation of the stratum corneum structure, along with the three recognised pathways of penetration through the skin, is shown in Fig. 9.1. Skin acts as a barrier between our body and the environment and covers an area of approximately 2 m^2 in an adult (Hadgraft 2001). It consists of three main layers: the epidermis, dermis and hypodermis (subcutaneous tissue). These layers are structurally different and contribute to the overall process of skin transport. The stratum corneum, the outermost layer of the epidermis, is a non-living layer, whereas the rest of the epidermis is viable tissue, called viable epidermis, comprised of nucleated cells, the keratinocytes. The stratum corneum is generally considered as the major barrier in skin delivery. It is composed of 15–25 layers of flattened, hexagonal and cornified cells called corneocytes. The stratum corneum layers are united by stratum corneum lipid bilayers assembled into a "brick and mortar" structure: corneocytes as the "bricks" and lipid bilayers as the "mortar" (Roberts et al. 2002a; Fluhr and Darlenski 2009). The viable epidermis is a site for drug binding and sequestration, drug metabolism and active transport. The dermis is an acellular layer, which is rich of collagen bundles. Below the dermis is the subcutaneous tissue connecting the skin to deeper body structures such as muscle and bone. It is composed of fat microlobules and collagen. The dermis and subcutaneous layers contain blood vessels, lymphatics and nerve fibres, as well as skin appendages (Jepps et al. 2013).

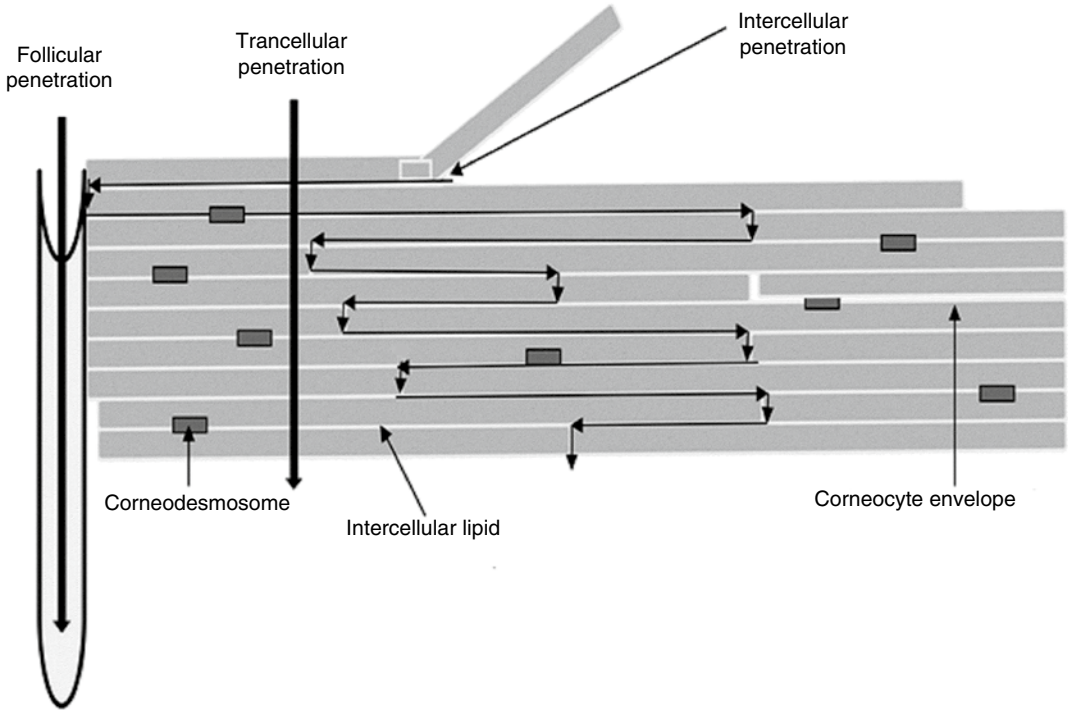

Fig. 9.1 Stratum corneum structure and main penetration pathways through skin (Adapted from Roberts et al. 2008)

Penetrants may cross the skin using a number of routes, i.e. intercellular and transcellular stratum corneum pathways, and appendageal routes (eccrine sweat glands or hair follicles). Whilst it was originally proposed that the transcellular route was the main penetration pathway in skin (Scheuplein and Blank 1971), it is now generally thought that most drugs penetrate via the intercellular pathway (Elias 1983; Barry 2001; Hadgraft 2001). Recent work by Nitsche et al. (Nitsche and Kasting 2008) has suggested that the relative transport through the two pathways is dependent on the nature of the compound. Compounds have long been shown to be taken up into the follicles but this uptake and penetration is believed to be dominant only at early times (Scheuplein and Blank 1971). This hypothesis has been recently confirmed in human skin in vivo by Liu et al., who showed that hair follicles contributed significantly to percutaneous absorption of caffeine only at times soon after application (Liu et al. 2011). Siddiqui et al. (1989) and Essa et al. (2002) have used pharmacokinetic modelling and a skin sandwich technique, respectively, to show that the extent of follicular transport at steady state is between about 5 and 30 % of the total flux. Grice et al. (2010) measured the stratum corneum and the follicles separately and showed that follicular transport for minoxidil was more important at early times. There are a number of conditions such as folliculitis, acne, where there is a need for direct follicular targeting. Topical follicular targeting can avoid systemic toxicity and possibly reduce the dose and frequency of application. The value of the follicular pathway for systemic drug delivery is not well established. Several advantages of follicular drug targeting are (1) bypassing the tortuous pathway of transepidermal absorption, (2) decreasing systemic toxicity and (3) possibly reducing the applied dose and/or frequency of its administration. Recently, there is a considerable interest in using the follicular pathway as a reservoir for localised therapy as well as a transport pathway for systemic drug delivery (Lademann et al. 2008).

9.3 Goals of Skin Delivery

Drugs are applied to skin for local, regional and systemic delivery. The intention of local delivery is to directly treat cutaneous disorders on the skin surface or within the skin directly beneath the application site, such as eczema or psoriasis. Any systemic absorption that may occur is undesirable (Roberts et al. 2002a). In contrast to local delivery, regional delivery requires deposition of the drug with deeper penetration beneath the site of application. Examples of target sites for regional delivery are muscles and joints, beneath and around the application site. Systemic absorption from this form of delivery is undesirable. High concentrations of drug can be localised directly at the target site, thereby reducing systemic drug concentrations and possible systemic side effects (Honeywell-Nguyen and Bouwstra 2005). Systemic delivery requires application of the drug to the skin to treat systemic disorders. Here, the drug should diffuse sufficiently across the skin to achieve therapeutic systemic concentrations (Roberts et al. 2002a). Ideally, there should not be any accumulation of the drug within the skin. As an alternative to oral drug delivery, transdermal delivery avoids gastrointestinal absorption–related problems and hepatic first-pass metabolism and can provide zero-order drug delivery and therefore avoid variability of plasma levels (Honeywell-Nguyen and Bouwstra 2005).

9.4 Mathematical Considerations in Percutaneous Absorption

9.4.1 Principles of Percutaneous Absorption

When a chemical is in contact with skin, quantification of the extent of penetrant absorption through the skin is usually related to Fick's first law. As has been mentioned before, the stratum corneum is considered as the main barrier to penetration of most drugs and barrier effects in the viable epidermis and dermis are considered negligible. In such cases, Fick's law relates the amount of solute penetrating across unit area of skin (Q) to the duration of application (t), solute diffusivity (D), effective path length for the diffusion (h), as well as gradient concentration difference between immediately below the area of application $C_{sc(o)}$ and the inner side of stratum corneum $C_{sc(i)}$:

$$Q = \frac{D\,t\left(C_{sc(o)} - C_{sc(i)}\right)}{h} \tag{9.1}$$

Steady state flux, J_{ss}, is calculated from Eq. 9.1 as

$$J_{ss} = \frac{dQ}{dt} = \frac{D\left(C_{sc(o)} - C_{sc(i)}\right)}{h} \tag{9.2}$$

Steady state in Eq. 9.2 is only achieved when the lag time of diffusion has been reached and it is assumed that (i) the skin barrier, usually the stratum corneum, behaves like a pseudo-homogenous membrane with no appreciable changes in its barrier properties; (ii) depletion of the product applied does not occur and (iii) the system has perfect sink conditions (i.e., $C_{sc(i)} << C_{sc(o)}$ and $C_{sc(i)}$ can therefore be disregarded). Accordingly, if the solute stratum corneum-vehicle partition coefficient (K) is the ratio of $C_{sc(o)}$ and the solute concentration in the vehicle C_v, and the permeability coefficient (k_p) is a product of KD/h, Eq. 9.2 can be written as

$$J_{ss} = \frac{DK\,C_v}{h} = k_p\,C_v \tag{9.3}$$

The stratum corneum k_p is related to the permeability of the available pathway in the stratum corneum, e.g. intercellular lipid, polar route and transcellular route. For most solutes, it seems that penetration is via the intercellular lipid; therefore, k_p usually refers to stratum corneum lipid permeability (Roberts and Walters 1998).

Normally, maximum flux, J_{max}, is achieved at the solute solubility in the stratum corneum ($C_{sc\,sat}$) which also corresponds when solute solubility in the vehicle achieves its saturation condition ($C_{v\,sat}$). Substituting $C_{sc\,sat}$ into Eq. 9.2 under sink condition or $C_{v\,sat}$ into Eq. 9.3 indicates that J_{max} is expressed by either k_p and $C_{v\,sat}$ or by D, h and $C_{sc\,sat}$:

$$J_{max} = k_p C_{v\,sat} = \frac{D}{h} C_{sc\,sat} \tag{9.4}$$

Flux and k_p values can be determined practically through in vitro or in vivo studies. In addition, a qualitative structure–penetration relationship approach uses physicochemical properties of the solute in terms of octanol-water partition coefficient (log *P*) and molecular weight (*MW*), to give an approximation of k_p. Based on published permeability coefficient data from aqueous vehicles, Potts and Guy (1992) generated a model to predict k_p (cm h^{-1}) :

$$\text{Log}\, k_p = -6.3 + 0.71 \log P - 0.0061 MW \quad (9.5)$$

Recognising that the solubility of solutes in water can also be expressed in terms of solute melting point (*MP*) and Log *P*, Eq. 9.5 can also be expressed in terms of maximum flux J_{max} (Milewski and Stinchcomb 2012; Pastore et al. 2014, in press):

$$\begin{aligned}&\text{Log}\ J_{max}(\mu g\ cm^{-2}\ h^{-1}) \\ &\quad = 1.6 + \log MW - 0.0086 MW \\ &\qquad -0.01(MP - 25) - 0.219 \log P \end{aligned} \quad (9.6)$$

In addition to the stratum corneum being a barrier in skin penetration, other skin layers might also affect the skin penetration process. Equation 9.5 is frequently modified to adjust for the viable epidermal resistance for lipophilic solutes (Cleek and Bunge 1993). Transport in the dermis occurs by both diffusion and convective transport by the blood and the lymphatics. It has been suggested that convective transport will dominate when the compound is highly protein bound and this explains why many non-steroidal drugs provide effective local analgesia after topical application (Dancik et al. 2012; Anissimov et al. 2013). When the viable epidermis or subsequent layers act as extra barriers, Eq. 9.3 for steady state flux can be modified as follows:

$$J_{ss} = k_p^i C_v \quad (9.7)$$

where

$$\frac{1}{k_p^i} = \frac{1}{k_p^{sc}} + \frac{1}{k_p^{ve}} + \frac{1}{k_p^d} \quad (9.8)$$

where k_p^i is total skin permeability coefficient and k_p^{sc}, k_p^{ve}, k_p^d represent stratum corneum, viable epidermis and dermis permeability coefficients, respectively. Now, taking into account the overall route available for penetration, then Eq. 9.8 can be modified to account for the permeability coefficient of appendages (k_p^{app}) (Jepps et al. 2013):

$$k_p^{skin} = \left(\frac{1}{k_p^{sc}} + \frac{1}{k_p^{ve}} + \frac{1}{k_p^d} \right)^{-1} + k_p^{app} \quad (9.9)$$

9.4.2 Requirements for Optimised Delivery and Targeting: An Alternative Approach

We have now described the various transport processes required to achieve either topical or systemic delivery. Whatever the intended target of the delivery, solute penetration through the stratum corneum is the prerequisite. During the formulation development process, particularly, the ability to predict the rate of solute penetration through the stratum corneum would be useful to minimise trial and error and shorten the optimisation process. Additionally, it is also highly relevant for assessing potential dermal exposure from industrial and environmental hazards.

Predictive models for percutaneous penetration based on the relationship of steady state flux, solute concentration in the vehicle and skin permeability (k_p) are up to now perhaps the most widely studied. They assume that steady state flux is directly proportional to the skin permeability coefficient and the concentration of the penetrant in the vehicle. When the penetrant is at maximum solubility in the vehicle, maximum flux is achieved, provided the system behaves ideally, i.e. no solute-vehicle-skin interactions exist. Under these conditions, flux at a concentration below saturation in a given vehicle can be approximated if the fractional solubility is known.

Conditions required for k_p-based validity, however, are not always met. A solute may interact with vehicle to some degree, resulting in an increased or decreased effective solute concentration in the

vehicle. When this situation occurs, flux may no longer be directly related to vehicle solute concentration. Another scenario potentially affecting k_p-based validity is when either the solute or the vehicle interacts with the stratum corneum, causing alteration in barrier properties. The vehicle may enter the stratum corneum altering solute solubility in the skin. Alternatively, the vehicle may influence stratum corneum lipid packing which in turn may alter solute diffusivity in the stratum corneum.

Another limitation for k_p-based prediction may be faced during formulation development or optimisation processes. Given the rapid development in the variety of the potential formulation types in topical and transdermal delivery, a formulator may be eager to compare directly which type of formulation best suits the properties of a given penetrant. The use of k_p-based prediction models may be difficult in real formulations where there are a number of solvents, as k_p is usually derived from aqueous solutions.

We have recently proposed an alternative way to overcome the limitations of a k_p-based approach in predicting the extent of penetration for various different formulations (Wiechers et al. 2012). It is derived from a more fundamental definition where the steady state flux through the SC is directly related to diffusivity (D), path length of diffusion (h) and solute concentration in the stratum corneum (C_{sc}). The solute concentration in the stratum corneum can be measured when the amount of solute retained in stratum corneum and the stratum corneum volume are known. A tape stripping technique can be used to measure the amount of solute retained in the stratum corneum. However, as it may be difficult to estimate the solute concentration in the strips, we have proposed the use of Eq. 9.10, which is a variation of Eq. 9.2:

$$J_{ss} = T_r R_m \tag{9.10}$$

where T_r is the rate constant of solute transfer in the stratum corneum, directly related to D/h, and R_m is the amount of solute that is retained in the stratum corneum, analogous to C_{sc}. As Eq. 9.10 measures what actually happens in the skin rather than what happens in the formulation, this equation enables us to compare the abilities of different formulations to enhance the skin delivery of solutes. When solutes are saturated in formulations and formulations do not affect the skin, their J_{ss} will be similar. A reduced flux will likely be caused by a lower solute solubility in the formulation (i.e. lower thermodynamic activity in the vehicle) and a higher value will be caused by formulation-skin interactions, to increase either T_r or R_m. Increased T_r can be achieved by increasing diffusion in the membrane, whereas R_m can be increased by promoting solute partitioning from the formulation into the stratum corneum.

Given that stratum corneum penetration is the first requirement to exert a local, regional or a systemic effect, an increase in J_{ss} is desirable. However, the relative merit of enhancing either T_r or R_m depends on the aim of delivery. Topical delivery is best achieved by a localised solute concentration at the target skin layer, with minimal systemic delivery to prevent unwanted systemic side effects. This is achieved by maximising solute retention in the target layer (R_m^{target}) and the rate of solute transfer to the target layer (T_r^{target}). For example, when the stratum corneum is the target layer, such as in sunscreens, insect repellents and cosmetic preparations, it would be desirable to have formulations that maximise solute retention in the stratum corneum $\left(R_m^{sc}\right)$, preferably without an increase in T_r^{sc}. Similar requirements can be then adopted for the other skin layers. For example, dermal targeting with anaesthetic formulations requires high solute transfer that can facilitate solute diffusion to the dermal layer (i.e. high T_r^{sc}, T_r^{ve} and T_r^{dermis}) and high solute retention in the epidermal/dermal layers to localise solute accumulation R_m^{dermis}. Systemic delivery is best achieved by formulations that can facilitate solute diffusion across the skin to achieve systemic therapeutic solute concentrations. In other words, this requires high T_r^{skin} with optimized R_m^{skin} ($R_m^{skin} = R_m^{sc} + R_m^{ve} + R_m^{dermis}$).

Table 9.1 Effective plasma concentration, clearances, required steady state flux and physicochemical data used to predict required solute transdermal flux for passive topical delivery systems (Pastore et al. 2014, in press)

Solute	Plasma level (ng/mL)	*Cl* systemic (L/h/70 kg)	Estimated J_{ss} required (μg/h)	$t_{1/2}$ (h)	*MW*	*MP* (°C)	Log *P*
Clonidine	0.2–2.0	15	3–30	8–13	230	130	2.7
Estradiol	0.04–0.06	600–800	24–48	~1	272	173–179	4.2
Fentanyl	1–3	27–75	27–225	3–12	337	83–84	3.9
Nicotine	5–30	77	385–2,310	2	162	−79	1.1
Nitroglycerin	0.02–0.4	216–3,270	4.32–1,308	0.03–0.05	227	13	1
Scopolamine	>0.05	65–121	3.25–6.05	1–5	303	55	0.8
Testosterone	3–10.5	41	123–430.5	0.17–1.7	288	155	3.6

9.4.3 Skin Pharmacokinetics: Drug Concentration in the Target

In relation to the goal of skin delivery, the concentration at the target site could be mathematically modelled based on the related transport process. In the viable epidermis, other processes that may affect solute penetration and distribution within the skin include epidermal metabolism, solute binding and sequestration. These variables may also affect solute availability (*F*) for solute penetration into the dermis and beyond. The steady state solute concentration at a site C_{ss} is approximated by accounting for input rate and local clearance (Anissimov et al. 2013; Jepps et al. 2013). In general, the concentration at any skin layer target site is determined by the steady state flux input to the target site $\left(J_{ss}^{target}\right)$, the bioavailability (*F*), the area of application (*A*) and the local clearance (Cl_{local}):

$$C_{skin} = \frac{J_{ss}^{target} F A}{Cl_{local}} = \frac{k_p C_v F A}{Cl_{local}} \qquad (9.11)$$

For systemic delivery, the concentration of solute in plasma after topical application, C_{ss}^{p}, can be approximated from Eq. 9.11, in which k_p^{skin} is defined as in Eq. 9.9:

$$C_{ss}^{p} = \frac{J_{ss}^{skin} F A}{Cl_{systemic}} = \frac{k_p^{skin} C_v F A}{Cl_{systemic}} \qquad (9.12)$$

Equation 9.12 can be used in finding the best drug candidate for achieving the required J_{ss}^{target} based on drug physicochemical properties, as shown in Table 9.1.

9.5 Drug Candidates

Despite the advantages of using skin as a drug application site, to the present only a few drugs are marketed commercially in transdermal delivery systems. Some applications include motion sickness, hypertension, angina, anaesthesia, hormone replacement therapy, contraception and smoking cessation therapy (Finnin and Morgan 1999; Thong et al. 2007). This limited application is due mainly to the limited permeability of the stratum corneum. Improved understanding of percutaneous transport and skin structure has led us to conclude that the best candidate drugs for passive skin delivery must have certain physicochemical properties: low molecular weight (<500 Da), moderate lipophilicity, melting point <250 °C and good potency (daily systemic dose ≤20 mg) (Finnin and Morgan 1999).

Studies on Quantitative Structure–Penetration Relationships (QSPR) (Anderson and Raykar 1989; Magnusson et al. 2004) have shown that solute size is important for facilitating the diffusion process while adequate lipophilicity is required to provide sufficient solubility in the stratum corneum. Maximum flux was shown to have an inverse relationship with molecular weight (Magnusson et al. 2004) and in aqueous vehicles reached a maximum when $2.7 < \log P < 3.1$ (Zhang et al. 2009). However, it does not necessarily follow that all lipophilic solutes with log $P > 3$ are poor candidates for skin delivery, because drug potency must

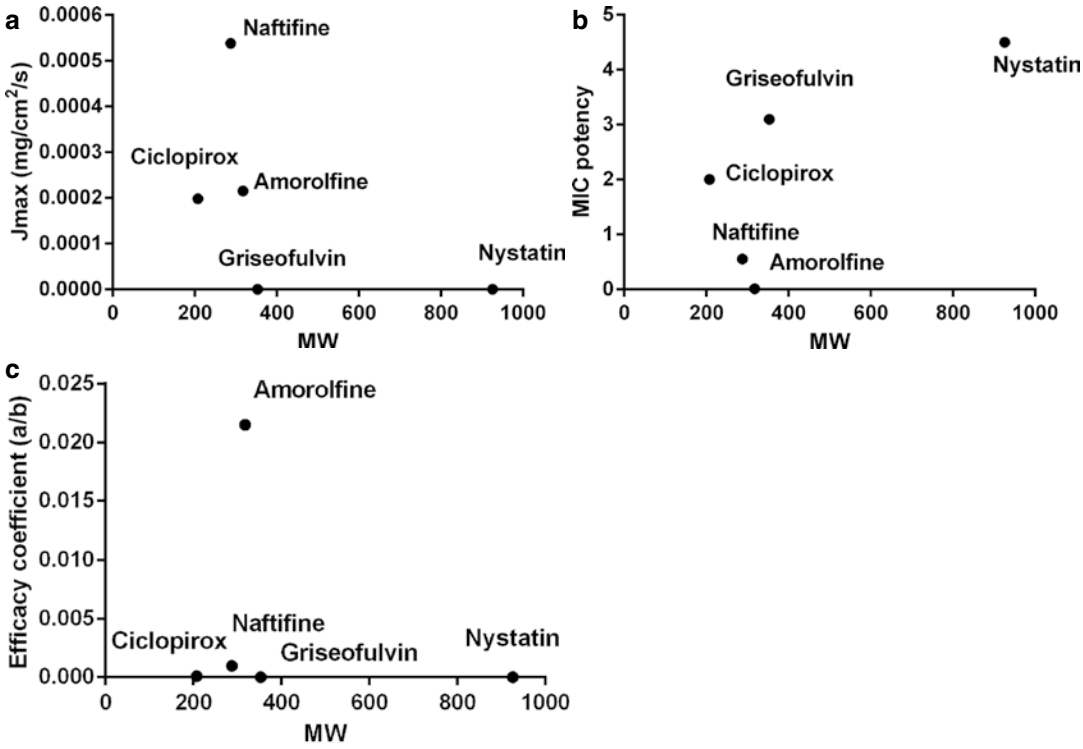

Fig. 9.2 Efficacy concept for antimycotics, whereby efficacy (**c**) is defined as the ratio of its maximum penetration flux (J_{max}, **a**) from a formulation to its potency defined in terms of its minimum inhibitory concentration (*MIC*, **b**) (Mertin and Lippold 1997)

also be considered. Lippold (Wenkers and Lippold 2000) tested the correlation between maximum flux and potency of a range of anti-inflammatory drugs with varying lipophilicities and showed diclofenac, with a log $P > 4$, to have the highest efficacy coefficient ratio (the ratio of maximum flux and drug potency dose). Even though the maximum flux of diclofenac was lower than drugs with log P in the range 2.7–3.1, its potency was greater, since the anti-inflammatory effect of a drug also depends on lipophilicity. A similar concept was also recognised for anti-mycotics as shown in Fig. 9.2 (Mertin and Lippold 1997). It is observed that nystatin is an inappropriate compound because it has a low J_{max} due to its large size and at the same time is not very potent. Griseofulvin also shows poor efficacy due to both poor penetration and a higher MIC. The most ideal compound for efficacy appears to be amorolfine, which has a lower J_{max} than naftifine but is much more potent (lower minimum inhibitory concentration or MIC).

9.6 Formulation-Based Strategies for Optimised Percutaneous Absorption

Formulation encompasses multiple processes that lead eventually to a successful product in the market. The demonstration of product efficacy and good customer acceptance would perhaps be key points for achieving such a goal. While in a broader sense, dosage form design, composition design, packaging design and industrial scaling-up are also essential parts of the formulation process, we will limit our discussion in this chapter to the choice of potential techniques and ingredients that can be adopted to optimise dermal and transdermal drug delivery.

Based on the theoretical requirements described in previous sections, improved skin delivery of a solute can be achieved by increasing its concentration in the stratum corneum and its rate of transport in this barrier. Table 9.2

Table 9.2 Formulation-based enhancement strategies and their possible effects on solute diffusivity and retention in stratum corneum

Approaches	Mechanisms	Potential effects
Thermodynamic activity	Supersaturation	Increase partitioning; may increase R_m
	Formulating for Efficacy	Increase partitioning and/or R_m; (potentially) increase D
Chemical penetration enhancer	Stratum corneum lipid alteration, e.g. fluidised lipid bilayer, lipid extraction, polar head group alteration, hydrophobic lipid	Increase D
	Stratum corneum desmosomes and protein alterations	Increase D
	Corneocytes alteration	Increase D
	Shift stratum corneum solubility closer to that of permeant	Increase R_m and/or partitioning
	Increase solute solubility in the stratum corneum because of solvent-drag mechanism	Increase partitioning and/or R_m
Ion pairs and complex coacervates	Increase lipophilicity	Increase partitioning
Eutectic mixture	Decrease melting point	Increase R_m

summarises formulation-based passive current approaches for percutaneous absorption enhancement and their possible effect on R_m and/or T_r. Other aspects considered later include thermodynamic activity, chemical enhancers, hydration, ion pairs, complex coacervation, pH adjustment and eutectic mixtures.

9.6.1 Escaping Tendency from Formulation (Thermodynamic Activity)

The thermodynamic activity of a solute can be estimated from the ability of the solute to escape from the formulation (i.e. its vapour pressure). Thermodynamic activity also has been referred to as the effective solute concentration in the applied formulation. Increasing the solute concentration is a common way to increase thermodynamic activity. When saturation conditions are reached, the condition in which the solute has maximum thermodynamic activity, the skin delivery rate will be the highest. This fundamental concept was clearly shown by Twist and Zatz (1986) with their flux comparison of saturated paraben from various solvents through a silicone membrane. The fluxes were similar, despite the concentration difference of paraben due to the variation of solubilities. As thermodynamic activity is based on the extent of solute solubility in the applied vehicle/formulation, the degree of solubility or saturation provides a useful way of comparing solute thermodynamic activity among different vehicles/formulations. Percutaneous absorption is directly related to the thermodynamic activity unless the skin is affected by solute– or vehicle–skin interactions (Twist and Zatz 1986).

The thermodynamic activity concept has been used to develop formulations for many years (Poulsen et al. 1968; Ostrenga et al. 1971; Woodford and Barry 1982; Ishii et al. 2010). For example, Poulsen et al. (1968) optimised the composition of propylene glycol-water to modulate the release of 0.025 % fluocinolone and its ester. It was shown that release of both of the drugs was maximal at the least amount of propylene glycol needed to completely dissolve the drug, i.e. 20 and 75 % of propylene glycol in water for fluocinolone and fluocinolone ester, respectively. A high proportion of propylene glycol in water beyond the required least amount to completely dissolve steroids increased the solubility of both steroids and thus decreased the partition coefficient, leading to lower thermodynamic activity. A dependency of drug release on the vehicle and the nature of the drug could be demonstrated. Similar results have been observed

by Ostrenga et al. (1971), who found that a relationship could be built between in vitro and in vivo data and emphasised the importance of thermodynamic activity considerations in the development of formulations.

Another example of the role of thermodynamic activity as a determinant of skin penetration flux was reported by Barry et al. (1985a, b). In their work, the thermodynamic activity of benzyl alcohol, a volatile hydrogen-bonding model penetrant, was measured using headspace chromatography from various binary solvents. The vapour flux across human abdominal skin was directly related to its thermodynamic activity (Barry et al. 1985b). However, when liquid flux was tested, the relationship was less consistent. It was found that some liquid vehicles, such as toluene, damaged the skin (Barry et al. 1985a). This result indicates that if skin is affected by vehicle-skin interactions, thermodynamic activity and flux relationship may not be linear. Expanding on the thermodynamic activity approach, concepts such as Formulating for Efficacy and Supersaturation have been developed and applied.

9.6.1.1 Formulating for Efficacy (FFE)

This concept was first introduced by the late Johann Wiechers in 2004 (2004). FFE was developed to combine two contradictory properties required of by a formulation, namely, (i) the formulation has maximum solubility for the active chemical to accommodate the required dose and (ii) the formulation should have, at the same time, minimum active chemical solubility so as to promote the active chemical leaving the formulation and partitioning into the stratum corneum. A Relative Polarity Index (RPI) is used to measure the polarity difference between the active ingredient and the vehicle. To achieve the first goal, a solvent or emollient is chosen based on a low RPI. Because the first chosen solvent or emollient has similar polarity to that of the active chemical, they are mutually soluble; however, the escaping tendency is low. Therefore, a second solvent or emollient is added to the formulation to reduce the active chemical solubility to just above the required solubility to solubilise the therapeutic dose. This second solvent or emollient has a large RPI. Wiechers recently published a practical application of the principles of FFE based on maximum flux considerations (Wiechers et al. 2012).

9.6.1.2 Supersaturation

Supersaturation is a condition in which an active chemical has greater thermodynamic activity than that occurring under saturation conditions. A supersaturated formulation can be designed by different methods, including co-solvent mixing. The major drawback for this system is its instability. Crystallisation may occur during storage, leading to a change in therapeutic performance of the formulation. An anti-nucleating agent such as a polymer is often added to reduce crystallisation. Supersaturated conditions can also sometimes be formed after application of the formulation to the skin surface. Volatile components such as ethanol or water may evaporate, leading to supersaturated conditions in the remaining formulation (Pellett et al. 1994, 1997). As seen in in vitro (Oliveira et al. 2012) and in vivo experiments (Stinchcomb et al. 1999), there was an initial rapid solute uptake when volatile solvent evaporated from the formulation, leading to increasing solute concentration and eventual supersaturation. However, after complete evaporation, penetration was markedly decreased as the solute was deposited as a crystallised film on the skin surface.

9.6.2 Chemical Penetration Enhancers

Chemical penetration enhancers are purposely incorporated in topical or transdermal formulations to alter solute skin solubility, partitioning from the formulation to the skin and/or diffusivity in the stratum corneum for promoting skin delivery. Ideally, they should also be non-irritant, cosmetically acceptable (e.g. odourless, tasteless and colourless), rapid acting with predictable duration, reversible and chemically stable. Potential mechanisms by which solvents and vehicles,

commonly used as enhancers, may affect stratum corneum properties have been summarised in earlier work (Roberts et al. 2002b). Briefly, altered skin barrier properties may result from the extraction of stratum corneum lipids, the penetration of enhancers into or through the stratum corneum and their interactions with stratum corneum components (Lane 2013). Either intercellular lipids or intracellular proteins of the stratum corneum, as proposed in the Lipid-Protein-Partitioning theory of Barry (1991) could be potential sites of action for enhancers. The effects of these interactions may alter stratum corneum-vehicle partitioning, skin solubility and/or penetrant diffusion in the stratum corneum.

There is no accepted consensus on the classification of enhancers. Nevertheless, simple classifications such as organic vs. inorganic or polar vs. non-polar have been used. In this review, enhancers are grouped according to the potential mechanisms by which enhancers affect D, K, R_m or combinations of those parameters.

9.6.2.1 Effects on Solute Diffusivity

Enhancers have been postulated to affect diffusivity in the stratum corneum in various ways. They can act on stratum corneum lipids as well as proteins. They may disturb the lipid organisation of the stratum corneum, making it fluidised. As the result of the disturbed stratum corneum lipid packing, D may be increased. Enhancers may act on the hydrophobic stratum corneum lipid tails and create free volume available for a penetrating solute to diffuse. Examples of common enhancers that work to increase diffusivity are laurocapram (Azone®, Whitby Research Inc., USA) and oleic acid. They may be dispersed in the stratum corneum intercellular lipid, such as Azone® (Hoogstraate et al. 1991), or may be located in separate domain pools within lipid domain, such as oleic acid (Ongpipattanakul et al. 1991). Terpenes such as limonene and cineole have also been shown to increase drug diffusivity (Moghimi et al. 1996a, b). In addition to disturbance of the stratum corneum lipid packing, solute diffusivity may also be increased as a result of stratum corneum lipid extraction. Some solvents such as dimethyl sulphoxide (DMSO) and ethanol may act by this mechanism although more systematic research needs to be conducted to confirm the mechanism (Barry 2001; Benson 2005). Alternatively, enhancers such as polar solvents may also interact near the polar head groups of the stratum corneum lipids. Interactions with keratin in the corneocytes may open the dense protein structure and thereby increase D. Surfactants, DMSO and urea interact with keratin. The peptide/protein in the lipid bilayer domain may also be affected by this type of enhancer (Benson 2005).

9.6.2.2 Effects on Solute Partitioning and Solubility in Stratum Corneum

Enhancers that increase solute solubility in the stratum corneum can promote partitioning into the skin and hence improve flux. If enhancers permeate into the stratum corneum and shift the skin polarity closer to that of the permeant, the permeant skin solubility and hence partitioning will increase (Cross et al. 2001). A similar result may be expected via a solvent-drag mechanism, in which vehicle entering into the stratum corneum carries solute with it (Zhang et al. 2009; 2011). A number of solvents have been reported to increase partitioning and stratum corneum solubility, e.g. ethanol, propylene glycol, Transcutol® and N-methyl pyrrolidone (Williams and Barry 2004; Benson 2005).

9.6.2.3 Synergistic Effects on Diffusivity and Solute Skin Solubility

Combinations of enhancers which increase D and K or stratum corneum solubility have been reported to give a synergistic effect of enhancement. Examples for this approach are Azone®/propylene glycol for metronidazole (Wotton et al. 1985) and Azone®/diethylene glycol monoethyl ether (Transcutol®, Gattefosse Co., France) for prostaglandin (Watkinson et al. 1991) as well as oleic acid and propylene glycol (Walker and Smith 1996; Benson 2005).

9.6.2.4 Formulation Dependency of Enhancement Effects

It is important to note that enhancers may affect skin properties through different mechanisms, resulting in either enhancement (Megrab et al. 1995; Watkinson et al. 2009), retardation (Hadgraft et al. 1996) or sometimes no significant effect (Sheth et al. 1986). The effect may be either drug dependent (Aungst et al. 1986), concentration dependent (Megrab et al. 1995; Watkinson et al. 2009), time dependent (Grice et al. 2010) or vehicle or formulation dependent (Sheth et al. 1986; Rhee et al. 2007). Sheth et al. (1986) compared the penetration enhancement of trifluorothymidine (TFT), an anti-viral compound, amongst propylene glycol, polyethylene glycol-300 (PEG 300) and water with and without the addition of Azone®. Without Azone®, TFT flux was ranked in the order of propylene glycol > water > PEG 300. With the addition of 5 % Azone®, TFT flux increased three- to fourfold as the ratio of PG : PEG 300 in the vehicle went from 0:100 to 100:0. This suggested that the extent of enhancement was dependent on the vehicles in which the enhancers were incorporated. In addition, it seemed that PEG 300 was not a good solvent for enhancement purposes (Hadgraft 1983).

9.6.3 Synergistic Thermodynamic Activity and Chemical Enhancers

Considering that percutaneous delivery involves two consecutive processes, each of which may be rate limiting, it would be beneficial if optimisation addressed both thermodynamic activity and stratum corneum modification (diffusivity, partitioning and solubility). Supersaturation coupled with oleic acid, a lipid fluidiser, has been shown to increase flurbiprofen flux (Pellett et al. 1997). Wiechers' Formulating for Efficacy concept might also offer potential for synergistic effects of thermodynamic activity and enhancers. If a second chosen solvent or emollient were also an enhancer, a multiplicative flux enhancement could be expected.

An example of how a formulation might be designed by maximising both thermodynamic activity and the use of enhancers was shown by Rhee et al. (2007) in the development of a transdermal gel formulation for the anti-emetic drug, clebopride. The first step was to investigate the best solvent from a range of vehicles. Using saturated drug solutions, the best enhancement was with isopropyl myristate (IPM), the solvent in which the drug had the lowest solubility. Because of clebopride's low solubility in IPM, Transcutol® was added as a co-solvent to increase drug solubility. However, adding too much Transcutol® led to a decreased clebopride flux. An optimal flux was achieved when 40–60 % Transcutol® was added, resulting in 80 times greater enhancement compared to IPM alone. Other studies showed similar results when combining hydrophilic and lipophilic penetration enhancers (Barry et al. 1995; Mayorga et al. 1996; Thomas and Panchagnula 2003; Suwanpidokkul et al. 2004; Karande et al. 2006).

9.6.4 Hydration

Skin hydration, the extent of hydration of the stratum corneum, is a major determinant in percutaneous absorption. At normal humidity, 15–20 % of the dry weight of stratum corneum is made up of water. The stratum corneum may expand by up to 300–400 % of its dry weight when exposed to occlusion, soaking or very high humidity (Roberts et al. 2008). It has been proposed that water taken up by the stratum corneum may interpolate and open up tightly packed intercellular lipid bilayer structure, resulting in an increased rate of skin delivery (Hikima and Maibach 2006). However, fluidisation of the intercellular lipid bilayer and partitioning modification have also been suggested to explain the effect of hydration on percutaneous absorption (Benson 2005). Hydration can be influenced by several means, including the presence of ingredients in the formulation such as humectants (glycerol, glycols). Urea is an example of a humectant, where as a 10 % cream, it increases the water-holding capacity of stratum corneum by 100 % (Williams and Barry 1989). Urea has been reported to be responsible for significantly

enhanced ketoprofen diffusion and permeability through excised rat skin, using water as vehicle (Kim et al. 1993). Another method to increase hydration is the application of occlusive systems like occlusive dressings, patches, ointments and water-in-oil emulsions (Barry 2001; Zhai and Maibach 2002). However, Treffel et al. (1992) have suggested that occlusion does not necessarily increase penetration, as enhancement was shown only for lipophilic compounds. In contrast, Wurster and Kramer in 1961 (1961) showed that hydration promoted the penetration of the more polar salicylate esters. Roberts et al. (2008) have reviewed a number of other studies exploring the effect of hydration on skin penetration and found conflicting results, with no apparent relationship between hydration and penetration in some cases. Following occlusion, reduced paraben partitioning from the formulation to stratum corneum, without significant changes in diffusivity, was responsible for the decreased flux from paraben-loaded ointment, whereas increased diffusivities, with relatively similar partitioning, occurred with paraben-loaded acetone or ethanol (Cross and Roberts 2000).

9.6.5 Ion Pairs

Many drugs are weak acids or bases that are ionised at normal physiological pH. Under these conditions, they are generally poorly absorbed by the membrane. Several techniques have been studied to increase skin absorption of such molecules, including prodrug design, iontophoresis, and ion pairing. In principle, ion pairing involves the addition of an oppositely charged counter-ion to form a neutral ion pair. This neutral species has increased lipophilicity and hence, membrane permeability is increased. The ion pair diffuses into the stratum corneum and dissociates to form the parent compound when it reaches the viable epidermis or deeper skin layers (Seung Jin et al. 1987; Hatanaka et al. 2000; Megwa et al. 2000a, b). Table 9.3 lists formulation studies using an ion-pair approach. The ion-pairing technique offers benefits over these two techniques because no chemical modification is required, as in the prodrug approach, and no external driving force, such as an applied current in iontophoresis, is used (Seung Jin et al. 1987).

9.6.6 Complex Coacervation

Another method to improve percutaneous flux of charged chemicals is by complex coacervation. Complex coacervation and ion pairing are similar in the use of counter-ions to increase chemical lipophilicity. However, the distinction lies in complex coacervates being two-phase systems, i.e. a dilute aqueous phase and an oil phase with a high concentration of lipophilic complex. The two systems are in equilibrium with interchange of charged chemical from the aqueous phase and complex in the oil phase. A study using antidepressants as model drugs suggested that the increased lipophilicity of complex coacervates can increase the transdermal flux of charged species (Stott et al. 1996).

9.6.7 pH Adjustment

As mentioned above, many drugs are ionisable and therefore sensitive to pH changes. Studies on the effect of pH on the penetration of ionisable drug such as salicylic acid (Smith and Irwin 2000), diclofenac (Obata et al. 1993) and ibuprofen (Watkinson et al. 2009) have been reported. Ionised species are considered to penetrate poorly compared to non-ionised species (Scheuplein and Blank 1971; Michaels et al. 1975). Kushla et al. (Kushla and Zatz 1991) investigated the influence of pH on lidocaine flux and permeability. It was found that the permeability coefficient of unionised lidocaine through human skin was 50 times greater than that of ionised lidocaine. It was suggested that pH adjustment in the vehicle can be one approach to alter the relative amount of non-ionised to ionised compound and hence alter the extent of penetration. Evidence has shown, however, that in some cases, the contribution of ionised species on the skin penetration cannot be neglected. For example, steady state flux of diclofenac (pKa of 4.7) increased abruptly as the

Table 9.3 Ion-pair studies in skin delivery

Chemical	Counterion	Membrane	Effect	References
Salicylate	Alkyl amines and quaternary ammonium ion	Human epidermis	Enhancement was shown using ternary amines as counterion	Megwa et al. (2000a)
	A series of amines	Human epidermis and anaesthetised rat	No enhancement of flux through human epidermis skin in vitro; but drug was localised up to the top rat muscle layer	Megwa et al. (2000b)
Methotrexate	Monooctyl phosphate, monodecyl phosphate, monodecyl glycerophosphate, taurodeoxycholate, dodecyl sulphate and dioctyl sulphosuccinate	Hairless mouse skin	Marked increase in flux using dodecyl sulphate and dioctyl sulphosuccinate	Trotta et al. (1996)
Cephalexin	Alkyl sulphonates, tetraalkylammonium	Rats	Enhanced flux	Hatanaka et al. (2000)
Piroxicam	Monoethanolamine, diethanolamine, triethanolamine	Hairless mouse skin	Enhanced skin permeability	Cheong and Choi (2002)
Retinoic acid	Phenylalanine methyl ester, phenylalanine ethyl ester, histidine methyl ester, tryptophan methyl ester, valine methyl ester	Polydimethylsiloxane	Permeation significantly increased	Trotta et al. (2003)
Risedronate	L-arginine, L-lysine, diethylenetriamine	Hairless mouse skin	Flux enhancement 36-fold higher	Nam et al. (2011)
Lornoxicam	Triethylamine, diethylamine, diethanolamine, triethanolamine	Rabbit skin in vitro	All amine counterions, especially triethanolamine, showed obvious enhancing effect	Xi et al. (2012)

pH of the vehicle went from 3 to 7. As the permeability coefficients decreased at the corresponding pHs, the increase of flux was considered to arise mainly from the increased solubility of the ionised diclofenac at higher pH (Obata et al. 1993).

9.6.8 Eutectic Mixtures

A eutectic mixture is a mixture of two or more components that do not interact to form a new chemical compound but which, in certain ratios, inhibit crystallisation of one another, resulting in a system with a lower melting point than either of the components. Since the decrease of melting point has been shown to be inversely proportional to lipophilicity, and hence transdermal flux, eutectic mixtures have been studied for enhancing the flux of drugs. Examples are ibuprofen (Stott et al. 1998) (from an ibuprofen-terpene eutectic mixture), testosterone (Kaplun-Frischoff and Touitou 1997) (from a testosterone-menthol eutectic mixture) and the β-blocker propranolol (Stott et al. 2001) (from a propranolol-fatty acids eutectic mixture).

9.7 Importance of Other Formulation Ingredients on Percutaneous Absorption

In addition to maximising skin delivery to improve bioavailability, formulation must also ensure physicochemical stability of the product

Table 9.4 Ingredients commonly added to achieve physically stable and cosmetically and aesthethically acceptable formulations

	Types of ingredient needed
Physicochemical stability of the active chemical and formulation	Dispersing and/or solubilising agent, e.g. water, propylene glycol, ethanol
	Emulsifier, e.g. polysorbates, cetrimide, sodium dodecyl sulphate
	Increase vesicles stability, e.g. cholesterol addition in conventional liposomes
	Preservatives, e.g. benzyl alcohol, phenol, chlorocresol
	Antioxidant
Improve cosmetic and aesthetic acceptance	Emollient, humectant, moisturiser, e.g. urea
	Colouring
	Fragrance, e.g. terpenes
	Viscosity modifiers, e.g. carbomer, carrageenan, acacia, alginates, xanthan

as well as cosmetic and aesthetic acceptance. Various ingredients are usually incorporated into the formulation to achieve these purposes. Table 9.4 shows examples of common ingredients for such purposes and their functions in the formulation. The effects of such ingredients on percutaneous absorption are discussed here.

9.7.1 Solvent and Co-solvent

One of the common ingredients needed in the formulation is a solubilising agent. Active ingredients may be hydrophilic or hydrophobic in nature. Due to the principle of "like dissolves like", while a hydrophilic chemical may be easily solubilised in a polar vehicle such as water, a hydrophobic chemical may face difficulty. The co-solvent method, where two miscible solvents are mixed, is a common technique used to increase solubility in a formulation (Rhee et al. 2007; Watkinson et al. 2009). To increase the solubility of ibuprofen, a lipophilic compound, ethanol : water co-solvent mixtures have been employed. Ibuprofen solubility was greatly increased by 5,500-fold relative to its aqueous solubility as the amount of ethanol was increased from 0 to 100 % (Watkinson et al. 2009). A similar result was found for oestradiol, where its solubility was increased by 30-fold (Megrab et al. 1995), and subsequently, the partition coefficient was decreased.

Many solvents and co-solvents are known to influence the barrier properties of the skin. Ethanol was used in a number of studies as a co-solvent (Pershing et al. 1990; Hatanaka et al. 1993; Obata et al. 1993). Megrab et al. (1995) investigated the mechanism behind the enhancement of oestradiol flux using saturated oestradiol in 0–90 % ethanol : water compositions tested using three different membranes, namely, excised human skin, silastic membrane and snake skin. Maximum flux was achieved at different ethanol concentrations, namely, 40–60 %, 80 % and 40 % (followed by a constant phase up to 90 % ethanol) for excised human skin, silastic membrane and snake skin, respectively. They have suggested that enhancement of oestradiol flux at low ethanol concentrations was due to the increased drug solubility in the stratum corneum. The decreased oestradiol fluxes at higher ethanol concentrations were due to the dehydration effects of ethanol on the stratum corneum. In agreement with this, Watkinson et al. (2009) found greater enhancement for ibuprofen flux with 75 % ethanol for human skin. This evidence emphasised a concentration dependence of ethanol for flux enhancement.

Propylene glycol (PG), a colourless and viscous liquid, is commonly used in topical formulations (Arellano et al. 1999; Nicolazzo et al. 2005). The binary system PG : water has been shown to enhance ibuprofen flux either in excised human skin or silicone membranes. A linear enhancement with increasing PG content was seen in human skin membranes, but in the case of silastic membrane, there was no linear relationship and the optimum enhancement was achieved at 70 % PG. The effect of PG on human skin was primarily on the solubility and partitioning of ibuprofen (Watkinson et al. 2009).

Another study investigated the effect of a ternary mixture of ethanol : PG : water on drug flux. Recent research by our colleagues Grice et al.

(2010) investigated the influence of a combination ethanol : PG : water, i.e. 60 : 20 : 20; 80 : 20 : 0 and 0 : 80 : 20 on the minoxidil uptake into appendages, stratum corneum and through human skin in vitro. A change in the transport mechanism of minoxidil uptake was noticed. At early times (before 12 h), formulations containing high amounts of ethanol with the least amount of PG gave higher minoxidil uptake. Evaporation of volatile solvents, e.g. ethanol, led to increased minoxidil concentrations due to the resulting reduction in volume. After 12 h, on the other hand, maximum flux was obtained from the formulation with the least amount of ethanol but the highest amount of PG. The authors suggested that PG was slowly taken up by the membrane, leading to its physical modification and enhanced minoxidil flux. A similar result was found by Tata et al. (1994).

Vehicle uptake into the membrane can strongly influence skin penetration. Our recent study, for example, showed that maximum flux from similar sized compounds was determined by solute solubility in the stratum corneum (Zhang et al. 2009) which was dependent on the amount of vehicle penetrating into the stratum corneum (Zhang et al. 2011). The ability to predict vehicle uptake into the membrane and the rate of solute penetration into the skin would have significant impact on clinical and toxicological applications (Sloan et al. 1986). Earlier work by Potts and Guy (1992) to predict the extent of percutaneous absorption was based on a QSPR approach. Using Flynn's (1990) database generated from in vitro data, it was shown that lipophilicity and molecular weight of the solute determine skin penetration (Mitragotri et al. 2011).

Attempts have been made to relate vehicle physicochemical properties and flux across the skin. The use of solubility parameters (δ) has been shown to be useful as a flux descriptor (Sloan et al. 1986; Jiang et al. 1998; Cross et al. 2001; Dias et al. 2007). Sloan suggested that uptake of vehicle into a membrane has its maximum effect in increasing solute flux when the solubility parameter of the vehicle, δ_v, is close to that of membrane, δ_s. Our work showed that the maximum flux of hydrocortisone was directly related to the volume of vehicle absorbed by the membrane. However, a dependency of the vehicle volume fraction sorbed into silastic membrane on δ_v for a wide range of structurally unrelated vehicles was not seen. A linear observed versus predicted solvent volume fraction relationship was shown after adjusting δ_v for vehicle molecular weight and hydrogen-bonding properties. This result indicates that it is not only δ_v but also vehicle molecular size and hydrogen bonding that determine vehicle-membrane interactions (Cross et al. 2001). In addition, vehicle molecular size has been shown to be an important determinant for mobility in the membrane (Most 1972). Further, the relative importance of solute diffusivity or membrane solubility as dominating factors responsible for the enhanced flux following solvent uptake is likely to be determined by the combination of solute and solvent used (Cross et al. 2001). For example, while Sloan found that partitioning was the dominant factor, in our hydrocortisone penetration study we observed that flux was dominated by neither diffusivity nor membrane vehicle partition. A combination of both diffusivity and membrane partitioning effects determined vehicle-membrane interactions.

9.7.2 Viscosity Modifiers

To increase product acceptance, it would be preferable to thicken low-viscosity formulations, such as co-solvent mixtures (Rhee et al. 2007) and microemulsions (Chen et al. 2006; 2007; Zhu et al. 2009). A number of viscosity modifiers have been investigated for this purpose including silicone dioxide (Rhee et al. 2007), carbomer (Carbopol® 940, Lubrizol Co., USA) (Chen et al. 2007) and carrageenan (Valenta and Schultz 2004).

One of the drawbacks of incorporating viscosity modifiers in a formulation is that they may hinder drug diffusion from the formulation into the skin. For example, addition of a 7 % gelling agent to thicken a Transcutol® : isopropyl myristate binary mixture (40:60) resulted in a

significantly reduced steady state clebopride flux, four times lower compared to the un-gelled optimised mixture. It was suggested that this was due to the increased viscosity of the clebopride gel formulation (Rhee et al. 2007). A significantly reduced permeability coefficient was also seen when 4 % Carbopol® 940 was added to thicken an oestradiol microemulsion, which could be partly due to the increased viscosity of the microemulsion (Peltola et al. 2003).

On the other hand, there are examples in the literature where the addition of viscosity modifiers has no effect on permeation rates. Chen et al. (2007) used a hydrogel-thickened microemulsion for the topical administration of tripolide at an extremely low concentration. Even though the microemulsion viscosity was increased significantly by the addition of Carbopol® 940, no significant difference in the tripolide steady state flux was observed compared to the microemulsion without Carbopol® 940. The authors suggested that close contact of microemulsion droplets to the skin promoted by the adhesiveness of Carbopol® 940 might play a role in tripolide penetration across mouse skin. A similar mechanism has been attributed to the facilitation of sodium fluorescein penetration from carrageenan-thickened microemulsions (Valenta and Schultz 2004).

9.7.3 Ingredients Used for Stability

Ingredients may be added to a formulation to aid its physical stability. For instance, emulsions are formed from mixtures of immiscible liquids, in which one phase will be dispersed in the other. This requires a third component such as an emulsifier to lower interfacial tension and ensure physical stability (Riviere et al. 2010).

The effects of surfactants on skin absorption have been extensively studied (Shokri et al. 2001; van der Merwe and Riviere 2005; Riviere et al. 2010). For example, van der Merwe et al. (van der Merwe and Riviere 2005) studied the influence of sodium lauryl sulphate (SLS) on the permeability and stratum corneum partitioning of 10 agricultural and industrial chemicals using porcine skin. Addition of SLS to a water vehicle was generally found to reduce partitioning and permeability of lipophilic compounds. The presence of SLS at a concentration above its critical micelle concentration caused the formation of micelles around the non-polar compounds and reduced their availability for absorption. In contrast, when SLS was incorporated into an ethanol vehicle, it did not affect partitioning. The authors hypothesised that since ethanol has a short carbon chain and a polar group, it is able to attract polar and non-polar molecules making partitioning from an ethanol vehicle system not sensitive to SLS addition. Increased permeability from an ethanol vehicle system was linked to changes in diffusivity.

9.7.4 Ingredients Used for Customer Acceptance

Product acceptance is another aspect that can be improved by the ingredients in formulation. Fragrance is the most commonly added in the topical formulations to provide an agreeable odour or mask an unacceptable one. They are usually lipophilic compounds and therefore more likely to be absorbed into the skin. Diethyl maleate, an example of a fragrance compound, was absorbed at 54 % of the applied dose over 24 h in a human in vivo under occluded conditions (Bronaugh et al. 1990). Other fragrance examples are benzyl derivatives (Bronaugh et al. 1990) and terpenes (Hotchkiss 1998). It is worth noting that terpenes used as fragrances have also been shown to increase percutaneous absorption. For instance, 1,8 cineole enhanced the diffusion coefficient of oestradiol, a lipophilic model drug, and 5-fluorouracil, a hydrophilic model drug (Moghimi et al. 1996a, b, 1997).

9.8 Formulation-Related Issues for Nanoparticulate Carriers

The range of dosage forms for skin delivery can be broadly divided into conventional and novel delivery systems. Conventional dosage forms

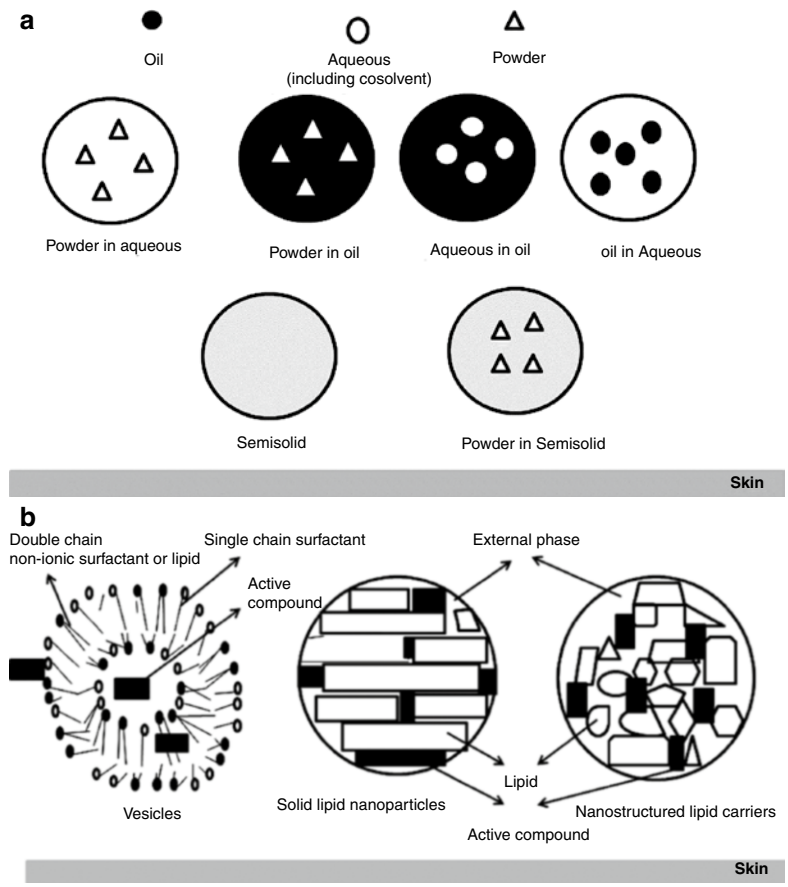

Fig. 9.3 Some representative of conventional (Adapted from Roberts et al. (2002a)) (**a**) and nanoparticle novel delivery systems used as dermatological carriers (Adapted from Honeywell-Nguyen and Bouwstra (2005), Pardeike et al. (2009)) (**b**)

encompass liquids (such as lotions, emulsions), semisolids (such as ointments, pastes, gels and creams) and solid preparations (such as powders or patches) (Epstein 2009; Narasimha Murthy and Shivakumar 2010). Besides these, there are different novel and more complicated systems that are used to optimise percutaneous absorption of drugs like patches, microneedles and nanoparticles. Among these systems, nanoparticles that have the ability to penetrate skin will be discussed in more detail below. Some examples of conventional formulations and nanoparticulate delivery systems used as dermatological carriers are shown in Fig. 9.3.

A number of review articles have been published summarising the application of novel delivery systems such as microemulsions (Lawrence and Rees 2000; Kogan and Garti 2006; Heuschkel et al. 2008; Santos et al. 2008), vesicle (liposomes and flexible liposomes) (Yarosh 2001; Cevc 2004; Honeywell-Nguyen and Bouwstra 2005; Elsayed et al. 2007) and nanoparticles (Souto et al. 2007; Souto and Müller 2008; Pardeike et al. 2009; Prow et al. 2011) for dermal and transdermal delivery. We will now give a general description of some novel delivery systems followed by a discussion of their application to percutaneous.

Microemulsions have gained interest as potential drug carriers due to several beneficial features, such as ease of preparation, long-term stability, high solubilisation capacity of hydrophilic and hydrophobic drugs and improved drug delivery. However, the mechanisms behind the enhancement are still unclear. Some published studies attributed the enhancement to the composition as well as its formulation characteristics (Heuschkel et al. 2008). Typically, a microemulsion consists of lipophilic and hydrophilic ingredients, surfactant and co-surfactant. Microemulsion formation

is highly dependent on the surfactant capacity to reduce interfacial tension between the hydrophilic and lipophilic ingredients. Often, surfactant alone is not sufficient to ensure physical stability of the mixture. Co-surfactant addition will further reduce interfacial tension to facilitate microemulsion formation. Non-ionic surfactants, such as polyglyceryl-6-isostearate (Plurol® Isostearique, Gattefosse Co., France), highly purified diethylene glycol monoethyl ether (Transcutol® P, Gattefosse Co., France) and caprylcaproyl macrogol glycerides (Labrasol®, Gattefosse Co., France), are usually considered safe for human skin application and less irritating than ionic surfactants. Examples of co-surfactants are short- or medium-chain alcohols such as ethanol, isopropanol and butanol. Isopropyl myristate, isopropyl palmitate, ethyl oleate and oleic acid are commonly used as lipophilic phases, whereas water, viscosity-enhancing agents, sodium chloride, buffer salts and preservatives are included in the hydrophilic phase. For topical applications, microemulsions have been used to deliver model chemicals such as retinoic acid (Trotta et al. 2003) and tea tree oil (Biju et al. 2005). For transdermal applications, steroids such as oestradiol (Peltola et al. 2003) and testosterone (Malcolmson and Lawrence 1993) as well as beta blockers (Kemken et al. 1991; 1992) have been studied. A major concern over the application of microemulsions, requiring further research, is the irritation potential due to the high surfactant/co-surfactant content (Santos et al. 2008).

Liposomes are colloidal structures consisting of a mixture of phosphatidylcholine and cholesterol, as typical ingredients, and other substances. First reported in 1980, liposomes showed superior percutaneous enhancement compared to conventional dosage forms such as lotions and creams. Despite the potential applications for delivering chemicals topically or systemically, the current weight of research suggests that liposomes remain accumulated in the stratum corneum and upper skin layers. The application to transdermal delivery is less promising because of generally minimal drug penetration into deeper skin layers and the systemic circulation. Numerous studies have modified the classical liposome formulation, into highly deformable liposomes, including Transfersomes (Cevc et al. 1998; 2002; Schätzlein and Cevc 1998; Jain et al. 2003), ethosomes (Dayan and Touitou 2000; Touitou et al. 2000; El Zaafarany et al. 2010), niosomes (Schreier and Bouwstra 1994; Fang et al. 2001) and SECosomes (Geusens et al. 2010) by the addition of other ingredients. Transfersomes are liposomes consisting of surfactant and ethanol, whereas ethosomes are liposomes with a high alcohol content. Niosomes result from the addition of a non-ionic surfactant, whereas SECosomes are produced by the addition of a combination of surfactant and ethanol (Geusens et al. 2010). Unlike classical liposomes, those highly deformable liposomes are reported to be able to penetrate stratum corneum and even deeper skin layers, carrying encapsulated molecules with them.

Solid lipid nanoparticles (SLN) consist of a mixture of solid lipids and surfactants. The ingredients are usually those commonly used in topical cosmetic and pharmaceutical products, which are considered as generally regarded as safe (GRAS) substances, providing a broad range of choices which may avoid safety issue problems. In addition, the concentrations of the ingredients are similar to those used in conventional formulations.

It was shown that incorporation of an active compound into SLN could increase its stability. The nature of the solid lipids and the concentration of surfactant in the formulation were important parameters contributing to the stability (Müller et al. 2002). The potential for occlusion due to the small particle size of SLN and the ability to control active compound release are other attractive features of SLN for topical application (Müller et al. 2002; Neubert 2011). Nanostructured lipid carriers (NLC) are a new generation of lipid nanoparticles developed to overcome some limitations of SLN. During storage, SLN transform into highly ordered crystal forms, resulting in drug expulsion. In NLC, however, lipid material consists of a blend of solid lipids and liquid lipids (oils) which reduces crystal order and hence deters drug expulsion. Lipid nanoparticles have been used as vehicles for the topical application of anti-acne drugs and

Table 9.5 Proposed mechanisms in promoting dermal and transdermal delivery from micellar systems and lipid nanoparticles

Microemulsion (Santos et al. 2008)	Liposomes (Touitou et al. 1994; Elsayed et al. 2007)	Flexible liposomes (Elsayed et al. 2007)	Lipid nanoparticles (Schafer-Korting et al. 2007)
High solubilisation capacity for hydrophilic and lipophilic drug	Liquid and gel state of liposomes	Liquid and gel state of liposomes	Component of formulation may penetrate into stratum corneum
Formulation component such as surfactant, cosurfactant and oil may act as penetration enhancer	Component of liposomes may penetrate into stratum corneum	Intact vesicles penetrate into stratum corneum	Release modulation from formulation
Increase of hydration		Component of liposomes may penetrate into stratum corneum	Occlusion
Very low interfacial tension ensures excellent contact with skin			

sunscreen agents (Schafer-Korting et al. 2007). Lipid particles have been tested in vitro, ex vivo and in vivo for transdermal delivery of flurbiprofen. Both SLN and NLC exhibited sustained drug release over a period of 24 h (Bhaskar et al. 2009).

Although these novel formulations have been shown to promote penetration across the skin, the exact mechanism is not yet clearly understood. In general, it is usually postulated that the effectiveness of drug delivery systems is related to a variety of factors depending on the composition of the formulation which also may strongly influence formulation characteristics. Table 9.5 presents proposed mechanisms for potential complex formulations in dermal and transdermal delivery.

The relationships between thermodynamic activity and percutaneous absorption are likely to be less well defined in such formulations. Ingredients that are required to achieve formulation stability could also affect solute thermodynamic activity in the formulation and, hence, skin delivery. Surfactant and co-surfactant, for examples, are required to reduce interfacial tension in microemulsion formation. At the same time, their inclusion in the formulation may increase solute solubility in the formulation. Indeed, there are reports indicating that an increased amount of surfactant in the microemulsion did not increase flux (Chen et al. 2004; Yuan et al. 2006). If a solute is incorporated at or near its saturation limit, solute thermodynamic activity will be enhanced. A similar situation is seen with NLC, in which liquid or oil lipid is incorporated into the formulation mixture to minimise drug expulsion during storage. While solute solubility in the NLC increases, the thermodynamic activity will also change, and hence, solute concentration might require adjustment.

Intact vesicles containing entrapped drug have also been proposed to penetrate into the stratum corneum. Studies performed with surfactant-based deformable vesicles indicate that intact vesicles may partition into the stratum corneum, but show very limited partitioning into the viable epidermis, if any (Honeywell-Nguyen and Bouwstra 2005). Interactions of the formulations with skin may also occur due to increased hydration following film formation (Müller et al. 2002), water penetration from oil in water systems and occlusive conditions in patch systems.

Despite rapid development in formulation technologies for topical and transdermal delivery, a reliable percutaneous absorption prediction from such formulations remains a challenge. Unlike simple formulations, where the dominant factors controlling partitioning from vehicle to skin, diffusivity and skin solubility could be distinguished relatively easily, complex formulations are more challenging. Expanding on approaches used for simple vehicle mixtures, Riviere et al. (Ghafourian et al. 2010a, b) recently employed a QSPR method to relate skin penetration to the

chemical properties of the mixture and the molecular structure of the penetrants. Permeability coefficients were obtained from 12 different penetrants each incorporated in 24 different mixtures of various solvents, a surfactant (sodium lauryl sulphate) and a vasodilator (methyl nicotinate) measured from finite-dose application through porcine skin. Prediction was performed to relate penetrant physicochemical properties (i.e. log *P* and the 9th order path molecular connectivity index) and solvent properties (i.e. the difference between boiling point and melting points). The result suggests that skin penetration from complex mixtures could be well predicted using a QSPR approach and that high skin penetration would be predicted from vehicle mixtures having small differences in their boiling and melting points.

Conclusion

Drug delivery into or through the skin is an attractive route for local or transdermal applications. The skin barrier, particularly of the stratum corneum, has yet to be predictably and consistently overcome to increase bioavailability. Formulation-based optimisation, using various techniques including thermodynamic activity, chemical penetration enhancers, hydration, ion pairs, complex coacervation, pH adjustment and eutectic mixtures, is employed to achieve efficacious skin delivery. In addition to this, ingredients commonly used for physicochemical formulation stability, aesthetic or cosmetic purposes might potentially alter drug penetration. Potential novel dermal and transdermal delivery systems, e.g. microemulsions, flexible liposomes and lipid nanoparticles, have been shown to promote skin penetration through various mechanisms requiring further systematic investigation. A reliable method to predict percutaneous absorption across all varieties of solutes, complex formulation types and conditions remains a challenge.

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Part III

Drug Manipulation Strategies in Penetration Enhancement

Selection of a Proper Prodrug for Penetration Enhancement

10

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10.1 Introduction

10.1.1 Push Versus Pull Mechanisms for Penetration Enhancers

From a mechanistic point of view, there are two general ways to accomplish the task of improving topical delivery using a chemical-based approach. The first approach is to increase the "push" of the vehicle components on the drug to drive it into the skin (Kadir et al. 1987). One way to increase the "push" of the vehicle is to use vehicle components in which the drug is more soluble but which are more volatile than the other components. Evaporation of the volatile components after application of the drug-vehicle combination leaves a supersaturated solution of the drug in a state of heightened thermodynamic activity in the vehicle (α_{VEH}) (Coldman et al. 1969), that is, α_{VEH} greater than one. The second approach is to increase the "pull" on the drug into the skin by components of the vehicle that have permeated the skin and have decreased the resistance of the skin to permeation by the drug (Kadir et al. 1987) or increased the solubility of the drug in the skin, S_{M1}: these components interact with the skin. Such components of the vehicle do not have to permeate the skin faster than the drug. However, another way to increase the "pull" on the drug by components of the vehicle is to use components that do permeate the skin faster than the drug and pull the drug along with them – a "drag" effect (Friend and Smedley 1993).

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The basis for the two chemical-based approaches to enhancing topical delivery (decreasing the solubility of the drug in the vehicle and increasing its solubility in the skin; "push" and "pull," respectively) lies in the form of the equation that describes flux. The flux, J, of the drug through skin is directly related to the concentration of the drug in the first layer of the skin, C_{M1}, from Fick's Law: $J=(C_{M1} - C_{Mn})\ D/L$ where C_{Mn} is the concentration of the drug in the last layer of skin (and is assumed to approach zero at steady-state), D is the diffusion coefficient of the drug in the skin, and L is the thickness of the membrane. The concentration of the drug in the skin, C_{M1}, is generated from its equilibrium with the concentration of the drug in the vehicle, C_{VEH}, through the product of its partition coefficient between the two phases $K_{M1:VEH}$ and C_{VEH}. The concentration of the drug in the skin approaches its saturated solubility in the skin, S_{M1}, and a thermodynamic activity (α_{M1}) of one when C_{VEH} approaches the saturated solubility of the drug in a noninteractive vehicle, S_{VEH}; that is, α_{VEH} also is one. The flux J is now the maximum possible flux from noninteractive vehicles, J_M, and Fick's law can be written as Eq. 10.1. Regardless of the value for S_{VEH}, the highest concentration of drug in the skin that is possible from a drug applied in a noninteractive vehicle is S_{M1}. As S_{VEH} increases $K_{M1:\ VEH}$ tends to decrease and as S_{VEH} decreases $K_{M1:\ VEH}$ tends to increase. S_{M1} can only be increased by using an interactive component in the vehicle that changes the solubilizing capacity of the skin, the "pull," or by increasing the thermodynamic activity of the drug in the vehicle, α_{VEH}, so that it is greater than one, the "push," and hence the activity of the drug in the skin, α_{M1}, is also greater than one.

$$J_M = (D/L)(S_{MI} - C_{Mn}) \qquad (10.1)$$

10.2 Basis for Prodrugs as Penetration Enhancers

Although increasing the "push" can be easily accomplished by manipulating the components of the vehicle in which the drug is applied (its formulation), increasing the "pull" can be more easily accomplished using a prodrug approach that changes the solubility properties of the drug. A prodrug is a chemically or enzymatically reversible derivative of a parent drug that improves the physicochemical or biological properties of the parent drug molecule to overcome some intrinsic problem associated with its therapeutic use: in this case, poor solubility in the skin and hence low topical delivery (Sloan 1992). The particular combination of functional groups that is added to the parent drug is called the promoiety, and the reversible connection between the promoiety and the parent drug is called the enabling functional group. A prodrug approach, then, can be envisaged as a 1:1 molecular combination of the drug and a promoiety that contains functional groups that will increase its solubility in the skin (Sloan and Wasdo 2003). This prodrug approach stands in sharp contrast to most formulation approaches where large molar excesses of penetration enhancers as vehicle components are routinely needed to increase S_{M1} for the drug.

What are the properties of the functional groups in the promoiety which, when added to the parent drug, could be reasonably expected to cause an increase in S_{M1} of the resulting prodrug compared to the parent drug and hence to cause an increase in its maximum flux, J_M? Since it is difficult to measure S_{M1} of the prodrug, it is more convenient to measure its J_M in diffusion cell experiments and assume, based on Fick's law Eq. 10.1, that there is a direct relationship between increased J_M and increased S_{M1}. Using increases in J_M as the criterion for increased S_{M1}, it has been observed for quite some time that for homologous series of more lipophilic prodrugs that the more water soluble members of the series gave the greatest increase in J_M and not the more lipid soluble members (Sloan 1989, 1992; Sloan et al. 1984). In order to account for these qualitative observations, S_{M1} in Fick's law Eq. 10.1 was expanded mathematically to include dependence on solubility in a lipid, S_{LIPID}, and in water, S_{AQ}. This form of Fick's law is the Roberts-Sloan (RS) Eq. 10.2 (Roberts and Sloan 1999): a transformation of the popular, but very specific, Potts-Guy (PG) Eq. 10.3 (Potts and Guy 1992) into more general, useful terms.

$$\text{Log}\, J_M = x + y \log S_{LIPID} + (1-y) \log S_{AQ} - z\text{MW} \quad (10.2)$$

$$\text{Log}\, P = x + y \log K_{OCT:AQ} - z\text{MW} \quad (10.3)$$

When a database of those homologous series of more lipid soluble prodrugs ($n=42$) comprised of their molecular weights, MW, their solubilities in isopropyl myristate (IPM), S_{IPM} ($S_{IPM}=S_{LIPID}$ in Eq. 10.2), and in water, S_{AQ}, and their maximum fluxes from IPM through hairless mouse skin, J_{MMIPM}, were collected and fitted to Eq. 10.2, the values for the coefficients were $x=-0.211$, $y=0.534$, $z=0.00364$, and $r^2=0.937$ (Roberts and Sloan 1999). The size of the J_{MMIPM} database has since been increased to $n=94$, and the values for the coefficients are now $x=-0.377$, $y=0.527$, $z=0.00346$, and $r^2=0.900$ (Majumdar et al. 2012). The maximum fluxes of prodrugs and non-prodrug through human skin in vitro and in vivo, respectively, from mineral oil (MO), J_{MHMO}, their solubilities in mineral oil, S_{MO} ($S_{MO}=S_{LIPID}$ in Eq. 10.2), and in water, S_{AQ}, and their MW also gave good fit to Eq. 10.2: $x=-1.83$, $y=0.462$, $z=0.00153$, and $r^2=0.80$ for $n=30$ prodrugs (Sloan et al. 2011); $x=-1.459$, $y=0.72$, $z=0.00013$, and $r^2=0.934$ for $n=10$ nonsteriodal anti-inflammatory drugs (Wenkers and Lippold 1999; Roberts and Sloan 2001). Thus, good fits to Eq. 10.2 are obtained if the vehicle is a lipid (IPM or MO) and the lipid solubility of the permeant, S_{LIPID}, and S_{AQ} are independent valuables.

A similar strong dependence of maximum flux through hairless mouse from water, J_{MMAQ}, on S_{IPM} ($S_{IPM}=S_{LIPID}$ in Eq. 10.2) and S_{AQ} for some of the members of the $n=94$ J_{MMIPM} database was observed where $x=-2.30$, $y=0.575$, $z=0.0016$, and $r^2=0.903$ for $n=32$ (Sloan et al. 2003; Wasdo et al. 2009). Also a strong dependence of maximum flux through human skin in vitro from water, J_{MHAQ}, on the solubilities of the permeants in octanol, S_{OCT} ($S_{OCT}=S_{LIPID}$ in Eq. 10.2) and S_{AQ}, was observed where $x=-2.506$, $y=0.538$, $z=0.00402$, and $r^2=0.839$ for $n=185$ (Juntunen et al. 2008). Even maximum flux through silicone membranes from water, J_{MPAQ}, for some of the members of the $n=94$ J_{MMIPM} database was found to be dependent on S_{IPM} ($S_{IPM}=S_{LIPID}$ in Eq. 10.2) and S_{AQ} where $x=-1.837$, $y=0.742$, $z=0.00435$, and $r^2=0.86$ for $n=38$ (Synovec et al. 2013). Thus, good fits to Eq. 10.2 are obtained regardless of whether the membrane is mouse, human, or silicone and regardless of whether the vehicle is a lipid or aqueous. Since the solubilities of the permeant in a lipid and in water are both necessary to define maximum flux, functional groups should be incorporated into the promoieties of prodrugs that can ideally increase both lipid and aqueous solubilities to increase maximum flux and by inference S_{M1}.

The reason that increasing both lipid and aqueous solubilities of the drug is important to increasing its solubility in skin, and hence its topical delivery, can be found in the structure of the barrier to topical delivery – the intercellular compartment of the stratum corneum (SC). The intercellular compartment consists of lamellar double bilayers comprised of lipid components such as ceramides, cholesterol, and fatty acids which have polar groups attached to them. These polar head groups have water associated with them so that for a permeant to cross these bilayers perpendicular to the axis of the bilayers, it must alternately cross lipid and aqueous phases (Sloan and Wasdo 2003; Sloan et al. 1984, 2011a, b). Thus, a balance of solubility in both lipid and aqueous phases by the drug (or increased lipid and aqueous solubility by its prodrug) is necessary for its most efficient permeation of the intercellular compartment of the SC. The agreement between the experimentally measurable physicochemical parameters in the theoretically derived Roberts-Sloan equation and in the biochemically based biphasic solubility model (Sloan et al. 2011a, b) for the barrier to permeation is encouraging.

10.3 Acyl Versus Soft Alkyl Promoieties

The promoieties that have been used to increase lipid and aqueous solubilities can be divided into two types based on whether they are attached directly to the functional group in the parent drug that is to be modified or indirectly through a

methylene or vinylogous methylene (aryl methylene) spacer (Sloan 1989, 1992; Sloan and Wasdo 2003). In each type, the enabling functional group is usually a carbonyl-type functional group because of its sensitivity to cleavage by chemical or enzymatic hydrolysis. Generally these types have been referred as acyl and soft alkyl-type promoieties, respectively. Cleavage of the acyl-type promoiety regenerates the parent drug directly while cleavage of the acyl group in the soft alkyl promoiety generates an intermediate drug–X–CHR–X′H from drug–X–CHR–X′–(C=X″)–X‴R′: X, X′, X″, and X‴ can be O, N, or S and R and R′ can be alkyl or aryl. The intermediate is designed to be intrinsically unstable and undergo rapid and complete chemical hydrolysis to the parent drug–X–H. The advantage of the soft alkyl prodrug approach is that the stability of the prodrug (as well as its attendant physicochemical properties) is not limited by the functional group in the parent drug to which it is attached. Generally, changing X will change the biochemical and/or pharmacological activity of the drug, but changing X′ to obtain a more or less stable or more or less soluble prodrug will not. Of course X″ and X‴ can be changed in the same ways that they could have been if an acyl prodrug approach had been used.

10.4 Mechanisms for Penetration Enhancement

10.4.1 Decrease Crystal Lattice Energy by Masking Hydrogen Bond Donor Functional Groups

Regardless of whether the prodrug is derived from an acyl or soft alkyl-type promoiety, there are two general mechanisms by which both types of promoieties can increase both lipid and aqueous solubilities. The first mechanism has its basis in decreasing the crystal lattice energy of the parent drug by modifying polar groups capable of forming intermolecular hydrogen bonds. In many if not most drug molecules, the X in drug–X–H is a heteroatom which causes X–H to be polarized because of the difference in electronegativities between X and H. This polarized drug–X–H bond is capable of forming intermolecular hydrogen bonds within the crystal lattice which leads to low solubilities especially in lipids but also frequently in water. The polarization is further attenuated if an electron withdrawing carbonyl-type functional group is attached to X–H to give drug–(O=C)–X–H. Examples of this type of drug molecule, which can be measurably but not highly ionized at physiological pH, include heterocycles such as 5-flurouracil (5-FU) (drug–(O=C)–NH) and 6-mercaptopurine (6-MP) (drug–(S=C)–NH) which are very high melting and exhibit low solubilities in both water and lipids. In other examples such as parent drugs containing a carboxylic acid functional group (drug–(O=C)–OH), the functional group is so highly polarized that it becomes highly ionized at physiological pH which does not allow it to readily cross the lipid phase of the alternating lipid-aqueous phases of the biological barrier. An important class of drugs that belong to this category is the nonsteroidal anti-inflammatory drugs. Another example of this class are the nucleotide-based drugs where the highly ionized functional group is a phosphate group. Simply masking the hydrogen bond donating abilities of the functional group by replacing the H in the drug–X–H with either an acyl or soft alkyl group decreases the melting point (*mp*) and increases the lipid solubility (S_{LIPID}) as well as frequently increasing the aqueous solubility (S_{AQ}) of the prodrug compared to the parent drug, especially for the shorter alkyl chain members of a homologous series (Sloan 1989).

Examples of the results that can be obtained by masking the polar functional groups in drugs to increase S_{LIPID} (S_{IPM}) and S_{AQ} and to increase topical delivery of the parent drug are several prodrugs of 5-FU.

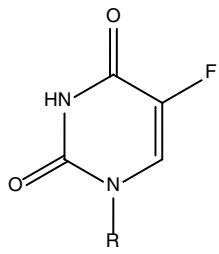
The *mp*, S_{AQ}, S_{IPM}, log partition coefficients between IPM and pH 4.0 buffer (log $K_{IPM:AQ}$), and rates of delivery of total 5-FU containing species through hairless mouse skin from an IPM vehicle in vitro (J_{MMIPM}) for four different series of prodrug of 5-FU are given in Table 10.1: three acyl types and a one soft alkyl type. The first acyl type of prodrug of 5-FU that was evaluated for its ability to increase the delivery of 5-FU was the alkylaminocarbonyl-5-FU (1-AAC-5-FU) prodrugs

Table 10.1 Prodrugs of 5-fluorouracil

Prodrugs, $R=$[a]	mp[b]	S_{IPM}[c]	S_{AQ}[c, d]	Log $K_{IPM:\ AQ}$[e]	J_{MMIPM}[f]
1-AAC-5-FU					
1, C1NHC=O	212	0.30	3.69	−1.09	0.208
2, C2NHC=O	180	2.79	7.76	−0.44	0.600
3, C3NHC=O	139	12.4	8.98	0.14	0.746
4, C4NHC=O	133	24.6	5.11	0.68	0.515
5, C6NHC=O	113	44.9	0.36	2.09	–
6, C8NHC=O	91	46.9	0.030	3.21	0.060
1-AOC-5-FU					
7, C1OC=O	160	2.13	112	−1.72	2.62
8, C2OC=O	128	13.1	175	−1.12	5.92
9, C3OC=O	126	15.2	42.2	−0.44	2.31
10, C4OC=O	98	33.8	24.1	0.15	2.23
11, C6OC=O	67	153	4.94	1.49	1.54
12, C8OC=O	98	36.2	0.13	2.45	0.29
1-AC-5-FU					
13, C1C=O	130	22.1	120	−0.73	9.3
14, C2C=O	131	36.4	47.6	−0.12	4.3
15, C3C=O	146	17.4	6.50	0.43	1.3
16, C4C=O	121	39.2	3.48	1.05	1.0
17, C5C=O	102	112.7	2.94	1.58	1.1
18, C7C=O	84	110.7	0.15	2.88	0.60
1-ACOM-5-FU					
19, C1(C=O)OCH_2	124	3.29	183	−1.74	2.88
20, C2(C=O)OCH_2	102	9.83	167	−1.23	3.82
21, C3(C=O)OCH_2	91	14.4	42.4	−0.47	2.57
22, C4(C=O)OCH_2	88	14.8	12.3	0.08	1.29
23, C5(C=O)OCH_2	91	14.7	2.23	0.82	0.56
24, C7(C=O)OCH_2	108	9.99	0.17	1.77	0.12
5-FU, **H**	284	0.049	85.4[g]	−3.24[h]	0.240

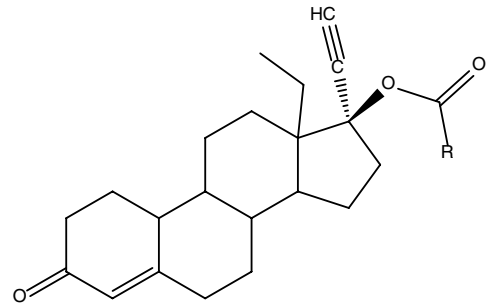
[a]C1, C2, etc., refer to the number of carbons in alkyl chain
[b]Units of °C
[c]Solubilities in units of m*M*
[d]Estimated from $S_{IPM}/K_{IPM:AQ}$
[e]Partition coefficient between IPM and pH4.0 buffer at 23 ± 1 °C
[f]Values for the delivery of total species containing 5-FU through hairless mouse skin from IPM in vitro in units of μmol cm^{-2} h^{-1}
[g]Solubility in pH 4.0 buffer
[h]Log solubility ratio between pH 4.0 buffer and IPM

(Table 10.1 and Fig. 10.1). Initially only the longer alkyl chain members of the series were evaluated (**4**–**6**) (Sasaki et al. 1990), but subsequently the shorter alkyl chain members (**1**–**3**) were evaluated, and one of them, **3**, was found to give the greatest increase in the delivery of the total 5-FU containing species, J_{MMIPM} (Sloan et al. 1993). All of the 1-AAC-5-FU prodrugs exhibited lower mp than 5-FU and all of them were more soluble in IPM than 5-FU: from 6 times for **1** to almost 1,000 times for **6**. However, the most lipid soluble member evaluated, **6**, gave only 0.25 times the flux of 5-FU. None of the 1-AAC-5-FU prodrugs was even as soluble in water as 5-FU, and the C3 member (**3**), not the shortest alkyl chain member of the series (**1**), gave the highest S_{AQ} value: only 0.11 times S_{AQ} for 5-FU. The C3 member also gave the greatest increase in J_{MMIPM} values for the series, albeit only three times. Thus, as predicted (Sloan 1992, 1989; Sloan and

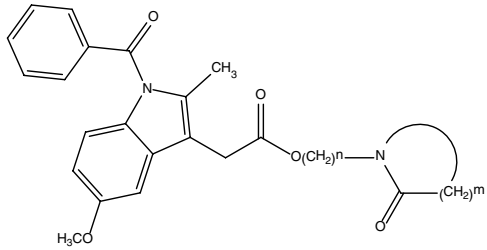


5-FU, R = H

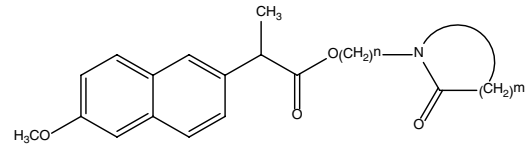
1-24



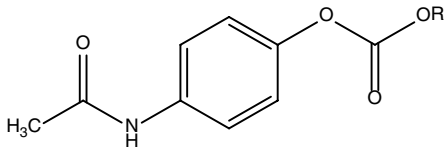
26-29



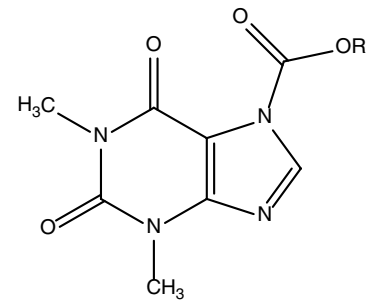
31-34



36-39



40-46



47-54

Fig. 10.1 Chemical structures of prodrugs for compounds 1-54

Wasdo 2003; Sloan et al. 1984), for a more lipid soluble homologous series of prodrugs, the more water soluble member gave the highest J_{MMIPM} value. The low increase in J_{MMIPM} can be attributed to the low S_{AQ} values exhibited by the 1-ACC-5-FU prodrugs compared to subsequent series, and the low S_{AQ} values can be attributed to the fact that one of the hydrogen bond donor functional groups, (O = C)–NH, in 5-FU was merely replaced with another hydrogen bond donor group, N–(O = C) –NH, in the promoiety. The potential for forming intermolecular

hydrogen bonds was not decreased significantly and the added alkyl group in the promoiety further depressed S_{AQ}.

The second acyl type of prodrug of 5-FU that was evaluated was the alkyloxycarbonyl-5-FU (1-AOC-5-FU) prodrugs (Table 10.1, Fig. 10.1) (Beall et al. 1994). In this series the hydrogen bond donating group in the parent drug has not been replaced with another hydrogen bond donating group in the promoiety so the *mp* are somewhat lower than the corresponding members in the 1-AAC-5-FU series except for the C8 member of the series. Consequently, the members of the 1-AOC-5-FU series were also somewhat more soluble in IPM than the members of the 1-AAC-5-FU series except for the C8 member, **12**; and the worst member of the series in terms of increased S_{IPM} was 43 times instead of 6 times more soluble in IPM than 5-FU. However, the big difference between the two series was in the S_{AQ} values. Not only were two members of the series more water soluble than 5-FU, **7** and **8** (1.3 and 2 times, respectively), but they were all more water soluble than the corresponding members of the 1-AAC5-FU series (from 30 to 4.3 times). Thus, since the 1-AOC-5-FU series was more soluble in lipids and in water, as predicted (Sloan 1989, 1992; Sloan et al. 1984), they delivered more total 5-FU species through hairless mouse skin than the 1-AAC-5-FU series (from 3 to 12.5 times). Also, as predicted (Sloan 1989, 1992; Sloan et al. 1984) the C2 member, **8**, which was the most water soluble member of the series gave the greatest increase in J_{MMIPM} compared to 5-FU (24.7 times), and not the most lipid soluble member of the series, **11**. The next most water soluble member, **7**, gave the next greatest increase in J_{MMIPM} compared to 5-FU (11 times).

Based on previous literature, the 1-AOC series was expected to be more stable than the 1-AAC series of prodrugs of 5-FU. Whereas the amount of intact prodrug delivered by the 1-AAC series was in the 6–10 % range, the amount delivered by the 1-AOC series was in the 40–70 % range and was up to 90 % for the best performing member of the series, **8**. If delivery through the skin and subsequent slower release of 5-FU systemically was the target of topical delivery, then the members of the 1-AOC-5-FU series performed well. On the other hand, if delivery into the skin was the target, then a more rapidly hydrolyzing type of prodrug of 5-FU would be required.

The third acyl type of prodrug 5-FU that was evaluated was the alkylcarbonyl-5-FU (1-AC-5-FU) prodrugs (Table 10.1, Fig. 10.1) (Beall et al. 1996). The members of this series were known to hydrolyze quite rapidly ($t_{1/2}$ = 3–5 min), so it was expected that only 5-FU would be delivered through the skin. This expectation was realized, and only 5-FU and no intact prodrug was observed in the receptor phase after application of 1-AC-5-FU prodrugs in IPM in diffusion cell experiments. All of the members of the 1-AC series were much more soluble in IPM than 5-FU (355–2,300 times), and one member, C1 (**13**), was more soluble in water than 5-FU (1.4 times). However, direct comparisons between the 1-AC series and either the 1-AOC or the 1-AAC series based only on the alkyl chain length in the promoiety would be misleading without taking into account the added heteroatom in the latter two series. For example, we will compare the OC1 member (**7**) of the 1-AOC series with the C2 member of the 1-AC series (**14**), the OC2 with the C3, the OC3 with the C4, the OC4 with the C5, and the OC6 with the C7. Using these interseries comparisons, the members of the 1-AC series were more soluble in IPM (1.3–17 times) than those of the 1-AOC series, except for **18** compared to **11**. On the other hand, the members of the 1-AOC series were more soluble in water (2.4–33 times) than those of the 1-AC series, and as predicted (Sloan 1989, 1992; Sloan et al. 1984) they all gave higher J_{MMIPM} values than the corresponding members of the 1-AC series, except for OC1, **7**, versus C2, **14**. Prodrug **7** was only 2.4 times more soluble in water than **14,** while **14** was 17 times more soluble in IPM than **7**. Prodrug **14** exhibited a somewhat better balance of S_{AQ} and S_{IPM} than **7** and gave a higher J_{MMIPM} value (1.6 times). However, within the 1-AC series the C1 member, **13**, which was the more water soluble member of the series and not one of the more lipid soluble members, gave the

greatest enhancement in J_{MMIPM} (39 times that of 5-FU).

In the 1-AC series the effect of the *mp* on solubilities and ultimately on flux can be readily illustrated. The C3 member of the series, **15**, exhibited a higher *mp* than either the shorter, **14**, or longer alkyl chain member, **16**, and hence exhibited a lower S_{IPM} value than those members. The S_{AQ} value for **15** also dropped off more rapidly than expected as did its J_{MMIPM} value. On the other hand, the log *K* values appeared normally spaced and the methylene π values derived from the log *K* values only varied by 10 %: $\pi = 0.59 \pm 0.05$. Thus, log *K* values are no substitute for experimental solubilities for purposes of predicting trends in J_M.

The example of the use of a soft alkyl prodrug in the designs of prodrugs to increase S_{IPM} and S_{AQ} and to increase the topical delivery of the parent drug is also a 5-FU prodrug: the 1-alkylcarbonyloxymethyl-5-FU (1-ACOM-5-FU) prodrugs (Table 10.1, Fig. 10.1) (Taylor and Sloan 1998). As expected each of the 1-ACOM-5-FU prodrugs exhibited a lower *mp* than 5-FU since a hydrogen bond donor group had been masked in the prodrug. Also as expected each was much more soluble in IPM than 5-FU (67–302 times), and there were members, **19** and **20**, that were more soluble in water than 5-FU (2.1 and 1.9 times, respectively). As predicted (Sloan 1989, 1992; Sloan et al. 1984) **19** and **20** were the members that gave the greatest enhancement in J_{MMIPM} (12 and 16 times, respectively) and not the more lipid soluble, longer alkyl chain members of the series. However, to compare members of the 1-ACOM series with members of any one of the 1-acyl series, the added heteroatom and methylene spacer in the 1-ACOM series needs to be taken into account. Thus, comparison should be made between the C1 member of the 1-ACOM series, **19**, and the C3 member of the 1-AC, **15**; or the C2 member of the 1-AOC series, **8**, the C2 member of the 1-ACOM series, **20**, and the C4 member of the 1-AC series, **16**; or the C3 member of the 1-AOC series, **9**, etc. Using these interseries comparisons, the members of the 1-ACOM series were less soluble in IPM but much more soluble in water (15.0–48.0 times) than the members of the 1-AC series, and their J_{MMIPM} values were greater except for the comparison between **23** and **18** where the J_{MMIPM} values were equivalent. On the other hand, although the members of the 1-ACOM series were less soluble in IPM than the members of the 1-AOC series, in this comparison only two members of the 1-ACOM series, **20** and **21**, were substantially more soluble in water (4.0 and 1.8 times, respectively) and hence gave greater J_{MMIPM} values than the corresponding members of the 1-AOC series. In the comparison of **19** and **8**, the S_{AQ} values were very close and **8** was four times more lipid soluble, so **8** gave a two times greater increase in J_{MMIPM}. Similarly, **11** was 2.2 times more water soluble and ten times more IPM soluble than **23**, so **11** gave a three times greater increase in J_{MMIPM}.

Thus, the general mechanism for increasing lipid and aqueous solubilities of a drug by decreasing its ability to form intermolecular hydrogen bonds in the crystal lattice can be very effective (11–40 times enhancement of flux). But it is essential to evaluate the shorter alkyl chain members of any series to be considered because those are the members that are most likely to be more water soluble as well as more lipid soluble. In the examples based on 5-FU, the increases in flux realized with these acyl and soft alkyl prodrug approaches are more than sufficient to enlarge the indicated use of topical 5-FU from treating only actinic keratoses of the scalp (Dillaha et al. 1965) to treating recalcitrant psoriasis on less permeable areas of the body (Tsuji and Sugai 1972).

10.4.2 Incorporation of Water Solubility Enhancing Functional Groups into Promoiety

The second general mechanism by which acyl and soft alkyl promoieties can be used to increase the lipid and aqueous solubilities of prodrugs compared to their parent drugs is to incorporate polar, water solubilizing groups into their promoieties. In the examples illustrating the previous mechanism, the primary effect of the prodrug

modification was to increase lipid solubility because the promoiety contained only an enabling functional group and a simple alkyl group. Although large increases in S_{IPM} were realized for all members of homologous series, increases in S_{AQ} were usually modest (less than two times) and only for the shorter alkyl chain members. In the examples illustrating the second general mechanism, the promoiety contains an additional amine, amide, ether, or diol functional group which in retrospect could have been designed specifically to increase S_{AQ}. However, in most examples S_{AQ} values were not available from the original references.

The first example is the use of a diol functional group in the promoiety to increase the S_{AQ} of the prodrug and hence J_M for the delivery of the parent drug.

Although the stated rationale was that more hydrophilic prodrugs could overcome the perceived rate limiting contribution of the aqueous viable epidermis part of the barrier to permeation of the skin by highly lipophlic drugs (Friend et al. 1988), the success of such prodrugs would also support a model for permeation where alternating lipid-aqueous barriers must be crossed in the intercellular compartment of the SC (Sloan et al. 1984, Sloan et al. 2011a, b. In Table 10.2 the *mp* (°C), solubilities in mixtures of ethanol and water (S_{VEH}), log *K* between octanol, and pH 7.4 buffer (log $K_{OCT:AQ}$) and fluxes of total species delivered from suspensions in ethanol and water (VEH) through rat skin in vitro (J_{MHVEH}) are given for the evaluation of four acyl prodrugs of levonorgestrel.

Two of the prodrugs in Table 10.2 (Fig. 10.1) were simple alkylcarbonyl prodrugs: **26** and **27**. Neither was representative of the shorter alkyl chain members of the series which would have had the greatest potential for increased aqueous as well as lipid solubility. Since **26** and **27** were both more soluble in 95 % ethanol than levonorgestrel, **25**, was soluble in 100 % ethanol, it is reasonable to assume they would also be more soluble in octanol and hence be defined as more lipophilic than **25**. Since partition coefficients for **26** and **27** could not be obtained because no **26** or **27** could be measured in the aqueous phase (while **25** could), it is reasonable to assume that

Table 10.2 Prodrugs of levonorgestrel

Prodrugs, *R* =	*mp*[a]	S_{VEH}[b]	Log $K_{OCT:AQ}$[c]	J_{MHVEH}[d]
25, levonorgestrel	240	19.2 (100)	3.70	0.00019
26, C_5H_{11}	86	604 (95)		
		12.9 (62)		0.00058
27, C_4H_9	170	28.3 (95)		0.00026
28, $OCH_2CH(OH)CH_2OH$	148	30.2 (40)	3.22	0.0063
29, $O(CH_2)_4CH(OH)CH_2OH$	53	396 (40)	3.75	0.0030

[a]Units of °C

[b]Solubilities in mixtures of ethanol:water in units of m*M* where the value in parenthesis is percentage of ethanol in the mixture

[c]Partition coefficient between octanol and water at 24 °C

[d]Values for delivery of total species containing levonorgestrel from suspensions in mixtures of ethanol:water (given in the S_{VEH} column) through rat skin in vitro in units of μmol cm^{-2} h^{-1}

26 and **27** were less hydrophilic than **25**. Finally, since the flux of **25** from various ethanol and water (40–100 %) mixtures did not vary significantly (applications of ethanol and water mixtures did not change S_{M1}), it can be assumed that delivery of total species containing **25** by the prodrugs from widely different ethanol and water mixtures can be compared to the average flux generated by the application of **25** (0.00020 μmol cm^{-2} h^{-1}) in ethanol and water mixtures. Thus, **26** and **27**, which were more soluble in lipids but estimated to be less soluble in water, gave 3 and 1.3 times greater J_{MHVEH} values, respectively, than **25**. Only **25** was observed in the receptor phases.

By comparison, since the two prodrugs containing a diol functional group in the promoiety, **28** and **29**, were both more soluble in an ethanol and water mixture that was primarily aqueous in composition (40 % ethanol) than **25** was in 100 % ethanol, it can be reasonably assumed that **28** and **29** were more soluble in water than **25**. In addition, since **28** and **29** exhibit log $K_{OCT:AQ}$ that were comparable to that of **25** and were more soluble in water than **25**, it can be reasonably assumed that **28** and **29** were more soluble in octanol than **25**, that is, more lipophilic. Thus, since **28** and **29** were

more soluble in a lipid and in water than their parent drug, as predicted (Sloan 1989, 1992; Sloan et al. 1984), they gave much larger increases in J_{MHVEH} than the simple alkylcarbonyl prodrugs that were only more soluble in a lipid (31 and 15 times, respectively). However, because of their greater stabilities as carbonate esters, they delivered mostly intact prodrug through the skin (80 and 96 %, respectively).

The second example is the use of an amide functional group in the promoiety to increase S_{AQ} of the prodrug and hence J_M for the delivery of the parent drug. The first report of the synthesis of a promoiety containing an amide functional group as part of an effort to increase topical delivery was for theophylline: 7-(*N*, *N*-diethysuccinamoyloxymethyl) theophylline (Sloan and Bodor 1982). However, the prodrug was never completely evaluated. More recently 1-alkylazacycloalkan-2-one esters of indomethacin, **30** (Bonina et al. 1991), and naproxen, **35** (Bonina et al. 1993), have been synthesized and evaluated.

In Table 10.3 (Fig. 10.1) the values of S_{IPM}, S_{AQ}, and rates of delivery of total species containing **30** or **35** from water through human skin in vitro (J_{Mt}) are given. For the indomethacin series, the second member of the series, **32**, was the only member of the series that exhibited a greater S_{AQ} than indomethacin, and although it was barely as soluble in IPM as indomethacin, it caused the greatest enhancement of J_{MHEV} (4 times). The more lipid soluble but less water soluble members gave lower enhancement of J_{MHAQ}. For the naproxen series, the first member of the series, **36**, was more soluble in water (8 times) than naproxen and was more soluble in water than the other members of the series. Prodrug **36** was also more soluble in IPM than the other members of the series but none were as soluble as naproxen. Thus, **36**, which was more soluble in lipids and water than the other members of the series, gave the greatest enhancement in J_{MHAQ} (2.7 times) as would be predicted (Sloan 1989, 1992; Sloan et al. 1984).

Table 10.3 Prodrugs of indomethacin and naproxen

Prodrugs	S_{IPM}[a]	S_{AQ}[a]	J_{MHAQ}[b]
30, indomethacin	7.82	0.011	0.23
31, $n=2$, $m=3$	6.00	0.0096	0.80
32, $n=2$, $m=4$	7.34	0.016	0.96
33, $n=2$, $m=5$	19.0	0.012	0.77
34, $n=2$, $m=6$	27.5	0.0074	0.19
35, naproxen	23.5	0.045	5.1
36, $n=2$, $m=3$	21.1	0.355	13.8
37, $n=2$, $m=4$	18.8	0.249	8.9
38, $n=2$, $m=5$	16.7	0.032	4.0
39, $n=2$, $m=6$	7.64	0.011	2.8

[a]Solubilities in units of m*M*

[b]Values for delivery of total species containing parent drug from water through human skin in vitro in nmol cm^{-2} h^{-1}

There are two additional observations that can be made about these two series of prodrugs which have an amide functional incorporated into the promoiety. First, although the S_{IPM} values for the two series are comparable, the S_{AQ} values for the naproxen series (**36**–**39**) are almost uniformly ten times greater than those for the indomethacin series (**31**–**34**), and consequently the J_{MHAQ} values for the naproxen series are almost uniformly ten times greater. Second, although more labile soft alkyl-type prodrugs ($n=1$) had been synthesized, they were never evaluated because they were considered to be too labile. On the other hand, the $n=2$ prodrugs were too stable, and only 10–12 % of either parent drug was observed in the receptor phases of the diffusion cell experiments in which they were evaluated. It would have been interesting to have evaluated the $n=1$ series of prodrugs using an IPM vehicle, in which they would have been stable, to determine how effective they might have been at delivering the parent drug.

The third example is the use of an amine functional group in the promoiety to increase the S_{AQ} of the prodrug and hence J_M. Again the first report of the synthesis of a promoiety containing an amine functional group as part of an effort to increase the topical delivery of a parent drug was for theophylline: 7-(*N*, *N*-dimethylaminoacetyloxymethyl) theophylline (Sloan and Bodor 1982). However, again the prodrug was never completely evaluated. More recently the 17-(4′-dimethylaminobutyrate) ester prodrug of testosterone was evaluated using a 10 % solution of the prodrug in pH 7.4 buffer (Milosovich et al. 1993). Compared to the

delivery from a suspension of testosterone in pH 7.4 buffer, the prodrug was 60 times more effective at delivering testosterone. Although no solubility data were reported, a 10 % solution of the prodrug was evaluated which suggests that it is substantially more soluble in water than testosterone which was soluble only to the extent of 0.004 %. The 2-diethylaminoethyl ester prodrug of indomethacin was also evaluated by the same group (Jona et al. 1995). It was reported that the prodrug drug was 3.7 times more soluble in pH 7.4 buffer and its partition coefficient between octanol and pH 7.4 buffer was 6.2 times greater than that of indomethacin so the prodrug was also much more soluble in octanol (23 times). Thus, it was entirely predictable (Sloan 1989, 1992; Sloan et al. 1984) that the prodrug gave a 4.3 times enhancement in the delivery of total indomethacin containing species through human skin in vitro.

The fourth example is the use of an ether functional group in the promoiety to increase the S_{AQ} of the prodrug and hence J_M. There are numerous reports in the literature where polyoxyethylene (POE) esters have been used as prodrugs to enhance oral delivery (Greenwald 2001) but only a few where POE esters have been used to enhance topical delivery. One of the limiting factors associated with using data from previous reports on the use of prodrugs containing oxyethylene groups in their promoieties to enhance the topical delivery of their parent drugs to design new prodrugs is the lack of experimental values for S_{LIPID} (S_{OCT}, S_{MO}, S_{IPM}), S_{AQ}, and $K_{LIPID:AQ}$ in the literature (Bonina et al. 2001). This lack of experimental solubility and K data makes it impossible to predict changes in the solubility of the prodrug, attributable to the properties of the promoiety, compared to its parent in the membrane, S_{M1}, and hence J_M in Eq. 10.2.

However, there are several examples where those experimental S_{LIPID} and S_{AQ} values for prodrugs containing oxyethylene groups in their promoieties have been reported together with their corresponding maximum flux values, J_M. In the first example, the effect of incorporating one oxyethylene group into carbonate derivatives of acetaminophen, APAP (Fig. 10.1), on their S_{LIPID} and S_{AQ} was compared with the effect of incorporating an alkyl group into carbonate derivatives of APAP on their S_{LIPID} and S_{AQ} (Table 10.4) (Wasdo and Sloan 2004).

The resulting effect on experimental J_{MMIPM} was predictable based on the fit of the data to Eq. 10.2 (Roberts and Sloan 1999). The best alkyl carbonate in terms of enhancing J_{MMIPM} was the C1 derivative, and the best oxyethylene carbonate was $CH_2CH_2OCH_3$. Although the $CH_2CH_2OCH_3$ carbonate was equally soluble in IPM and somewhat more soluble in water than the C1 carbonate, the C1 carbonate produced the greater J_M. The slightly better S_{AQ} of the $CH_2CH_2OCH_3$ carbonate was offset by its higher molecular weight which was predicted by Eq. 10.2 (Roberts and Sloan 1999) to reduce the value of J_M. Note that the solubility ratio (SR) for

Table 10.4 Acetaminophen, APAP, prodrugs

4-AOC-APAP	MW	mp[a]	Log S_{IPM}[b]	Log S_{AQ}[b]	Log J_{MMIPM}[c]
40, C1[d]	209	115	1.076	1.314	0.00
41, C2[d]	223	122	0.968	0.577	−0.76
42, C3[d]	237	106	1.375	0.427	−0.45
43, C4[d]	251	120	1.143	−0.377	−1.01
44, C6[d]	279	110	1.220	−1.328	−1.49
45, $CH_2CH_2OCH_3$	253	81	1.013	1.537	−0.11
46, $CH(CH_3)CH_2OCH_3$	267	123	0.529	0.516	−1.06
APAP	151	170	0.279	2.000	−0.29

[a]°C

[b]Units of mM

[c]Units of μmole cm^{-2} h^{-1}

[d]C1, C2 indicates the numbers of carbons in alkyl group

the $CH(CH_3)CH_2OCH_3$ carbonate was greater than that for the $CH_2CH_2OCH_3$ carbonate derivative (log $SR = 0.013$ and -0.52, respectively), but it was less soluble in both IPM and water than the $CH_2CH_2OCH_3$ carbonate derivative so it only produced about one tenth the maximum flux. This illustrates how misleading *SR* or *K* can be in predicting flux and indesigning optimized topical products.

Similarly, in the second example the effect of incorporating one or two oxyethylene groups into carbamate derivatives of theophylline, Th-H (Fig. 10.1), on their experimental S_{IPM} and S_{AQ} values was compared with the effect of incorporating alkyl groups into carbamate derivatives of Th-H on their S_{IPM} and S_{AQ} values (Table 10.5) (Majumdar et al. 2012).

Again the resulting effect on experimental J_{MMIPM} was predicted based on the fit of the data to Eq. 10.2 (Roberts and Sloan 1999). The best alkyl carbamate in terms of increasing J_{MMIPM} was the C3 derivative and the best oxyethylene carbamate derivative was the $(CH_2CH_2O)_2CH_3$ derivative. The C3 alkyl carbamate was essentially equal in solubility in water to the C2 alkyl carbamate, but it was about 20 times more soluble in IPM. Therefore, the J_{MMIPM} for the C3 alkyl carbamate was about four times that of the C1 regardless of the negative effect of its increased molecular weight predicted by Eq. 10.2 (Roberts and Sloan 1999). Although the $(CH_2CH_2O)_2CH_3$ carbamate derivative was only about 0.25 times as soluble in IPM as the C3 alkyl carbamate derivative, it was 11 times more soluble in water. Therefore, the J_{MMIPM} for the $(CH_2CH_2O)_2CH_3$ carbamate derivative was about three times that of the C3 alkyl derivative regardless of the negative effect of its increased molecular weight. Among the oxyethylene carbamate derivatives, the $(CH_2CH_2O)_2CH_3$ carbamate derivative was three times more soluble in water and 30 % more soluble in IPM than the $CH_2CH_2OCH_3$ carbamate derivative so, as predicted by Eq. 10.2 (Roberts and Sloan 1999), its J_M value was about two times that of the $CH_2CH_2OCH_3$ carbamate derivative. Although the $CH(CH_3)CH_2OCH_3$ carbamate derivative was almost two times more soluble in IPM, it was only 0.40 times as soluble in water as the $CH_2CH_2OCH_3$ carbamate derivative so, together with its increased molecular weight, the effect of its solubilities on J_{MMIPM} led to its lower J_{MMIPM} value. Again, the log *SR* value for the $CH(CH_3)CH_2OCH_3$ carbamate derivative was much more positive than that of the other oxyethylene carbamate derivatives, but its J_{MMIPM} value was lower, illustrating the misleading effect of *SR* and *K* in predicting flux.

In both examples, the incorporation of oxyethylene groups into the promoieties of prodrugs led to enhanced solubility properties of the prodrugs compared to their parent compounds that led to higher J_M values.

Table 10.5 Theophylline, Th-H, prodrugs

7-AOC-Th	MW	mp[a]	Log S_{IPM}[b]	Log S_{AQ}[b]	Log J_{MMIPM}[c]
47, C1[d]	238	175	0.28	1.45	−0.54
48, C2[d]	252	141	0.65	1.18	−0.68
49, C3[d]	266	87	1.59	1.43	0.03
50, C4[d]	280	82	1.70	0.93	−0.19
51, C6[d]	294	79	1.69	−0.27	−0.82
52, $CH_2CH_2OCH_3$	282	96	0.87	1.99	0.21
53, $(CH_2CH_2O)_2CH_3$	326	64	0.97	2.49	0.56
54, $CH(CH_3)CH_2OCH_3$	296	104	1.15	1.61	−0.31
Th-H	180	170	−0.47	1.66	−0.32

[a]°C

[b]Units of mM

[c]Units of μmole cm^{-2} h^{-1}

[d]C1, C2 indicates the numbers of carbons in alkyl group

Conclusion

Recognizing that one of the mechanisms for topical penetration enhancement involves increasing the solubility of the drug in the skin and that prodrugs increase the delivery of drugs into and through the skin by achieving the same, then it is quite clear that prodrugs constitute one type of penetration enhancer separate from formulation approaches. An even more powerful approach to enhancing topical delivery would be to use combinations of prodrugs with formulation approaches to enhancing topical delivery. So far there have been no reports of the use of such combinations except for simple one-component vehicles which have obviously not been optimized (Waranis and Sloan 1987). However, the possibilities with the use of such a combination approach would seem to be limitless.

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Transdermal Drug Delivery Systems Using Supersaturation

11

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Contents

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11.1 Introduction

The stratum corneum (SC) is the outermost layer of the skin and acts as a primary barrier to the permeation of chemicals across the skin. In order to achieve transdermal penetration enhancement across SC, physical and chemical methods are applied for effective drug delivery. The physical enhancement techniques include iontophoresis, sonophoresis, electroporation, microneedle-based devices, liquid jet injectors, powder injectors, ultrasound, laser radiation, radiofrequency, magnetophoresis, and temperature. The chemical penetration enhancement methods involve either disrupting the stratum corneum by fluidizing or disrupting the intercellular lipids and are achieved through the use of different chemical classes including sulfoxides, azone, pyrrolidones, fatty acids, alcohols, glycols, and terpenes (Rai 2010). The physical and chemical enhancement techniques mentioned above can be expensive and can potentially give rise to toxicity and irritation (due to disruption in the SC integrity). Supersaturation provides a potentially safe, inexpensive mechanism for penetration enhancement of drugs. Also the use of supersaturated systems does not interfere with the SC integrity.

A supersaturated system, by definition, is a system where the concentration of a chemical compound in solution exceeds that of a saturated solution. This system is thermodynamically unstable and, in many cases, results in the formation

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of crystals spontaneously (labile state). If the compound remains in solution under supersaturated conditions, the state is called metastable state. Because of the nature of metastable state, addition of foreign particles or external forces such as ultrasound, nucleation results in crystallization. The boundary between the metastable and labile states is called the critical degree of supersaturation (Fig. 11.1). The degree of supersaturation (DS) is then dependent on the concentration of the drug on the solution and the temperature of the system.

The advantages of supersaturated systems for percutaneous penetration were first recognized by Higuchi (Higuchi 1960) (Fig. 11.2). The role of supersaturation in transdermal delivery has been tested and found effective consistently in the past (Coldman et al. 1969; Guy 2007; Morrow 2007); however, in order to achieve supersaturation, the choice of optimal solvents or co-solvents is critical (Poulsen et al. 1968). The concept and the understanding of supersaturated systems were borne out of the crystallization theory, where a solution must be first of all supersaturated in order for crystals to form. The precise mechanism of nucleation and subsequent crystal growth is not fully understood. There are multiple theories proposed in the literature for predicting crystal growth (Frank 1949; Mehta et al. 1970; Rodríguez-Hornedo and Wu 1991). It has been observed that the choice of solvent is critical for the type of crystals (e.g., hydrate, alcoholate) and polymorphs formed in the solution (Corrigan and Timoney 1974; Khoshkoo 1991). It has also been observed that different polymorphs of a compound can have different saturated solubilities in a solvent and, therefore, one can be supersaturated with respect to the other. Solvate forms of drugs, sometimes called pseudopolymorphs, can also have different solubilities and dissolution rates to their corresponding non-solvate forms. Similar to polymorphs, an anhydrous form of a drug can be supersaturated with respect to its hydrate form (de Smidt et al. 1986; Davis 1993).

11.2 Method of Preparation for Supersaturated Solutions

There are four primary methods of preparing the supersaturated solutions, viz., biphasic or binary mixing (addition of a substance to a solution which reduces the solubility of the solute); evaporation; heating and then cooling; and via chemical reactions (of two or more solutes to produce a new compound which is less soluble in solution than the original starting solutes), e.g., melt extrusion technology, granulation. The supersaturated

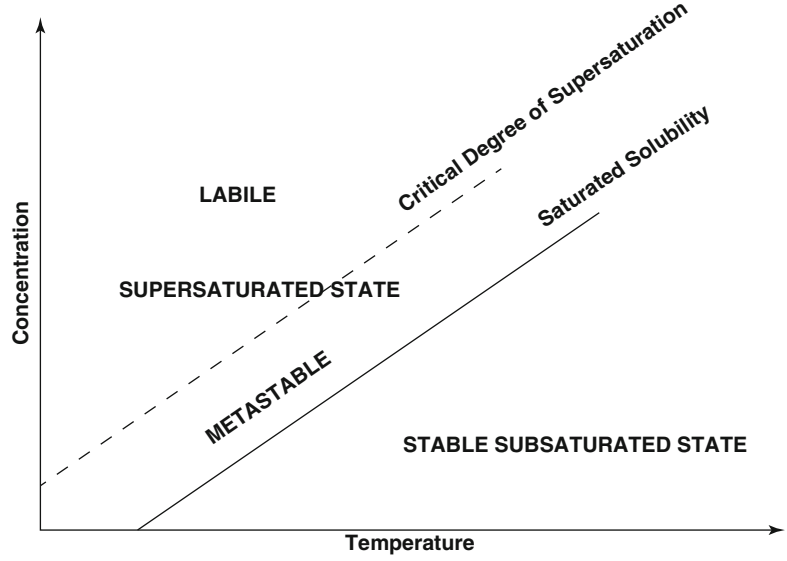

Fig. 11.1 Diagram showing the critical degree of supersaturation (*DS*) and the different stability states of supersaturated systems

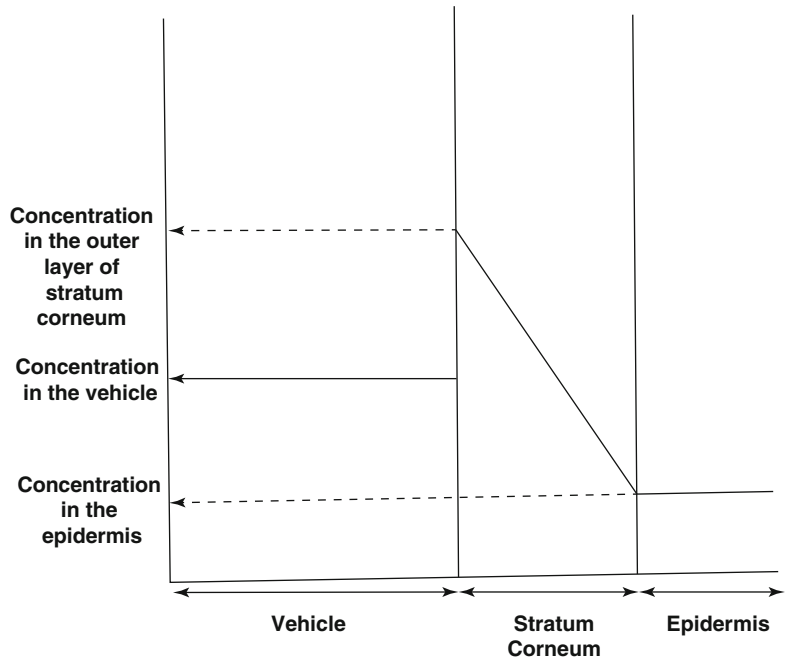

Fig. 11.2 Higuchi's model of percutaneous absorption (Higuchi 1960)

solutions prepared using all the above techniques have shown increased percutaneous permeation of drugs in the past (Adrian et al. 2002).

The preparation of biphasic mixtures can be explained by constructing a curve for the saturated solubility of the drug in a binary co-solvent system where the drug is more soluble in one of the solvents than the other (Fig. 11.3). By preparing a saturated solution of the drug in solvent B and diluting with solvent A, a point, E, is obtained along the line CD, where C represents 100 % of solvent A and D represents 100 % solvent B. This is a supersaturated solution and its degree of saturation is calculated by dividing the amount of drug in solution by its saturated solubility in the same co-solvent mixture. In this example, the solution E has two degrees of saturation. Crystallization in a biphasic system is inhibited (or retarded) by addition of an anti-nucleant polymer to solvent A (Adrian 2002).

The diffusion studies of the biphasic mixture of water to the solutions of hydrocortisone acetate in propylene glycol (PG) and the anti-nucleant polymers polyacrylate, PVP, and HPMC indicated that the supersaturated solutions gave an increase in flux when compared with the saturated solutions and that the flux was proportional to the degree of saturation. It was also found that a 0.02 % supersaturated gel of hydrocortisone acetate was bioequivalent to a 1 % cream in reducing a surfactant-induced erythema (Davis and Hadgraft 1991; Davis 1993). Similar observation with increase in flux from increasing DS has also been observed in fentanyl delivery when supersaturated solutions with different DS were prepared using PG/water and PG/ethanol systems (Santos et al. 2011). An 18-fold increase in the concentration of estradiol in the stratum corneum was observed within ten minutes of exposure to a supersaturated solution when compared with a saturated solution in the same co-solvent mixture (Megrab et al. 1995). A testosterone-supersaturated solution with 2.5° of supersaturation in 1/1 ethanol/PG was found to show flux (across hairless rat skin) similar to that of metered dose transdermal spray system under commercial development (MDTS®, Acrux Ltd., Australia, www.acrux.com.au) (Leichtnam 2006). The delivery of piroxicam from a fourfold supersaturated solution across silicone and human skin in vitro was increased four times compared to the drug delivery of a saturated solution in the

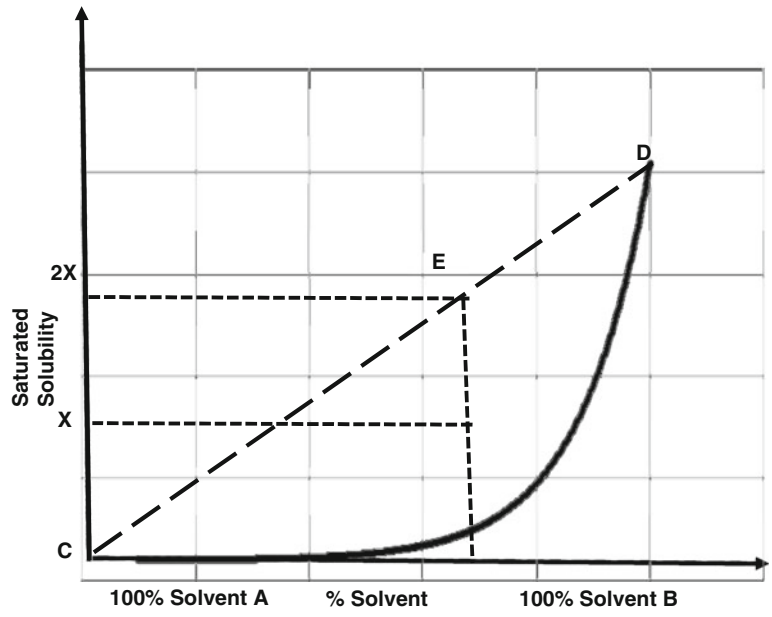

Fig. 11.3 Graph showing the solubility of a drug in a binary co-solvent system and the method used to obtain a supersaturated solution

same vehicle. The solutions up to 4° of saturation were found to be stable for 16 h and then crystallization was observed (Pellett et al. 1997). It was also demonstrated via tape stripping technique that the intercellular regions of the stratum corneum were capable of maintaining supersaturated systems as the drug is transported across the stratum corneum (Pellett et al. 1997). In another study with delivery of fluocinonide across silicone membranes using in vitro diffusion cells, supersaturated solutions up to 3.8° of saturation were prepared using PVP and a vehicle composition of ethanol, water, glycerol, and propylene glycol, and it was found that fluocinonide permeation across silicone membranes was linearly related to degree of saturation (Schwarb et al. 1999). Permeation studies of ketotifen from silicon-based pressure-sensitive adhesive (PSA) with n-hexane and another solvent dichloromethane, tetrahydrofuran, acetone, ethyl acetate, or toluene demonstrated that the formulation was in a supersaturated state (Inoue et al. 2005).

Supersaturation can also be achieved by evaporation of the volatile components in the formulation resulting in decreased solubility in the residual component of the skin surface leading to increased thermodynamic activity. However, the only drawback of supersaturation achieved via evaporation is the lack of control on concentration of the supersaturated solution thereby having less control on flux via that solution. The evaporation theory can be tested by occluding/unoccluding the formulation when volatile solvents are present in the vehicle (Tanaka et al. 1985). Just like binary systems, control of crystallization is important to maintain the supersaturated state of drug in solution. In a study where nifedipine was dissolved in combination of vehicles like isopropyl myristate, PG, and acetone and studies for permeation across an ethylene-vinyl acetate copolymer membrane, the initial flux values in the system were found to be greater than the final steady-state fluxes attributing to the crystallization of the drug component. The incorporation of anti-nucleant polymers into the IPM:PG:acetone vehicle led to a three to five times enhancement in flux (Kondo and Sugimoto 1987). These findings of increased permeation across the lipoidal membrane were confirmed in an in vivo/in vitro correlation study where similar observations were reported across excised male Wistar rat skin in vivo using the nifedipine solution in 75:25 ethanol and diethyl sebacate combinations (Kondo et al. 1987). In another study, hydrocortisone was dissolved in a mixture of acetone and water, and up to 3.9° of saturation was achieved

giving a proportional increase in flux, which was monitored across an ethylene-vinyl acetate copolymer membrane after the evaporation of volatile component (Theeuwes et al. 1976). The increase in delivery of sodium nonivamide acetate has also been tested across rat skin from supersaturated solutions prepared by evaporation of the volatile components of an aqueous ethanolic vehicle, and two components, methyl cellulose and hydroxypropyl cellulose, were found to have a stabilizing effect as an anti-nucleant preventing crystallization within the skin membrane (Fang et al. 1999).

When a solution is prepared from cooling it to a temperature below that of the saturated solubility, the solution may crystallize or a stable supersaturated system may result. A tenfold increase in the permeation was observed when an indomethacin gel was prepared by heating a mixture of the drug with hydrogenated soybean phospholipid and liquid paraffin to 95 °C, cooling to room temperature, placing it in an air incubator at 40 °C for 3 days, and then storing at room temperature, compared to that prepared at room temperature in the same vehicle components. Supersaturated solutions of flurbiprofen and ketoprofen also showed an increase in the permeation rates from the heated and cooled formulations, but no difference in the rates for ibuprofen formulations was observed (Henmi et al. 1994).

11.3 Role of Anti-nucleant Polymers

Supersaturated systems, by definitions, are unstable in their native state. In order to stabilize a supersaturated system and to achieve effective transdermal enhancement, it is absolutely crucial to keep the process of crystal formation to its minimum. For this purpose, anti-nucleant polymers are used in the supersaturated system. Some of the common polymers used as effective anti-nucleants are hydroxypropyl methyl cellulose (HPMC), polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA), polyethylene glycol (PEG), and Eudragits (acrylic polymers). These polymers have been shown to inhibit the crystal growth of compounds like paracetamol, nifedipine, spironolactone, sulfamethiazole, hydroflumethiazide, n-paraffin, estradiol, and griseofulvin from supersaturated solutions in a particular solvent and delivery systems (Simonelli et al. 1970; Corrigan and Timoney 1975; Sekikawa et al. 1978; Holden 1979; Hasegawa et al. 1985; Femi-Oyewo and Spring 1994; Kotiyan and Vavia 2001; Valenta and Auner 2004). Use of copolymers of methacrylic acid (Eudragit® E, EuE, and Eudragit® RL, EuRL) as ibuprofen crystal inhibitors in the matrices was found to prevent the drug crystallization for more than 12 months (Cilurzo et al. 2005). In another study, supersaturated solutions of ibuprofen were prepared using 25 % PG, 5 % vitamin ETPGS (d-alpha tocopheryl polyethylene glycol 1,000 succinate), and 5 % ethylene oxide/propylene oxide block copolymer (pluronic F127) solvents and their combinations to achieve different DS (0.5, 1, 2.5, 5.0, 10.0, 25.0, and 50), and it was found in that study that vitamin ETPGS improved the flux better compared to PG and pluronic F127. The optimization of the vitamin ETPGS/ibuprofen formulation with polymeric stabilizers like HPMC and PVP K-30 resulted in inhibiting crystal growth (HPMC showed better crystal growth inhibition compared to that of PVP K-30). However, the use of PVP K-30 increased the permeation rate of drug through the skin relative to the HPMC (Ghosh and Michniak-Kohn 2012).

How to select an appropriate polymer for a particular drug-solvent system is of a concern. The concept of nucleation and crystallization is not completely understood but efforts have been made to study the process using techniques like differential scanning calorimetry (DSC). In a study involving isothermal crystallization of lidocaine (LC) in supersaturated polyacrylate pressure-sensitive adhesive (LC/Duro-Tak® 87-2287 (DT2287)) system, some of the common reasons for crystallization are given (Cui and Frank 2005). Multiple theories have been proposed for the inhibition of crystal formation by the use of polymer additives and therefore stabilization of supersaturated solution of a compound in a solvent. In a study involving the preparation

of supersaturated sulfamethiazole solution in presence of PVP, it was suggested that PVP formed a netlike structure over a growing crystal face with fingerlike crystal growths occurring between the pores of the PVP network, and due to the curvature of these protrusions, a higher degree of supersaturation was required to continue crystal growth (Simonelli et al. 1970). In another study involving the investigation of crystallization and morphology of hydrocortisone acetate crystal formation, it was proposed and observed via IR spectroscopy that crystal growth was inhibited by absorption of polymer, PVP into the growing crystal surface through hydrogen bonding (Raghavan et al. 2001). Similar observation of presence of hydrogen bond was also observed in an ibuprofen – HPMC system (Iervolino et al. 2001). In yet another study, the preparation and stabilization of colloidal dispersions of triclosan, an antimicrobial agent used up to 2 % in cosmetic and detergent formulations for disinfection of the skin, were performed from supersaturated solutions of triclosan either in the absence or presence of the polymer, HPMC, and it was observed that the particle sizes of triclosan were stabilized in the range of 90–250 nm after addition of HPMC in a water-propylene glycol co-solvent system, whereas large-shaped crystal morphology was observed in the absence of polymer (Raghavan et al. 2003). Stabilization of fentanyl in the supersaturated PG/water formulation with 3DS was achieved by addition of 1 % hydroxypropyl cellulose (HPC) leading to improved permeation flux across the skin. Fentanyl crystal growth was retarded minimally using HPMC or PVP K90 at 1 % (w/v) for the 5 DS formulation compared with HPC (Santos et al. 2011).

However, presence of a polymer in the drug solution does not necessarily affect drug permeation in all cases. In a study of the effect of hydroxypropyl-β-cyclodextrin (HPβCD) on in vitro transdermal permeation of corticosterone through hairless mouse skin, contradictory evidences have been observed between the groups. Shaker et al. have been observed that use of HPβCD, PVP, or the HPβCD/PVP combination does not affect the permeation of corticosterone across synthetic cellulose membrane or the hairless mouse skin, whereas Loftson et al. have reported an increase in permeation of corticosterone in presence of HPβCD (Loftsson and Sigurðardóttir 1994; Shaker et al. 2003; Torres-Labandeira et al. 1991). Similarly, use of 2-hydroxypropyl-ß-cyclodextrin has also been found not to alter crystal growth in ibuprofen solution (Iervolino et al. 2001). A very similar observation was observed in a study of testosterone, added in excess to 4:1:1 (v/v) ethanol/PG/water along with multiple antinucleant polymers (PVP Kollidon®30, vinylpyrrolidone-vinyl acetate copolymer Kollidon® VA64, a randomly methylated cyclodextrin RAMEB, methacrylic acid and ethyl acrylate copolymer Eudragit®L100-55, nonionic poly (ethylene oxide) polymer Polyox®WSR N-10, hydroxypropyl methyl cellulose Methocel®E5, hydroxypropyl methyl cellulose phthalate HPMCP®50, and hydroxypropyl cellulose Klucel®EF) to prevent crystallization. It was found that only Kollidon®VA64 and a cyclodextrin derivative, RAMEB, at 5 % conc., showed promise in the preparation of supersaturated solution with a degree of supersaturation between 1.4 and 2.6, but the supersaturated state existed only for 6 h (Leichtnam et al. 2006).

11.4 Determination of Supersaturation and Crystal Growth

The easiest way to determine crystal formation is via spreading the solution into a thin film on a slide and visually observing the crystals under the microscope. Other methods to determine the solubility of the drug and crystal formation are via methods like isothermal heat conduction, microcalorimetry, X-ray diffraction, and DSC (Theeuwes et al. 1974; Latsch et al. 2004a, b). Along from experimental techniques, computational techniques like kinetic Monte Carlo method has been used for predicting of Oswald ripening and evolution in crystal formation/growth of a supersaturated solution (Zeng et al. 2004, 2006).

11.5 Synergistic Use of Supersaturation Along with Other Enhancement Technologies/Methods

Synergistic effect of supersaturation along with the use of the chemical enhancer, oleic acid, was observed on the flurbiprofen permeation across human skin. The stratum corneum of human skin was pretreated for 1 h with a 2.8 % ethanolic (EtOH) solution of oleic acid (OA), and a supersaturated solution of flurbiprofen with six degrees of saturation was applied on the skin. Compared to ER=1.0 with saturated solution of flurbiprofen in EtOH, the 2.8 % OA in EtOH showed an ER of 2.1, whereas the sixfold supersaturated solution showed an ER of 4.5, whereas in presence of both supersaturation and oleic acid, the enhancement ratio (ER) of 9.9 was observed (Pellett 2012).

Transdermal films of caffeine and sumatriptan have been shown to result in generation of supersaturated state of drug on the skin surface leading to increased permeation (Nicoli 2005; Femenía-Font et al. 2006).

Non-entrapped hydrocortisone (HC) liposomal supersaturated system has been shown to increase the transdermal flux due to increase in thermodynamic activity of the drug caused by accumulation of HC in the skin creating a supersaturated state (Barichello et al. 2006). Another similar approach is applied in delivery of paclitaxel using paclitaxel-loaded methoxy poly(ethylene glycol)-*block*-poly(D, L lactic acid (PEDELLA) diblock copolymer nanoparticles (PNPs), synthesized from polycondensation of D,L-lactic acid and mPEG. In this study, PNPs showed better permeation compared to that of the supersaturated solution of paclitaxel in water. However, such approach can be combined along with the supersaturation to obtain an effective transdermal penetration (Li et al. 2008).

Similar observation was observed with estradiol permeation across human skin and silastic membrane when the effect of propylene glycol-induced enhancement was observed along with the effect of supersaturated solution. Both the methods increased the percutaneous permeation of estradiol separately and showed synergism in enhancement when used together. Propylene glycol was believed to increase the partitioning of estradiol into the membrane whereas supersaturation was believed to increase the concentration of compound in the vehicle beyond that of the normally limited saturated solubility leading to enhanced drug permeation (Megrab et al. 1995). Similarly, maintenance of supersaturated state of estradiol in a drug-adhesive patch system is important to be controlled and monitored for proper drug release from patch system. This can be achieved by use of an appropriate polymer, casting solvent, drug loading, and thickness (μm) of the adhesive layer (Imani et al. 2010; Jain and Banga 2010: Pattnaik et al. 2011).

It has been observed that it is difficult to maintain the stability of the testosterone-supersaturated solution in a mechanical aerosol delivery system. In a study involving preparation/delivery of stable testosterone solution in 3:1 ethanol/PG mixture containing a high percentage of propellant (~50 %), crystallization was observed on the skin surface from the aerosol and spray system, whereas the solution did not exhibit this problem after application (Leichtnam et al. 2006). In another metered dose aerosol (MDA) system, consisting of hydrofluoroalkane 134a, ethanol, poly(vinyl pyrrolidone) K90, and beclomethasone dipropionate (BDP), with different concentration (0, 5 or 10 % w/w) of polyethylene glycol (PEG) 400, different degree of saturation of 6 for 0 and 5 % PG and DS = 10 for 10 % PG formulation was achieved. It was found that the rate of drug release was controlled by the DS and the increased mobility of the PEG films and slower supersaturation kinetics allowed a more sustained drug release (Reid et al. 2008). It was observed that the formulations with higher DS show rapid recrystallization on the skin. However, in a highly volatile spray system, BDP supersaturation for extended periods of time has been considered less important than generating instantaneous, high levels of supersaturation to enhance drug release (Reid et al. 2009).

11.6 Use of Supersaturation in Other Dosage Forms

Some of the other polymers used for prevention of crystallization in non-transdermal systems are 2-hydroxypropyl-ß-cyclodextrin (to prevent

crystallization of amorphous nifedipine in spray-dried powders) (Uekama et al. 1992), and ß-cyclodextrins (for the inhibition of crystal growth of isosorbide 5-mononitrate in tablets or powders) (Uekama et al. 1985). Hydroxypropyl cyclodextrins have also been used to supersaturate pancratistatin, an anticancer drug, for parenteral use (Torres-Labandeira et al. 1991). In a study involving permeation of supersaturated diazepam (DZP) across polydimethylsiloxane (PDMS) membranes, chosen as an in vitro model for nasal mucosa, it was observed that there was a proportional increase in DZP flux across PDMS membrane up to 3× supersaturation, and beyond this point only minor increment in flux was observed. Such supersaturated solutions of DZP in [glycofurol (GF)/water] cosolvent systems can result in rapid response to epileptic seizure emergencies (Hou and Siegel 2006). The role of excipient selection in the solid dosage form formulations is also very important. Some of the important properties taken into consideration for excipient selection are molecular weight, topological polar surface area (TPSA), log P, pKa, and molecular volume for screening the excipient materials. Some of the known excipients used in the solid dosage systems to achieve supersaturation – PEG400 in solution, HPMC E5 in coated bead, hydrophilic cyclodextrin, HPβBD in tablets, polyoxyl 40 hydrogenated castor oil CremophorRH40, and tocopheryl polyethylene glycol 1,000 succinate TPGS in capsule – have been tested and found efficient in increasing the oral bioavailability of the formulation (Vandecruys et al. 2007).

11.7 Commercialization of Supersaturated Transdermal Drug Delivery Systems

Increasing thermodynamic activity beyond saturation is an attractive methodology to enhance penetration of actives across skin since other technologies that utilize chemical and physical enhancement technique might not be feasible due to bulkiness of devices, higher cost, lack of patient compliance, chances of irritation, etc. Review of the literature suggests that supersaturation as an enhancement technique has been evaluated intensely in the last 20+ years. Most of the work on supersaturated systems to date has come from academic groups and minimal efforts have gone into developing supersaturated systems into a commercially viable product. The main bottleneck for a product that contains the drug in a supersaturated state is the stability of the product during (1) manufacture, (2) storage, (3) supply chain management, and (4) end use by the patients.

11.7.1 Manufacture

The process for manufacturing the product might involve heating and cooling at different temperatures, which is conducive to nucleation. Extreme care must be taken to control the process in order to avoid any nucleation. Further, mixing usually involves high shear mixing that can cause crystallization to occur. Other processes that can induce nucleation are packaging and analytical testing.

11.7.2 Storage

The stability of a product under the storage conditions specified in the label claim of a product determines its shelf life. It is known that supersaturated systems can become unstable upon storage for long periods of time. In addition, there are some formulations that require storage at refrigerated temperatures, which can cause thermodynamic instability. Based on the stability studies, a shorter shelf life might be required.

11.7.3 Supply Chain Management

Following manufacture, the products have to be transported from the manufacturer to the sales and distribution warehouses and to retail stores upon sales. Extreme care would be required to monitor and conduct thorough stability studies under different conditions of transport and storage

in order to make sure the product is thermodynamically stable under these conditions.

11.7.4 Patient Compliance

Upon receiving a prescription, the patient can potentially carry the product during travel or can store under different conditions of temperature and humidity. Extensive stability studies would be required to provide sufficient guidance and warnings to patients on how to store and use the product. In addition, some drug molecules, when used excessively, can cause skin irritation and hence might not be practical for the use of supersaturated concentrations.

Most of these challenges can be overcome through proper design of product including packaging. For example, the drug solution can be stored in the stable form in a container separated from the co-solvent and can be mixed at the time of use. Such a methodology has been used in products such as benzoyl peroxide – clindamycin gel (Benzaclin) and benzoyl peroxide-erythromycin gel (Benzamycin), where the benzyl peroxide gel is stored in a separate jar and clindamycin or erythromycin is compounded with the gel by the pharmacist. Extensive research needs to be undertaken by the industry in order to overcome these challenges in order to successfully bring this technology to the product. There is a huge potential for this technology to be exploited with proper choice of drug, optimization of the development and manufacturing processes, and innovative design.

Conclusions

Presently, it is well known that supersaturated solutions of different chemical compounds and drugs molecules have been shown to improve transdermal flux (Davis and Hadgraft 1991; Moser et al. 2001). The real challenge in maintaining a supersaturated state of a chemical compound in solution is to tailoring the solvent system and use of anti-nucleant polymers to achieve the maximum thermodynamic activity while still maintaining the compound's stability in solution. Use of anti-nucleant polymers has been tested and found effective; however, the concepts behind selection of an appropriate polymer for a particular supersaturated system in order to achieve maximum drug delivery are still not well understood. In spite of the huge challenges in developing a stable supersaturated product that can be commercialized, extensive research and development efforts as well as innovative designs can exploit the potential advantages of the technology.

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Eutectic Systems for Penetration Enhancement

12

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Contents

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12.1 Introduction

The skin is an extremely important organ of the body, it forms a highly restrictive barrier between soft human tissues and the external environment, and it maintains body fluid homeostasis. In addition, it protects other internal organs from noxious insults. As a consequence, when breaches in the skin's barrier occur, they are repaired quickly using an effective system of enzymes and immune cells which are within close proximity to the skin surface. If a medical condition requires treatment using the skin as a means of drug administration, this biological context must be understood, the properties of the applied chemical must be characterized, and the site of action of the applied agent must be known in order to facilitate effective clinical therapy.

When a discrepancy exists in the native physicochemical properties displayed by a drug applied to the skin and the desired properties that theoretically allow the agent to passively reach its intended site of action, enhancement strategies may be sought to try and optimize the delivery process (Suhonen et al. 1999). A wide range of percutaneous penetration enhancement strategies have been developed over the last six decades, and presenting a molecule to the skin as a eutectic mixture is one that falls within the category known as chemical enhancement techniques. A eutectic system is most typically a binary system that when mixed exhibits a melting point that is lower

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than either of the component agents (Benson 2005). Studies using several drugs including ibuprofen (Stott et al. 1998), propranolol (Stott et al. 2001), testosterone (Kaplun-Frischoff and Touitou 1997), and lidocaine (Kang et al. 2000), combined with a second component (which may be a drug or excipient) to form a eutectic system, have been shown to enhance drug penetration into the skin compared to control systems containing only the single therapeutic agent. The enhanced permeation into the skin has been attributed to the lower melting point of the combined agents, which has been said to increase the solubility of penetrants in the lipids of the skin's outermost layer, the *stratum corneum* (SC). EMLA cream® (AstraZeneca, UK), a product that displays superior clinical efficacy compared to either prilocaine or lidocaine applied alone (Juhlin et al. 1980), is the oft-cited example used to highlight the utility of the eutectic system approach, but the deliberate formation of these systems remains an underused means to enhance drug delivery into the skin. This could be due to the challenges of formulating a product that contains two important functional molecules. In addition to the regulatory and manufacturing challenges posed by the use of two functional components in a topical formulation, the presence of two important diffusing species, which can enter the body simultaneously, at different rates, renders the production of new eutectic systems problematic.

According to the Higuchi equation (Higuchi 1962), the flux of a compound from a saturated solution is constant, regardless of the saturated concentration in a given vehicle because all saturated solutions have a thermodynamic activity of one (Twist and Zatz 1986). This theory was developed using the context of highly restrictive barriers such as the skin, and it has shown to be a reliable model for a number of therapeutic delivery systems (Davis and Hadgraft 1991; Dias et al. 2007; Iervolino et al. 2001). However, this mathematical model was designed to describe the mass transfer of a single agent. Tracking the movement of two agents across the human skin generates a three-dimensional problem as not only do the molecules have the capacity to change their behavior in a ratio-dependent manner, but they also encounter a series of different diffusional barriers which have the ability to independently influence molecular transport. In the discourse that follows, an attempt has been made to review the experimental studies that have investigated the manner in which agents are transported through hydrophobic barriers when applied as eutectic systems. In addition, using the lidocaine and prilocaine eutectic system as a central theme, what appears to be the most important concepts in this field have been highlighted in an attempt to advance the current understanding of how eutectic systems function to enhance drug delivery into the skin.

12.2 Dual Drug Diffusion

The effects of applying multiple drug molecules on the process of diffusion through membranes can be studied using porous regenerated cellulose membranes (RCM). Regenerated cellulose is an inert material that when formed into a membrane has been shown to provide a diffusion barrier with minimal membrane-drug interactions with non-ionized agents (Reid et al. 2008; Fiala 2008). In addition, it has been found that when the polarity of the application vehicle is well matched to the membrane, the residence time of the applied drug molecules in the membrane is short, drug partitioning is limited, and hence the transport rate can be directly related to the rate of molecular diffusion in the membrane (Reid et al. 2008; Fiala 2008). Using this basis, previous experimental data using RCM has been interpreted to suggest that the simultaneous membrane diffusion of lidocaine and prilocaine can be considered as a competitive process (Fig. 12.1, Fiala et al. 2008).

Relating the correlation observed for lidocaine and prilocaine between the normalized drug ratio in the applied bulk solution and the species transport back to the principles of the Higuchi equation allows the reduced diffusion of one agent in the presence of the second to be predicted by the initial composition of the applied saturated solutions using Eq. 12.1, where the rate of transport for an individual molecule (dq/dt) was related to

the membrane surface area (A), the membrane diffusion coefficient (D), the thermodynamic activity (α), the activity coefficient in the membrane (γbar), the diffusion path length (L), and the normalized ratio of the applied agent (N):

$$\frac{\mathrm{d}q}{\mathrm{d}t} = A\frac{ND}{\gamma_{\mathrm{bar}}}\frac{\alpha}{L} \tag{12.1}$$

In the case of lidocaine and prilocaine combinations, the normalized solubility was calculated using Eqs. 12.2 and 12.3, respectively:

$$N_{\mathrm{lido}} = \frac{C_{\mathrm{lido}}}{C_{\mathrm{lido}} + \left(C_{\mathrm{prilo}} \cdot S_{\mathrm{lido}} / S_{\mathrm{prilo}}\right)} \tag{12.2}$$

$$N_{\mathrm{prilo}} = \frac{C_{\mathrm{prilo}}}{C_{\mathrm{prilo}} + \left(C_{\mathrm{lido}} \cdot S_{\mathrm{prilo}} / S_{\mathrm{lido}}\right)} \tag{12.3}$$

where S_{prilo}, S_{lido} was defined as the solubility of prilocaine and lidocaine in individually saturated solutions and C_{prilo}, C_{lido} the concentrations of prilocaine and lidocaine in the application solutions. The self-diffusion coefficient measurements (which do not change upon the mixing of lidocaine and prilocaine at equimolar ratios ~ $7.5 \times 10-6$ cm^2/s) were cited in the paper by Fiala et al. (2008) to suggest the trends in the data were due to a reduction in the capacity of the membrane to allow unhindered diffusion of one compound in the presence of the second and not drug-drug interactions (Nyqvist-Mayer et al. 1986).

12.3 Dual Drug Partitioning

Building the effects of drug partitioning into the dual drug transport process is very important in percutaneous penetration. The partition coefficient (log P) of a molecule is typically measured as a ratio between the affinity for a standard oil phase (often octanol) and water. It can be used to predict the ability of a molecule applied to a hydrophobic barrier to pass into it. In terms of the human skin, if the log P is high for a particular drug, it will move across the first energy barrier created by the water/SC interface and into the outer layers of the skin. However, when a polar vehicle is used to administer a compound to the skin's surface, the hydrophobic SC is sandwiched between two hydrophilic phases. The epidermal tissue underlying the SC contains more water and fewer lipids than the skin's outermost layer, and as a consequence, it is distinctly more hydrophilic than the SC (Scheuplein and Blank 1971). Therefore, even if an agent applied topically to the surface of the skin passes into the SC, its subsequent transport through the tissue into deeper layers may be retarded by a second energy barrier (Williams and Barry 2004). As a consequence, a more detailed understanding of the transport process can be obtained if the effects of partitioning into a confluent barrier from a specific application vehicle are recorded and compared to predicted values using transport models that employ log P rather than relying on the latter alone. Human skin can be used in such studies, but it suffers from the problem of structural breakdown when long equilibration times are required to gain the measurements. Silicone membranes can be a useful alternative to human skin in partitioning experiments. Many types of silicone membranes do not have the same structural stratifications as skin, but they form a continuous hydrophobic barrier that is relatively inert and immiscible with polar solvents. They can be sandwiched between two water phases using a Franz cell setup in vitro; thus, they can generate transport and partitioning data using equivalent experimental parameters.

Transport studies using binary mixtures of lidocaine and prilocaine dissolved at different ratios in water (at a pH that retains both agents in the unionized state) have shown that the linear relationship between drug transport and normalized applied concentration observed in the RCM studies (Fig. 12.1) was not conserved when the barrier was switched to silicone (Fig. 12.2). The reason for this was assigned to the changes that occurred during the act of partitioning, which for the confluent silicone membrane, unlike the porous RCM, was predicted to have a significant effect on the transport process (Fiala et al. 2008). The data reported by Fiala et al. (2008) indicated

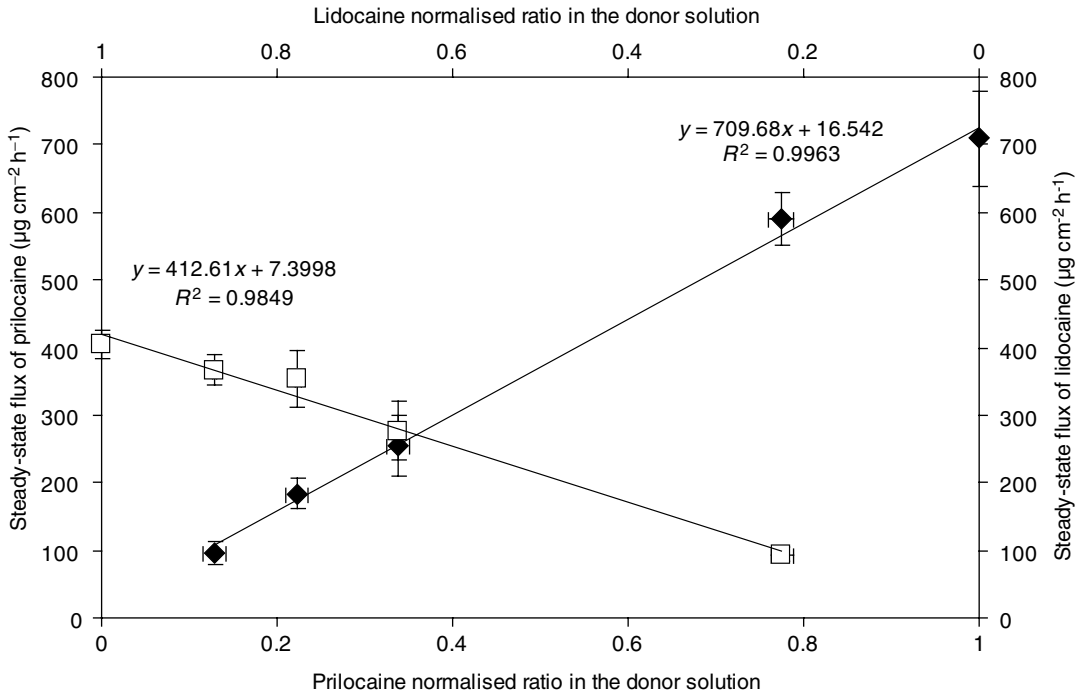

Fig. 12.1 Relationship between the steady-state flux of lidocaine and prilocaine through regenerated cellulose membrane and their solubility-normalized ratio in phosphate buffer solution: prilocaine (♦), lidocaine (□). Each point represents mean ± 1 standard deviation ($n=5$). A linear trend was observed between the steady-state flux of prilocaine and lidocaine and their solubility-normalized ratios in the donor solution and was plotted (represented by *black line*) (Reproduced with permission from Fiala et al. (2008))

that prilocaine partitioning remained unchanged irrespective of the concentration of lidocaine present in the binary drug mixtures, while lidocaine partitioning was enhanced when increasing amounts of prilocaine were present in the applied solutions. The solubility parameters of silicone, lidocaine, and prilocaine are 7.3 (cal $cm^{-3})^{1/2}$, 10.68 (cal $cm^{-3})^{1/2}$ and 11.05 (cal $cm^{-3})^{1/2}$, respectively (Fedors 1974). According to these values, the presence of either prilocaine or lidocaine in the membrane could theoretically alter the solubility parameter of silicone in a manner which may facilitate the passage of the second agent into the barrier when a binary mixture was applied onto its surface. Experimentally, a more efficient partitioning was only observed for lidocaine during the dual drug application process (Fiala et al. 2008). This is difficult to logically explain using the standard transport theories outlined in the literature. Furthermore, the transport data generated by Fiala et al. (2008) suggested that influence of the dual drug application on partitioning was relatively minor in comparison to drug diffusion when an aqueous application vehicle was used. These findings are not in agreement with other published work that has suggested eutectic system enhancement of drug delivery to the skin was mediated through changes in the partitioning process (Benson 2005; Stott et al. 2001). This raises a question as to the particular context in which the previous hypothesis regarding eutectic penetration enhancement was generated and the generality of the conclusions from any of the aforementioned studies.

12.4 Eutectic Combinations

Previous literature has suggested that the skin penetration enhancement effects of eutectic systems can be attributed to superior solubility of the active molecules presented by theses systems in

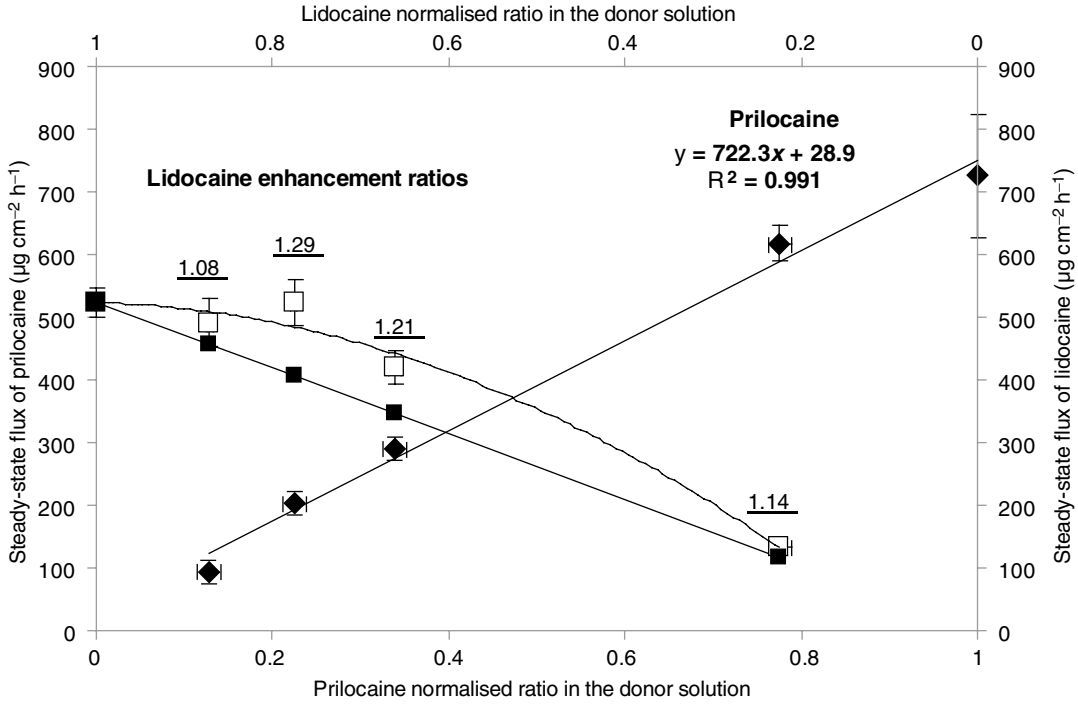

Fig. 12.2 Relationship between the steady-state flux of lidocaine and prilocaine through silicone membrane and their solubility-normalized ratio in phosphate buffer solution: prilocaine (◆), lidocaine (□). Theoretical lidocaine flux (■) was calculated assuming that the diffusion volume was changing as a function of the normalized ratio. Each point represents mean ± 1 standard deviation ($n = 5$). Enhancement ratios of lidocaine were calculated as the ratio of the actual to the theoretical steady-state flux and are indicated by the numbers on the graphs (Reproduced with permission from Fiala et al. (2008))

SC lipids (Alexander et al. 2012; Kaplun-Frischoff and Touitou 1997). This has been linked to the "melting point theory" which suggests that agents with a lower melting point (a key characteristic of all eutectic systems) will often have a greater propensity to associate with solvent molecules (Benson 2005). It has also been suggested in the literature that once the agents delivered by eutectic systems are within the skin, the co-localization of the diffusing species aids barrier penetration by interacting and modifying the skin structure (Watanabe et al. 2009; Woolfson et al. 2000). The facilitation of transport by eutectic systems through drug-skin interaction seems logical from both the perspective of increasing drug partitioning into the barrier and facilitating the movement of drugs through the barrier as such effects seem to be similar in nature to when the skin barrier is heated (Stott et al. 1998). According to Wood et al. (2012), heating the skin has a greater consequential effect on the process of drug partitioning compared to drug diffusion, but a similar conclusion is difficult to substantiate for eutectic systems. Both Pugh et al. (1996) and Fiala (2008) suggest that eutectic systems may have a negative influence on drug diffusion due to species competition and interaction in the solution state. This raises a question as to how, in the context of a topical formulation applied to the surface of the skin, the issues of competitive transport, molecular interactions, and facilitative partitioning function to generate the final transport rate generated from different eutectic systems. In an attempt to provide a critique on this matter, there is a need to highlight the specific details of how the various hypotheses surrounding the eutectic systems have been derived.

One model that relates melting point to SC solubility was proposed in the 1980s by Kasting et al. who suggested a relationship between transdermal

flux and melting point of the drug based on the concept of ideal solution state chemistry (Kasting et al. 1987). Using this model, the ideal solubility of a drug (S_{ideal} assuming thermodynamic ideality in terms of intermolecular interactions) in the skin lipids can be obtained using Eq. 12.4:

$$S_{ideal} = \frac{\rho}{1-\left\{1-\exp\left[\frac{\Delta S_f}{RT}\left(T_m - T\right)\right]\right\}\frac{M_1}{M_w}} \tag{12.4}$$

where ρ is the density of the skin lipids, M_1 is their average molecular weight, M_w is the molecular weight, T_m is the melting point in degrees Kelvin, and ΔS_f is the entropy of fusion of the drug. The ideal solubility was then used to predict a maximum flux, J_m (Eq. 12.5):

$$\log\left(J_m / S_{ideal}\right) = 1.80 - \left(0.0216 / 2.303\right) M_w \tag{12.5}$$

where M_w is the molecular weight. ΔS_f shows limited change as a function of melting point, but S_{ideal} increases exponentially with decreasing melting point for any given molecular weight, and this relationship is thought to drive flux across the skin, which would also theoretically increase exponentially. Touitou et al. (1994) used melting temperatures as indices to predict the relative transdermal fluxes of a series of enantiomeric eutectic mixtures. Solubility, expressed as solute mole fraction (X) in a given solvent, was related to the melting temperature (T_m) and the enthalpy of fusion (ΔH) using Eq. 12.6:

$$\ln X = -\frac{\Delta H}{R}\left(\frac{T_m - T}{T \cdot T_m}\right) \tag{12.6}$$

From this calculation, the maximum fluxes of one pure enantiomer ($J_{max,\,s}$) compared to the racemic mix ($J_{max,\,rs}$) were predicted (Eq. 12.7):

$$\ln\frac{J_{max,s}}{J_{max,rs}} = \ln\frac{X_{max,s}}{X_{max,rs}} = \frac{\Delta H_{rs}\left(T_{m,rs} - T\right)}{R \cdot T_{m,rs} \cdot T} - \frac{\Delta H_s\left(T_{m,s} - T\right)}{R \cdot T_{m,s} \cdot T} \tag{12.7}$$

Stott et al. (2001) generated data to suggest that the Touitou model (Eq. 12.7) was in good agreement with the skin permeation behavior of a beta-blocker eutectic system and from this concluded that the compound's melting point was an important factor in eutectic system enhancement effects. However, there are two critical details noted by Stott et al. (2001) that do not appear in some of the subsequent work that cites this study. First, Stott et al. (2001) showed that the correlation between melting point reduction and drug flux enhancement using Eq. 12.7 required normalizing to take account of the physicochemical properties of the compounds. This process of normalization involved generating an enhancement ratio against a standard chemical in the data set and effectively negated any effects of the eutectic combination on drug diffusion. Second, Stott et al. (2001) attempted to use the eutectic systems in their pure form, that is, without the addition of solvent molecules. Hence, the relationship between melting point and drug flux in Stott's work was particular in this context, i.e., the data was derived in the absence of a traditional semisolid delivery vehicle and when diffusion effects were negated through a process of mathematical "normalization."

In the case of the lidocaine and prilocaine eutectic system, in vitro transport studies using silicone membrane have shown that prilocaine-rich mixtures demonstrate superior total transport when compared to the 1:1 composition (the latter has the lowest melting point according to Brodin et al. 1984). This data was generated when lidocaine and prilocaine were presented to the membrane in a predominantly unionized state using a pH-modified aqueous solvent (Figs. 12.1 and 12.2). In addition, the TEMPE® spray (Topical Eutectic Mixture for Premature Ejaculation), a product in Phase III development by Plethora Solutions Plc (London, UK), uses a 3:1 lidocaine-to-prilocaine ratio when presenting the compounds as an oil (Henry 1999; Henry and Morales 2003; Henry et al. 2008). These two studies suggest that the melting point theory does not underpin the delivery of molecules from all eutectic systems and that the administration vehicle can have an important influence on the permeation of compounds from eutectic systems. The effects of

the delivery vehicle can be further exemplified through comparison of the data generated in the two papers by Fiala et al. (2008, 2011) in Fig. 12.3.

If the melting point theory cannot be used to adequately describe the transport rate changes observed from the lidocaine and prilocaine system, it would suggest that in the context of a pharmaceutical preparation the means by which eutectic systems enhance transdermal penetration of molecules is a little more complex than first suggested. There have been two additional theoretical concepts that have been proposed to play a role in eutectic system enhancement, these are centered on how the system's chemical potential and association of the drug molecules change when presented as topical formulation.

12.4.1 Chemical Potential

When presented as the pure molten oil, thermal analysis of the lidocaine and prilocaine eutectic system has shown that it can take approximately 7 days, if stored under refrigeration, to reach a state of physical equilibrium, i.e., a constant solid-liquid ratio is attained for the mixtures (Brodin et al. 1984). Chemical analysis of the liquid phase composition at room temperature has confirmed that lidocaine and prilocaine do not attain physical equilibrium after 24 h when mixed at room temperature (Fiala et al. 2011). Previous work has also shown when either prilocaine- or lidocaine-rich ratios of the pure molten oils are prepared at room temperature; a second phase, composed of microparticulate matter, which is presumably non-melted drug product suspended within the eutectic system, can be observed at several ratios of the two drugs (Fiala et al. 2011). It is possible that the suspension formed immediately upon mixing a eutectic system composed of only the two drugs lidocaine and prilocaine may exhibit properties that are similar to a supersaturated solution, in that a state of heightened chemical potential may be temporarily induced. If this was the case, the thermodynamic activity of the molecule in the eutectic mixture that was present in excess may increase transiently and thus generate an unexpectedly high rate of membrane transport. This hypothesis is supported by the observation that eutectic combinations of ibuprofen with terpenes enhanced membrane transport of ibuprofen when a second solid phase existed in the applied molten oil (Stott et al. 1998). If drug flux through human skin was

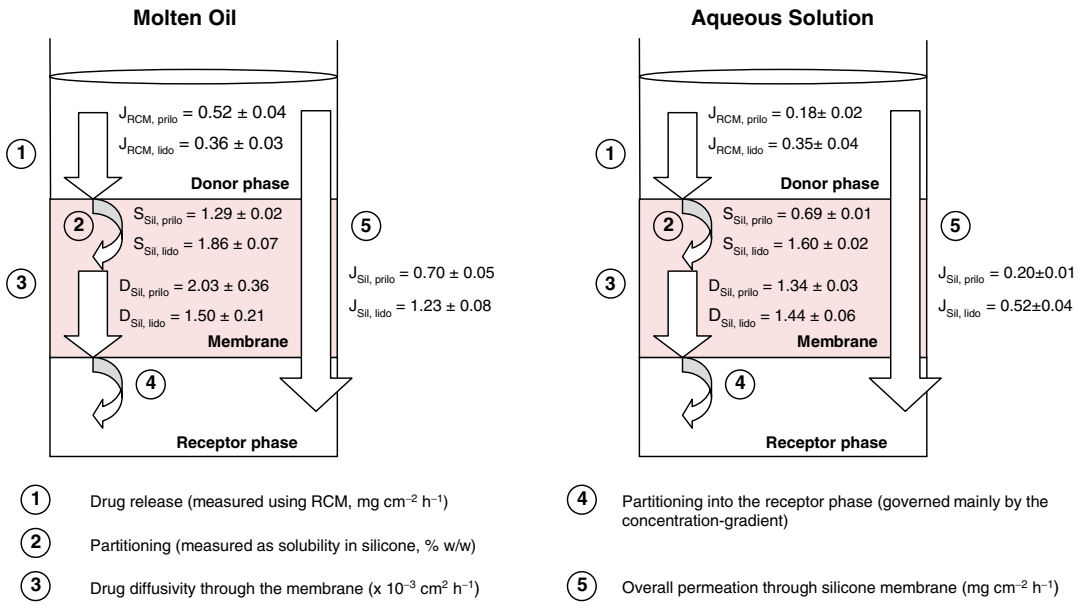

Fig. 12.3 Comparative summary of transport results from a series of studies that used a eutectic prilocaine/lidocaine mixture at a ratio of ca. 2:3 in the form of a molten oil and an aqueous solution

significantly influenced by the physical equilibration state of the pure oils, this may provide one explanation as to why the TEMPE® and EMLA® systems are formulated using different drug ratios. The former forms a eutectic system only upon its application to the skin, and thus it will most likely not reach a state of solid-liquid equilibrium during clinical use, while the latter is produced from a pre-equilibrated oil mixture free from solid particles. More experimental studies using human skin and a deeper theoretical consideration as to the chemical potential of pure molten oils are needed to understand the influence of physical equilibrium on eutectic enhancement, but it is possible that this could be an important factor in the development of eutectic systems.

12.4.2 Association Complexes

The principal components used to form a eutectic system must interact in their molten state in order to generate their unique melting point-lowering properties. H-bonding interactions are known to be important in the formation of eutectic systems between ibuprofen and terpenes (Stott et al. 1998) as well as between urethane and various polymers (Isama et al. 1993); however, the precise interactions between prilocaine and lidocaine have not been identified. As noted previously, Nyqvist-Mayer et al. (1986) suggested that in aqueous solvents the lidocaine and prilocaine molecules had an equivalent self-diffusion coefficient, and therefore it was deemed that a complex was not formed between the two molecules in an aqueous solvent. In polar vehicles, the molecular interactions could be influenced by the ionization of the molecules, and hence in the work by Fiala et al. (2008), the drug ionization was suppressed in order to reduce the impact of ion-pair formation and/or molecular association. The Nyqvist-Mayer study does not provide the details of drug ionization, and thus it is not clear what microspecies were presented in the test formulations, and this complicates the review of the data in this area. Even if the association of the compounds was considered to occur, there is no spectroscopy evidence to show if this association would be strong enough to form a pure 1:1 complex at equimolar levels of the two drugs or if there would be significant levels of free unassociated drug present in the mixtures. At ratios other than the eutectic, some levels of unassociated molecules are likely to exist, and this provides a complex solution state chemistry landscape where several species are penetrating the membrane and contributing to the total steady-state flux. It is interesting to note that silicone membrane solubility measurements using pure molten lidocaine and prilocaine oils at a ratio of 1:1 showed a higher lidocaine solubility compared to prilocaine, which suggests that either a pure 1:1 mixture does not exist in the donor oil or the silicone-lidocaine interaction is stronger than the 1:1 complex interaction.

For the lidocaine and prilocaine eutectic system, assuming a strong 1:1 complex is formed, an average log *P* for the 1:1 complex can be used with an additive molecule mass in the Potts and Guy (1992) equation to predict the penetration rate of the drugs across the skin when applied as a molten oil (Fig. 12.3). As only two diffusing species are assumed to be present (i.e., free and complexed), the overall permeability can be calculated as the sum of transport of the two species relative to their ratio in the mixture (Eqs. 12.8 and 12.9).

If $R_{\text{prilo}} > R_{\text{lido}}$:

$$K_p = \left[\left(K_{p,\text{comp}}\right)\cdot\left(2R_{\text{lido}}\right)\right] + \left[\left(K_{p,\text{prilo}}\right)\cdot\left(1-2R_{\text{lido}}\right)\right] \tag{12.8}$$

If $R_{\text{lido}} > R_{\text{prilo}}$:

$$K_p = \left[\left(K_{p,\text{comp}}\right)\cdot\left(2R_{\text{prilo}}\right)\right] + \left[\left(K_{p,\text{lido}}\right)\cdot\left(1-2R_{\text{prilo}}\right)\right] \tag{12.9}$$

where R_{prilo} and R_{lido} are prilocaine and lidocaine w/w ratios, respectively; $K_{\text{p, prilo}}$ and $K_{\text{p, lido}}$ are the individual permeability coefficients of prilocaine and lidocaine, respectively; $K_{\text{p, comp}}$ is the permeability coefficient of the complex; and K_{p} is the overall permeability coefficient of the eutectic

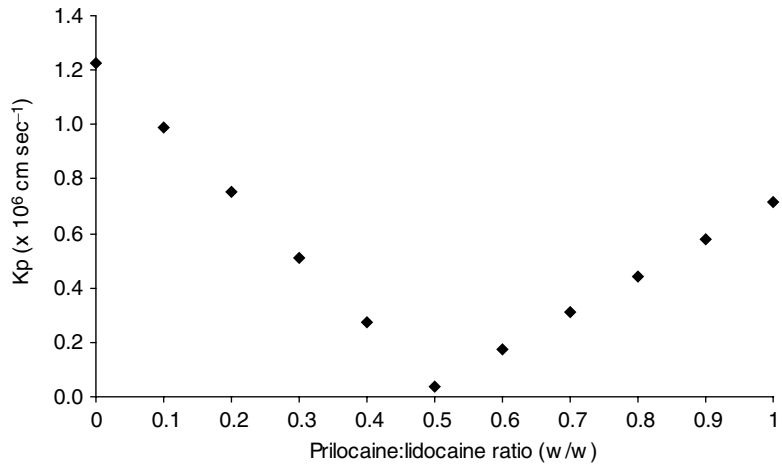

Fig. 12.4 Theoretical K_p values for prilocaine/lidocaine mixtures as calculated using the Potts and Guy equation

mixture. The overall trend of the calculated K_p values (Fig. 12.4) fits well with the competitive transport hypothesis applied to the two anesthetics (Figs. 12.1 and 12.2). However, the Potts and Guy K_p values indicate that when lidocaine was in excess, the free lidocaine molecules drive the total drug permeation to be higher at high lidocaine ratios compared to the high prilocaine ratios. This was not observed practically with the silicone membrane (Fiala et al. 2010), but the Potts and Guy model does support the lidocaine-rich mixture that was employed in the TEMPE® spray. At the time of writing this chapter, there are no publically available data using human skin to suggest the ratio for the TEMPE® system was selected based upon experimental rather than theoretical observations. In addition, the modeling performed in this discourse is extremely simplistic, and again, as stated previously, more transport data using human skin needs to be generated to probe further the issues of complexation. However, what this discussion highlights is that drug-drug complexation must be considered to be a potential factor in the manner in which eutectic systems enhance skin delivery. Furthermore, the strength of the complexes must be measured in the different application vehicles in order for the nature of the permeating species to be identified and allow the systems to be optimized.

Conclusions

Combining two therapeutic agents in a medicine is becoming more attractive to both clinicians and patients as the comorbidity rates continue to increase through the twenty-first century. It would therefore seem sensible to understand how the inclusion of a second agent functions to influence the properties of the first agent in relation to the functional activities of the dosage form. In products that are applied to the skin, if the agents form a eutectic system, current literature suggests that this provides additional advantages as eutectic systems can "enhance the penetration" of topically applied medicines into the skin. The dominant discourse in the literature to this point seems to suggest that a eutectic system reduces the melting point of the drug combination and increases the transport into the skin by facilitating the act of drug partitioning. However, a number of studies, including those which have tested the oft-cited eutectic example of lidocaine and prilocaine, seem to suggest that a direct link between melting point and transport cannot be applied to a number of contexts important to pharmaceutical dosage forms.

The data reviewed in this chapter seems to suggest that the ability to present actives to the skin as a molten oil, without additional formu-

lation excipients, is the special property that underpins eutectic system's induced skin penetration enhancement. When the molten mixture of the lidocaine and prilocaine eutectic system was specifically considered, then a two-phase mixture that was rich in the molecule which penetrates the barrier most effectively was shown to be the most efficient topical formulation and not the system with the lowest melting point. The TEMPE® spray which uses a 3:1 lidocaine-to-prilocaine ratio seems to be a more logical presentation format for these systems compared to a traditional cream, e.g., EMLA®. However, further work needs to be undertaken to understand how the molecules in a eutectic system interact in the molten and solution states and how presentation to the skin affects this in order to advance this very interesting field of research.

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Formation of Ion Pairs and Complex Coacervates

13

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13.1 Ion-Pair Formation for Penetration Enhancement

A diffusing molecule has to cross the skin barrier composed of the highly lipophilic stratum corneum (SC) and the hydrophilic viable epidermis (ED) in order to reach the deep skin layer as well as the layers under the skin. Therefore, only active compounds with ideal physicochemical properties, e.g., low molecular weight, suitable solubility in oil and water, moderate partition coefficient, and low melting point, can permeate through both the lipid and polar microenvironments in the skin (Barry 2001). Obviously, hydrophilic ionized drugs do not readily distribute into or penetrate through the lipophilic SC membranes. On the contrary, the problem for those very lipophilic drugs, which can easily distribute into SC membranes, is that they do not readily translocate from the SC to the relative hydrophilic ED due to their high solubility in the intercellular lipids, thus limiting their skin permeation. Ion pairing provides a possible approach to adjust the physicochemical properties of drugs without any changes on the structure and pharmacologic actions of the drug compound and consequently facilitate the penetration of drugs across the skin barrier (Neubert 1989). Some products using ion-pair technique, such as Flector® Patch (diclofenac epolamine topical

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patch) from IBSA Institut Biochimique SA (Switzerland) and Voltarol® emulgel (diclofenac diethylamine emulgel) from Novartis Consumer Health UK Ltd. (UK) are commercially available.

13.1.1 The Concept of Ion Pairs

An ion pair is defined as neutral species which consists of a pair of oppositely charged ions held together by coulomb attraction without formation of a covalent bond (McNaught and Wilkinson 1997). Practically, an ion pair behaves as one unit in determining conductivity, kinetic behavior, osmotic properties, etc.

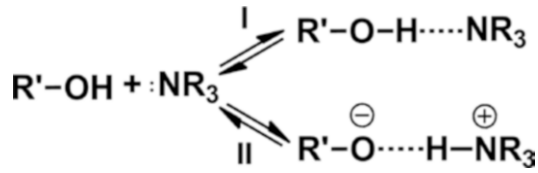
In 1926, Bjerrum developed a theory that took the interaction of ions into account. He introduced, for the first time, the concept of ion pairs and showed how the mass action constant of the equilibrium between ions and ion pairs is dependent on the dielectric constant of the solvent as well as on temperature and the size of the ions (Kraus 1956). According to the Bjerrum theory, oppositely charged ions with their centers closer together than a distance (q) (pm) are considered to constitute an ion pair:

$$q = 8.36\times10^{6} z^{+}z^{-} / \left(\varepsilon_{r} T\right) \qquad (13.1)$$

Where z^{+} and z^{-} are the charge numbers of the ions, ε_{r} is the relative dielectric constant of the medium and T is the absolute temperature. The resulting ion pairs exhibit stable, thermodynamically distinct species. Depending on the strength of the solvent–ion interactions, ion pairs can be classified into two types: contact (tight, intimate) and solvent-separated (loose) ion pairs (Nagy and Takacs-Novak 2000).

In polar solvents with high dielectric constant such as water, ion-pair formation is also possibly obtained. Diamond (1963) demonstrated that the existing of so-called hydrophobic ion pairing (HIP). HIP is composed of two large hydrophobic ions self-assembled together by coulomb attraction, hydrophobic forces, and hydrogen bonding in polar solvents. Even though the HIP complexes display enhanced lipophilicity and thus are more suited for their potential application, it has not been paid extensive attention yet compared to the classical ion pairs mentioned above.

Hydrogen-bonded ion pairs are a special type of ion pairs. The concept of hydrogen-bonded ion pairs was brought out for better understanding the nature of the protonic acid–base interaction occurring in non-dissociating solvents (Barrow 1956). The interaction between sufficiently strong protonic acids and bases in a solvent environment, especially in solvents with low dielectric constant, tends to promote the formation of ion pairs accompanied by proton transfer of a hydrogen bond. According to the Brønsted-Lowry concept of acids and bases in aprotic solvents, two forms of simple prototropic equilibrium exist as follows (Hudson et al. 1972):



The classical electrostatic attraction was predominant in weak hydrogen bonds, so-called classical hydrogen bonds (Equilibrium I). However, on going to stronger hydrogen bonds, the contribution of the proton transfer led to the formation of ion pairs, so-called hydrogen-bonded ion pairs (Equilibrium II) (Arunan et al. 2011; Ratajczak 1972). Such a hydrogen bond is extremely polarizable. The formation of a hydrogen-bonded ion pair between a carboxylic acid and a pyridine base in benzene solution is a typical example of acid and base interaction (Barrow 1956).

13.1.2 Molecules Suitable for Ion-Pair Formation

Both drugs and counter ions have to meet certain demands in order to form ion pairs successfully. For hydrophilic ionized drugs, Neubert (1989) pointed out that the ideal counter ions needed to possess high lipophilicity, sufficient solubility in physiological compatibility, and metabolic stability, which was suitable for ion-pair formation and crossing lipid membranes in

the form of ion pairs. The ion pairs formed by quaternary ammonium drugs and organic anions are typical examples of ion-pair formation (Takacs-Novak and Szasz 1999). Similarly, Miller et al. (2009) performed a study in which three lipophilic acidic counter ions were employed to give an understanding of the mechanism of ion-pair mediated membrane transport of low permeable drugs.

Besides ionized species, for stronger hydrogen bonds like OH···N, the contribution of the proton transfer, $OH\cdots N \rightleftharpoons O^{-}\cdots HN^{+}$, could lead to a hydrogen-bonded ion-pair formation. Sobczyk and Paweła (1974) have demonstrated the existence of proton-transfer equilibrium under appropriate conditions by measuring the dipole moment of carboxylic acid–pyridine base complexes. The results indicated that the dipole moments of these complexes were dependent on the pK_a difference (ΔpK_a) between carboxylic acid and pyridine base, and large dipole moment was induced by strong interaction of ion pairs. This type of interaction depends on the following factors: (a) ΔpK_a of protonic acid and base, (b) specific complex solvation by solvent molecules, and (c) the influence of solvent expressed by its macroscopic dielectric permittivity. Based on the ion-pair model established by Huyskens and Zeegers-Huyskens (1964), it was predicted that a ΔpK_a of 3.6–6 between protonic acid and base could lead to an almost complete shift to the proton-transfer equilibrium. A recent study (Gilli et al. 2009) also showed that ion-pair formation could be reliably predicted from ΔpK_a between the donor and acceptor groups.

13.1.3 The Confirmation of Ion-Pair Formation

In theory, ion pairs are defined as binary species which exist in solution and in solid in the salt form. Such intermolecular interaction can be qualitatively inferred from the spectral characteristics. A variety of spectroscopic techniques including infrared spectroscopy (IR), nuclear magnetic resonance (NMR), ultraviolet–visible spectroscopy (UV-Vis), electron spin resonance spectroscopy (ESR), and X-ray crystallography could provide insights into ion-pair formation.

IR and NMR spectroscopy often offer experimental proofs to directly indicate the formation of ion pairs, and they are especially pronounced for hydrogen-bonded ion pairs (Barthel and Deser 1994; Biliškov et al. 2011; Habeeb 1997; Pregosin 2009). Recently, by using IR and chemical exchange two-dimensional infrared (2DIR) spectroscopy, Lee et al. (2011) investigated the contact ion pairs (CIP) assembled by Li^{+} and SCN^{-}ions in *N*, *N*-dimethylformamide. In IR spectrum, the CIP formation led to a blue shift (~16 cm^{-1}) of the CN stretch frequency of Li–SCN CIP with respect to that of free SCN^{-} ion. Moreover, the temperature-dependent IR absorption spectra revealed that the CIP formation was an endothermic process. The CIP association and dissociation time constants (165 and 190 ps, respectively) were determined by chemical exchange 2DIR spectroscopy. The experimental results indicated that the ion-pair formation was a dynamic process in electrolyte solutions and in biological systems under physiological conditions. In the case of hydrogen-bonded ion pairs, a broad continuum, called as the Zundel continuum, is often observed in IR spectrum with extensive intermolecular hydrogen bonding, for which proton transfer is valuable. The broad band is caused by the strong hydrogen bonds in which a proton is distributed between the two hydrogen-bonded species by tunneling (Biliškov et al. 2011). According to the classical theory of hydrogen bond, a shift toward lower fields in the NMR spectrum is suggested as a criterion to confirm the formation of a hydrogen bond due to strong deshielding of the protons. Xi et al. (2012a, b) confirmed the formation of hydrogen-bonded ion pairs between weak acidic drugs and organic amines at 1:1 molar ratio by IR and ^{1}H-NMR. In this study, teriflunomide (TEF) and lornoxicam (LOX), two weak acidic drugs with OH groups, were used as the model drugs, and various organic amines including triethylamine (TEtA), diethylamine (DEtA), *N*-(2′-hydroxyethanol)-piperidine (NP), diethanolamine (DEA), and triethanolamine (TEA) were employed as the

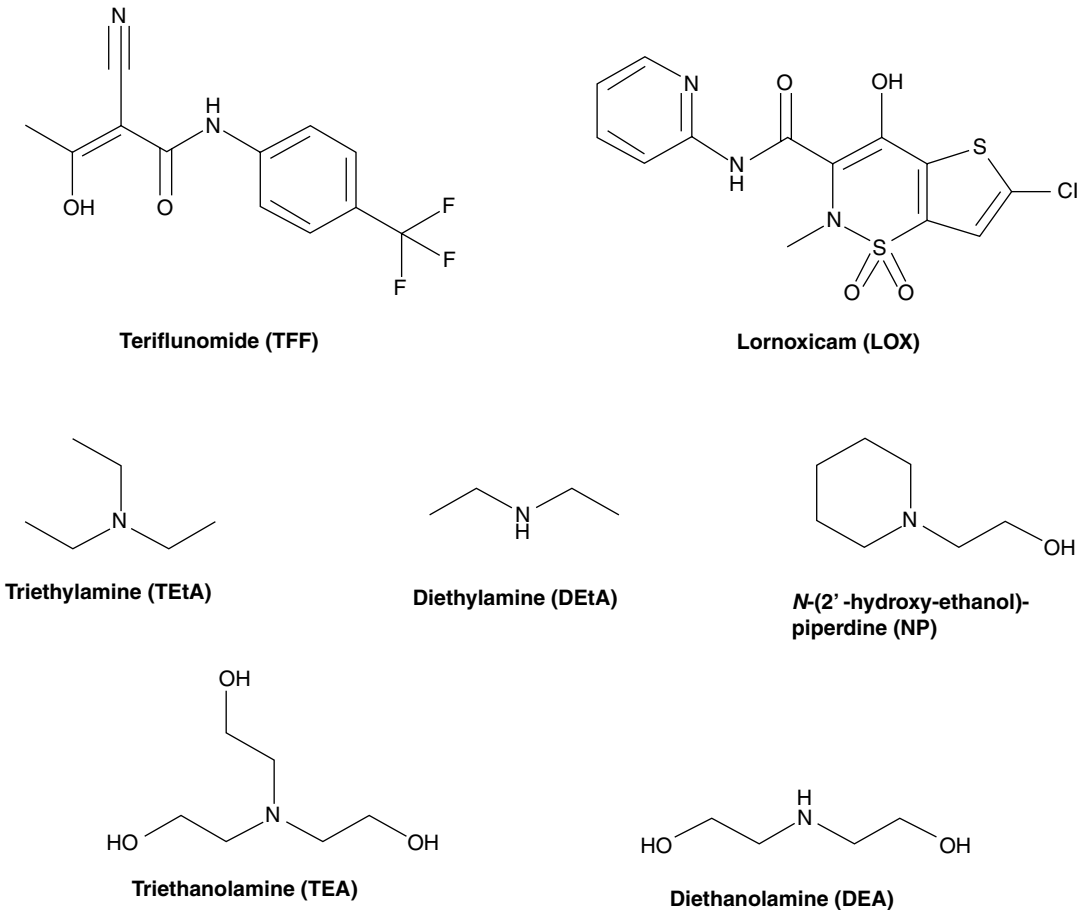


Fig. 13.1 The chemical structure of teriflunomide (TEF), lornoxicam (LOX), triethylamine (TEtA), diethylamine (DEtA), *N*-(2′-hydroxyethanol)-piperidine (NP), diethanolamine (DEA), and triethanolamine (TEA)

counter ions, whose structure was shown in Fig. 13.1. $CHCl_3$ and $CDCl_3$ solutions of TEF or LOX with or without the adding of equimolar organic amines were detected, respectively, by spectroscopic methods. In IR spectra (Fig. 13.2), the absorption at ~3,400 cm^{-1} was assigned to stretching vibration of OH group of the two drugs. A continuum gave rise to a very broad absorption in the 3,300–2,000 cm^{-1} range in the presence of most of organic amines. In ^{1}H-NMR study, compared to the signal of the proton from OH group of TEF or LOX itself (15.35 and 13.02 ppm, respectively), the proton magnetic resonance of OH in the complexes has moved toward higher field, as illustrated in Fig. 13.3. It seemed that the results were contradictory to the abovementioned classical theory of hydrogen bond in NMR. However, actually this phenomenon may be caused by strong shielding of the proton, which was a direct consequence of the intermolecular hydrogen bond interaction between drugs and organic amines instead of intramolecular hydrogen bonds in drugs. Notably, the chemical shift of OH group kept almost constant when the stoichiometric ratios of drug to organic amine were varied from 1:1 to 1:100. These results suggested that TEF and LOX have been integrated sufficiently into ion pairs at the equimolar ratio.

In addition, UV-Vis, ESR spectra in solution, and X-ray crystallography also have been employed for measuring the electronic changes in ion-pair formation process (Lü et al. 2005;

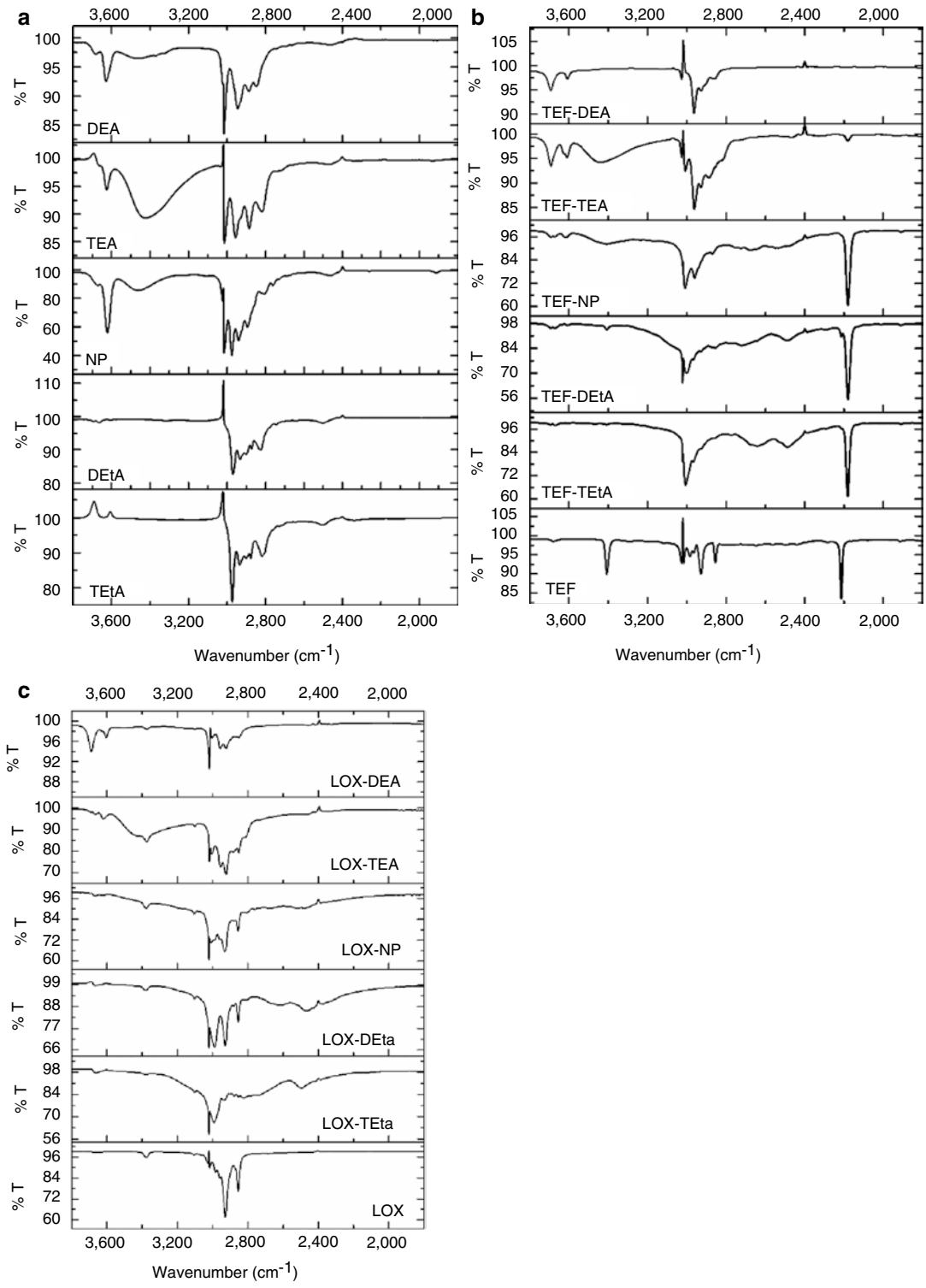


Fig. 13.2 Infrared spectra of organic amines, TEF and LOX in $CHCl_3$: (**a**) organic amines which was equimolar with test drug; (**b**) TEF with or without equimolar organic amines; (**c**) LOX with or without equimolar organic amines

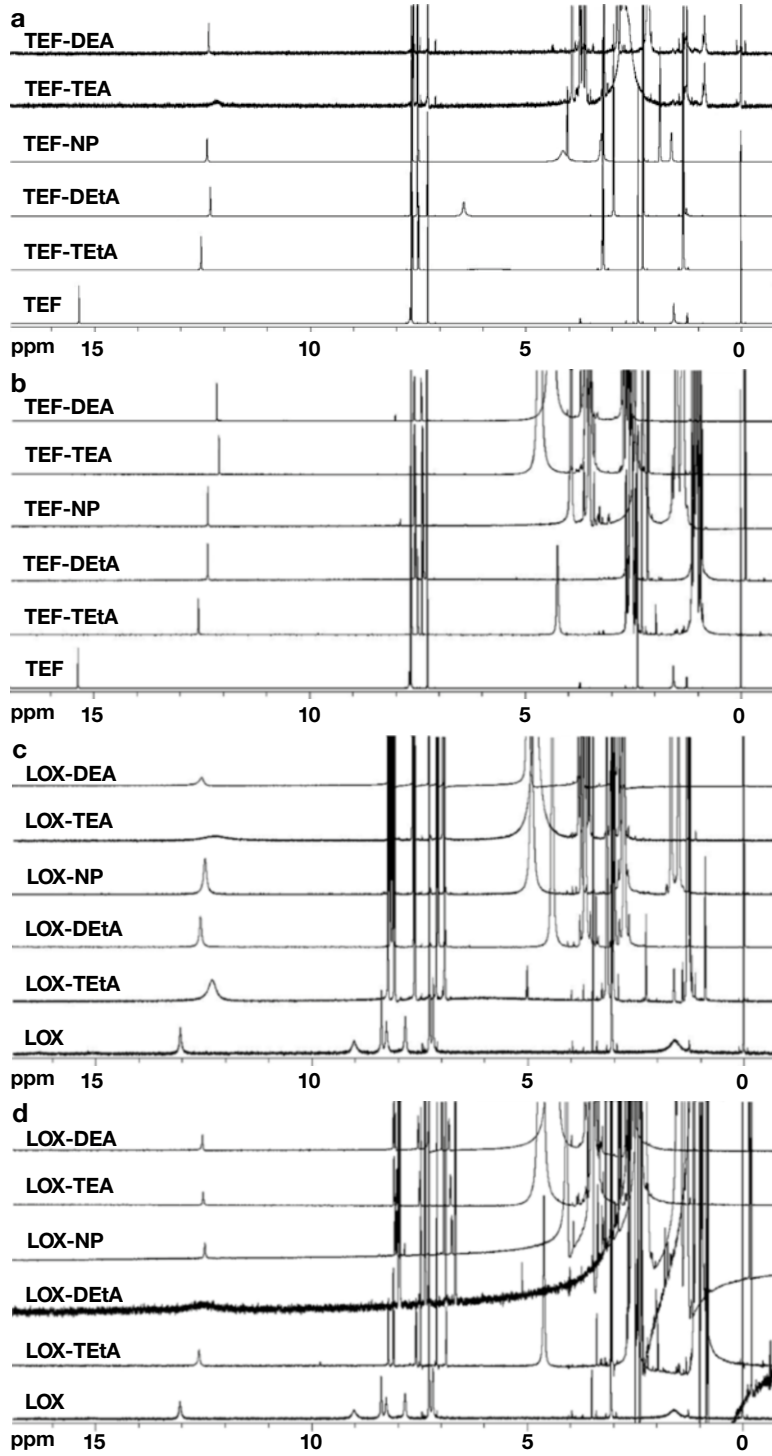


Fig. 13.3 ^{1}H-NMR spectra of TEF and LOX with or without organic amines in $CDCl_3$ at different molar ratios: (**a**) TEF, amines = 1:1; (**b**) TEF, amines = 1:100; (**c**) LOX, amines = 1:1; (**d**) LOX, amines = 1:100

Pal et al. 2010). Hudson et al. (1972) found that when weak acidic 3,4-dinitrophenol encountered organic amines at different molar ratio, a series of characteristic bathochromic shifts in UV absorption spectra were presented in going from free acid to a hydrogen-bonded complex, to an ion pair, to a solvated ion pair or a solvated anion. Lü et al. (2005) determined the crystal structures

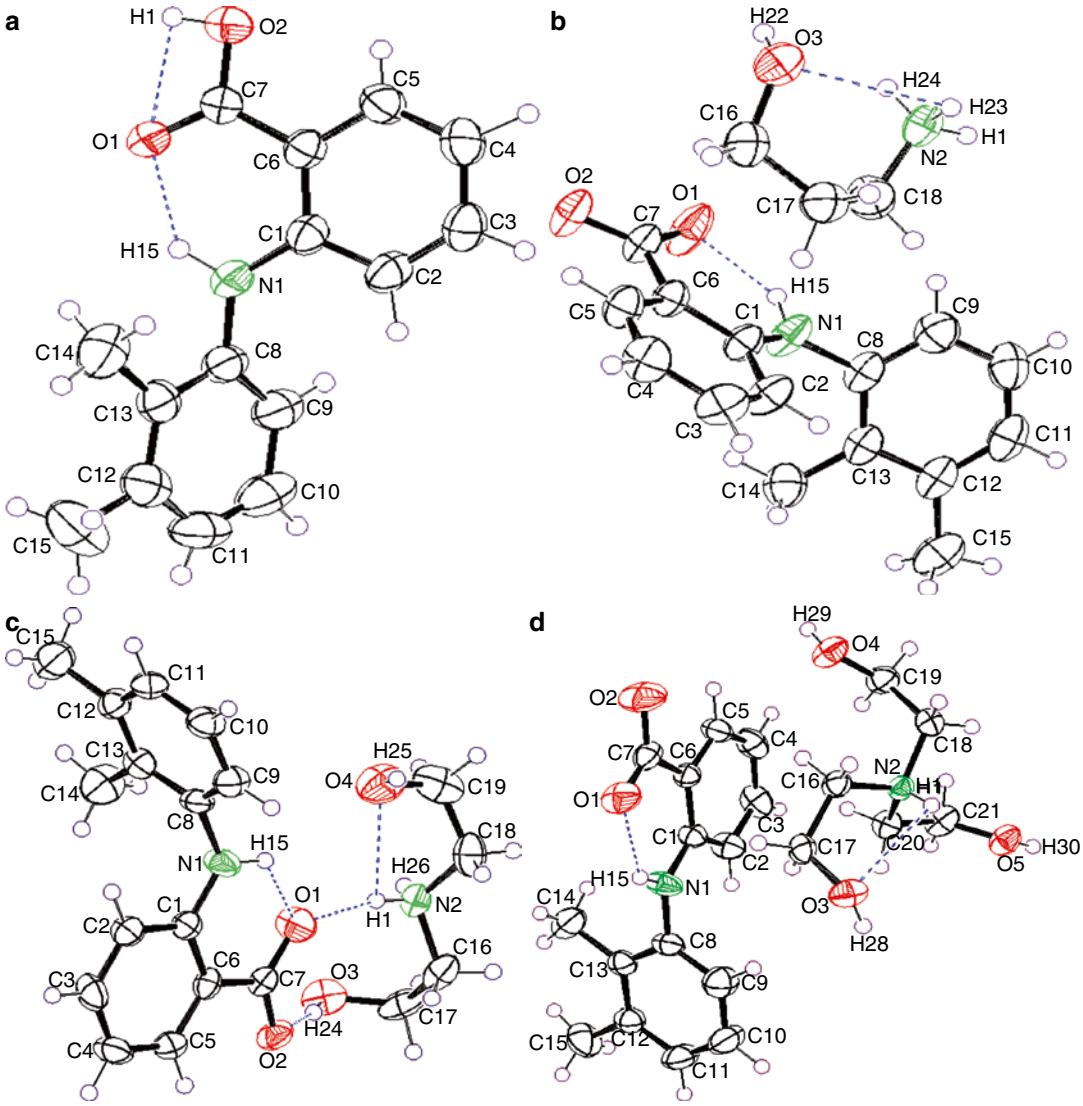


Fig. 13.4 Stereoscopic view of (**a**) MH, (**b**) MH-propanolamine, (**c**) MH-DEA, and (**d**) MH-TEA, showing atom numbering, 50 % thermal probability ellipsoids, and hydrogen bonds. *Dashed lines* indicate the intermolecular hydrogen bonding

of pure ion-pair salts $K(L_C)^+//DNB^-$ and $K(L_E)^+DNB^-$ by X-ray crystallography. In the near-IR spectral analysis, they found that there were the same patterns of vibronic progressions for distinguishing the "separated" from the "contact" ion pair in both of the crystalline solid state and THF solution state, which ensured that the same X-ray structures persist in solution. Most importantly, in this study, the labilities of these dynamic ion pairs in solution were thoroughly elucidated by the temperature-dependent ESR spectral changes. Compared with other methods, X-ray crystallography can provide definitive structural information via analyzing the diffraction pattern of single crystal ion-pair salts. In another study, Fang et al. (2004) prepared the crystals of ion-pair complexes with an equimolar ratio of mefenamic acid (MH) and alkanolamines by removing the solvent in vacuo and subsequently confirmed that these complexes were associated with hydrogen bonds using X-ray crystallography (Fig. 13.4).

13.1.4 The Effect of Ion-Pair Formation on Skin Permeation

13.1.4.1 The Mechanism of Skin Penetration Enhancement by Ion-Pair Formation

The effect of ion-pair formation on skin permeation is complex, and its mechanism has not been thoroughly clarified. Generally, the skin penetration enhancement by ion-pair approach with suitable counter ions is mainly dependent on the physicochemical properties of the counter ions (e.g., lipophilicity, pK_a, and structure) and the solubility of ion pairs in donor medium.

The extent of penetration enhancement by ion-pair formation is strongly related to the lipophilicity of the ion pairs and the properties that depend on the lipophilicity of the selected counter ions. A series of studies performed by Neubert et al. (Neubert et al. 1984; Neubert and Dittrich 1989; Neubert and Fischer 1991) have made great contribution to the understanding of how hydrophilic ionized drugs penetrate across lipid membranes together with lipophilic counter ions. These studies showed that the partition coefficient of the hydrophilic drugs, buformine, quinine, pholedrine, and bretylium, was markedly increased by more than twofold after the formation of ion pairs with lipophilic ions, and thereby the transport of ionized drugs across an artificial lipid membrane (dodecanol collodion membrane) could be enhanced. Moreover, it was found that the counter ions could be accumulated in the lipid membrane due to their high lipid solubility and that they acted as carriers for the ionized drugs. Besides the increased transport of ionized drugs, the counter transport of protons and lithium ions, respectively, was also observed. Nam et al. (2011) also provided a similar result in the skin permeation of hydrophilic and highly ionized risedronate (RIS) with three lipophilic basic counter ions, L-arginine, L-lysine, and diethylenetriamine, at different molar ratios. To varying degree, all the counter ions could enhance the solubility of RIS in xylene, a lipophilic solvent. Although RIS ion pairs are slightly unstable in the aqueous solution, they had a remarkable enhancing effect on RIS penetration from the aqueous solution into hairless mouse skin, and RIS-diethylenetriamine ion pair brought out the largest enhancement ratio (ER), up to 36-fold compared to only RIS.

As to lipophilic drugs possessing some polar functional groups, e.g., –COOH, –OH, and –NH_2, their skin permeation can also be enhanced by hydrogen-bonded ion-pair formation (Cheong and Choi 2002; Green et al. 1989; Kamal et al. 2007; Nogueira et al. 2011). However, for those lipophilic drugs, their lipophilicity can be decreased by ion-pair formation with small molecular weight relative hydrophilic counter ions (Fang et al. 2003). The effect of the organic amines including monoethanolamine (MEA), DEA, TEA, and propanolamine (PPA) on the penetration of mefenamic acid (MH) across hairless rat skin from the lipophilic mixed solvent of isopropyl myristate (IPM) and ethanol (9:1). The *n*-octanol/water partition coefficients (log $K_{o/w}$) at 32 °C of MH and its corresponding ion pairs, MH-MEA, MH-DEA, MH-TEA, and MH-PPA, were 3.31, 0.79, 0.74, 1.99, and 0.66, respectively, which indicated that these complexes were relatively hydrophilic compared with MH. Hence, the transdermal delivery of MH was significantly enhanced by the formation of hydrogen-bonded ion pairs, and the ER values of these ion pairs were 279, 48, 84, and 357, respectively. Obviously, the reduced lipophilicity of the complexes has facilitated the partition from the SC to the ED and consequently enhanced drug delivery through the skin. These results suggested that a major part of ion pairs remained the integrity of ion pair during the process of crossing the lipophilic SC and the hydrophilic ED until they reach the receptor compartment. The subsequent studies done by Fang's group further confirmed this point of view (Table 13.1).

In donor medium, the pK_a of the counter ions is another main factor that influences the skin permeation of ion pairs, especially the hydrogen-bonded ion pairs. A positive correlation was found between the skin permeation of ion pairs and the pK_a of the counter ions was found (Ma et al. 2010; Xi et al. 2012a, b). In other words, the ΔpK_a between the drug and the counter ion (they are acids and bases,

Table 13.1 The enhancement ratio (ER) of ion pairs of drugs with different amines

Drugs	Counter ions	ER[a]
Teriflunomide in isopropyl palmitate[b]	None	1.00
	Diethylamine	2.47
	Triethylamine	12.69
	Triethanolamine	1.44
	Diethanolamine	1.15
	N-(2′-hydroxyethanol)-piperidine	4.54
Lornoxicam in isopropyl palmitate[c]	None	1.00
	Diethylamine	13.63
	Triethylamine	19.52
	Triethanolamine	4.92
	Diethanolamine	13.77
	N-(2′-hydroxyethanol)-piperidine	12.08
Flurbiprofen in 10 % EtOH/isopropyl myristate[d]	None	1.00
	Diethylamine	2.02
	Triethylamine	2.27
	Triethanolamine	2.93
	Diethanolamine	3.76
	Ethanolamine	2.60
	N-(2′-hydroxyethanol)-piperidine	1.86
Glipizide in 20 % EtOH/isopropyl myristate[e]	Na salt	1.00
	Diethylamine	6.79
	Triethylamine	19.61
	Triethanolamine	2.34
	Diethanolamine	5.77
	Ethanolamine	11.92
	N-(2′-hydroxyethanol)-piperidine (NP)	3.90

[a]ER, the enhancement ratio of the cumulative amounts of drug permeated between with and without organic amines
[b,c]Xi et al. (2012a, b)
[d]Ma et al. (2010)
[e]Tan et al. (2009)

correspondingly) can directly influence the strengths (stability) of hydrogen-bonded ion pairs in a nonpolar medium.

Most intermolecular hydrogen bonds in the liquid state are formed and broken on an extremely short timescale (e.g., ~10^{-5} s), even a picosecond scale (Becker 2007; Simon and Peters 1982). It has been widely accepted that there are abundant hydrogen bond acceptors and donors existing in the SC part of the skin and they may disturb the binding of ion pairs and further influence the stability of hydrogen bonds (Guy and Hadgraft 1984; Michaels et al. 1975). According to Xi et al. (2012a, b), the stability parameter of ion pairs (T_{life}) can be obtained, based on the following Eq. 13.2 and data of ^{1}H-NMR shown in Fig. 13.3.

$$T_{life} > \frac{1}{2\pi(\Delta\delta)v_0} \tag{13.2}$$

where ν_0 was the spectrometer frequency and $\Delta\delta$ was the chemical shift difference (Tubbs and Hoffmann 2004). The results in Fig. 13.5 showed that, in general, the ER values were increased with T_{life}. Therefore, the better stability of hydrogen-bond ion pairs notably facilitated transdermal drug delivery.

Megwa et al. (2000a, b) carried out some in vitro studies to evaluate the possibility of improving the skin permeation of salicylate through human epidermis from an aqueous solution by forming ion pairs with basic counter ions (alkylamines and quaternary ammonium ions). The skin permeation of the salicylate ion pairs with primary amines and quaternary ammonium ions was lower than that of salicylate itself (ER < 1), while all of secondary and tertiary amines except DEtA (ER = 0.83) significantly promoted the skin permeation of salicylate (ER: 1.34–4.80). The enhancement effect of these amines on the penetration of salicylate was in the following order: quaternary < primary < secondary < tertiary. This phenomenon was attributed to the fact that the complex of salicylate with tertiary amines had higher stability than that with primary or secondary amines.

The skin permeation rate is also dependent on the concentration of the soluble permeant in the applied vehicle. For lipophilic drugs with sparingly solubility in both oil and water like piroxicam, meloxicam, and LOX, the skin permeation is very poor (Cheong and Choi 2002; Xi et al. 2012b; Zhang et al. 2009). Improving the solubility in donor solution and the partition in the SC and the ED by ion-pair formation is the main mechanism of penetration enhancement for those drugs. A recent study (Song et al. 2012) showed that bisoprolol-maleate salt possessed a higher solubility in DURO-TAK® 87-4098 acrylate-vinyl acetate

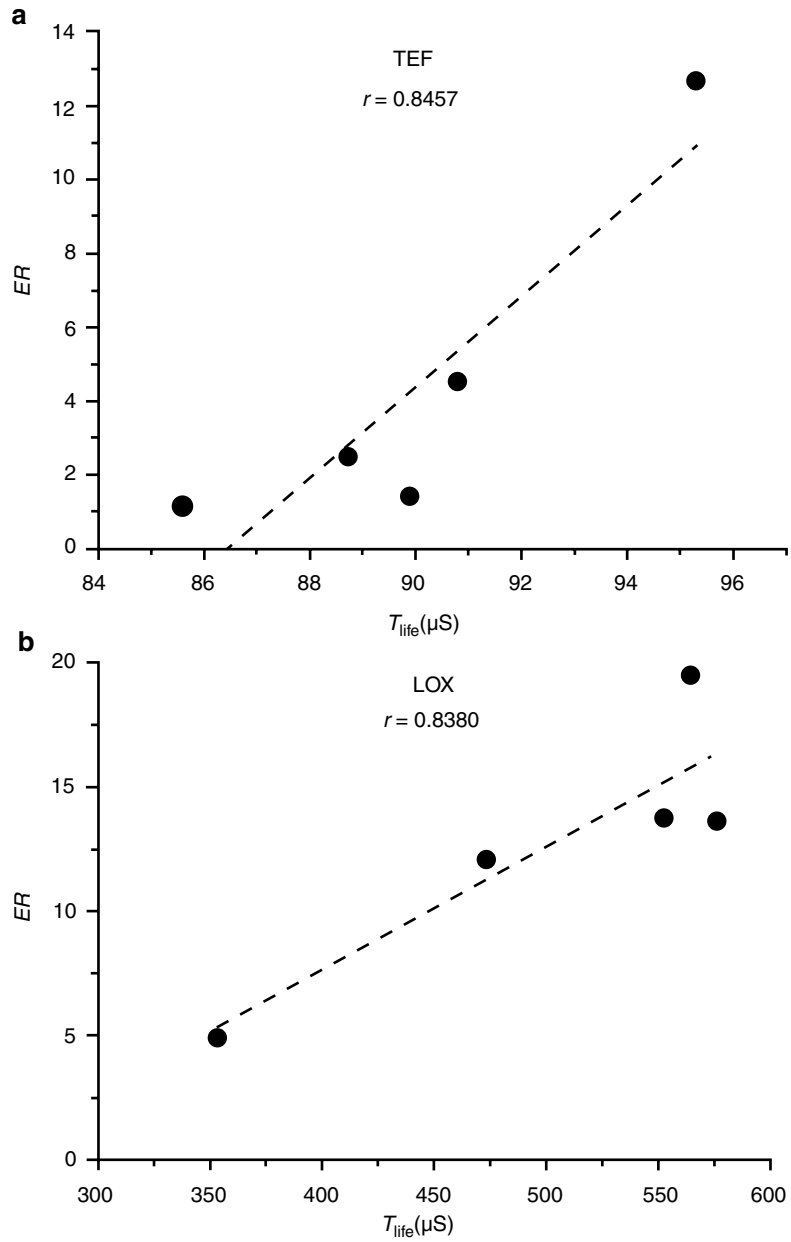


Fig. 13.5 The relationship between the skin enhancement of ion pairs of TEF and LOX and the stability parameter T_{life}

adhesive (National Starch & Chemical Co., USA) than bisoprolol-fumarate salt, which was responsible for the higher penetration enhancing effect of the maleate salt. In addition, ion pairs existing in salt form can obviously decrease the melting point of the parent drug, which is frequently used as a predictor of solubility, and thereby promotes the skin permeation of drugs from transdermal formulations, such as patches and emulgels (Cheong and Choi 2003; Wang and Fang 2008).

13.1.5 Ion-Pair Formation vs. Penetration Enhancers

Currently, the application of chemical enhancers is a frequently used approach to enhance the permeation of drugs through biomembranes. However, chemical enhancers are not omnipotent in the drug delivery across the skin. Tan et al. (2009) found that glipizide (GP) ion pairs with organic amines as the counter ions provided an

Table 13.2 The enhancement ratio (ER) of ion pairs of drugs with different counter ions and penetration enhancers

Drugs	Counter ions	ER[a]	Penetration enhancers	ER
Indapamide in 30 % (w/w) enthol/IPM [b]	None	1.00	None	1.00
	Acetic acid	2.74	5 % Azone®	4.83
	Maleic acid	4.34	5 % *l*-menthol	3.01
	Oxalic acid	2.85	5 % oleic acid	2.52
	Adipic acid	3.10	5 % *N*-methyl pyrrolidone	3.88
	Lactic acid	8.46		
	Citric acid	3.47		
	Succinic acid	5.13		
	Fumaric acid	6.93		
Scutellarin in 20 % enthol/IPM [c]	None	1.00	None	1.00
	Diethylamine	4.76	5 % Azone®	5.64
	Triethylamine	3.83	5 % *l*-menthol	3.28
	Ethanolamine	7.11	5 % oleic acid	2.60
	Diethanolamine	3.68	5 % *N*-methyl pyrrolidone	1.38
	Triethanolamine	1.66		
Meloxicam in 10 % propylene glycol /IPM [d]	None	1.00	None	1.00
	Diethylamine	3.71	5 % Azone®	2.89
	Triethylamine	1.99	5 % *l*-menthol	1.28
	Ethanolamine	1.399	5 % oleic acid	2.29
	Diethanolamine	4.20	5 % *N*-methyl pyrrolidone	5.77
	Triethanolamine	3.94		
	N-(2′-hydroxyethanol)-piperidine	5.78		

[a]ER, the enhancement ratio of the cumulative amounts of drug permeated between with and without counter ions
[b]Ren et al. (2007)
[c]Wang et al. (2008)
[d]Zhang et al. (2009)

obvious enhancement of the skin permeation of GP, while five common enhancers, IPM, propylene glycol, *N*-methyl-2-pyrrolidone (NMP), Azone® (AZ, Tianmen Kejie Pharmaceuticals Co., Ltd., China), and oleic acid (OA), had no enhancing effect. Moreover, Cheong and Choi (2002) investigated the effect of various enhancers on the permeation of piroxicam (PX) and its ethanolamine salts (PX-EAs). The results showed that, in general, ion-pair salts still exerted a great enhancement on the penetration of PX from various saturated solution in which the enhancers also work as the donor mediums. As illustrated in Table 13.2, some studies support the finding that ion-pair formation shows a better or comparable penetration enhancing effect compared to classical chemical penetration enhancers (Ren et al. 2008; Wang et al. 2008; Zhang et al. 2009).

13.2 Complex Coacervates for Penetration Enhancement

Complex coacervates are a specialized form of ion pairs, which represents the separation of an aqueous phase containing a mixture of oppositely, charged ions into a dense coacervate oil phase, rich in ionic complex, and a dilute equilibrium phase. The difference between complex coacervates and ion pairs is that a complex coacervate exists as a binary phase system. However, in transdermal delivery systems, complex coacervates behave like ion pairs. Stott et al. (1996) prepared complex coacervates composed of cationic amitriptyline (AMI) and counter ions including sodium deoxycholate (NaD) or sodium lauryl sulfate (SLS). The produced complex

coacervates AMI-NaD and AMI-SLS were employed to investigate their potentials to enhance transdermal flux of AMI. Although AMI-NaD was separated into two distinct phases (octanol and vehicle), while AMI-SLS was in sol state, both of the systems could obviously increase octanol/vehicle partition coefficients of AMI. However, in the skin permeation study, only the AMI-NaD coacervate provided a 2.2-fold increment in drug flux. On the contrary, the AMI-SLS coacervate showed a marked reduction in drug flux. The results indicated that the increased lipophilicity of the coacervate's oil phase could contribute to an increase in the transdermal flux of charged species.

13.3 Summary

In conclusion, ion-pair formation is a simple and useful method for enhancing percutaneous penetration of drugs by modifying the physicochemical properties of parent molecules and by regulating the partition of drugs between the dosage form, the SC, and the ED. Furthermore, hydrogen-bonded ion pairs make some unionizable molecules become suitable for ion-pair formation. In order to obtain a maximum enhancement in permeation of drugs, the combination of ion pairs and penetration enhancers is a feasible approach. The enhancement mechanism of ion pairs in transdermal drug delivery is worth for further study. As to complex coacervates, although the published data about their application in the field of transdermal drug delivery is limited, this technology is still a bright prospect for transdermal pharmaceutical formulations.

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Formulation of Drug-Cyclodextrin Complexes

14

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Contents

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14.1 Introduction

Drugs permeate intact skin as single molecules. When drug products are applied to the skin surface, dissolved drug molecules diffuse through the vehicle to the skin where the molecules partition from the vehicle into the skin and then permeate the skin barrier, stratum corneum, into the more permeable inner skin layers. Most penetration enhancers, chemical as well as physical, enhance drug delivery by making the skin barrier more permeable. Cyclodextrins are different. They enhance drug delivery into and through the skin by increasing the availability of dissolved drug molecules right at the skin surface. However, cyclodextrins can also hamper dermal and transdermal drug delivery by preventing drug molecules from partitioning from the surface into the skin. Thus, successful employment of cyclodextrins in topical drug formulations requires good understanding of their physicochemical properties and the way they enhance topical drug bioavailability.

Numerous books and reviews have been written on cyclodextrins, their industrial applications, and usage in drug formulations (Loftsson and Brewster 1996; Dodziuk 2006; Douhal 2006; Brewster and Loftsson 2007; Loftsson and Brewster 2010; Loftsson and Duchêne 2007; Stella and He 2008; Uekama et al. 2006; Hedges 1998; Kurkov and Loftsson 2013; Bilensoy 2011).

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In this chapter, the effects of cyclodextrins on drug delivery through biological membranes are discussed with emphasis on dermal and transdermal drug delivery.

14.1.1 Cyclodextrins and Their Properties

Cyclodextrins are cyclic oligosaccharides containing 6 (αCD), 7 (βCD), 8 (γCD), or more glucopyranose monomers linked via α-1,4-glycoside bonds (Table 14.1). These parent cyclodextrins are natural products formed by microbial degradation of starch. The outer surface of the doughnut-shaped cyclodextrin molecules is hydrophilic, bearing numerous hydroxyl groups, but their central cavity is somewhat lipophilic. Although the parent cyclodextrins and their complexes are hydrophilic, their aqueous solubility is somewhat limited. This is thought to be due to relative strong intermolecular binding in their crystal state. Partial random substitution of the hydroxy groups will result in significant improvements in their solubility (Table 14.1). Cyclodextrins possess many of the same physicochemical and biological properties as their corresponding linear dextrins. In their solid state, cyclodextrins are as stable as starch and can be stored for a number of years at room temperature without any detectable degradation (Szejtli 1988). In aqueous solutions, their degradation follows specific acid-catalyzed hydrolysis of the glycoside bonds to form glucose, maltose, and linear dextrins. In pure aqueous solution, the half-life for ring opening of βCD was determined to be approximately 15 h at pH 1.1 and 70 °C (Hirayama et al. 1992). αCD is somewhat more stable and γCD somewhat less than βCD (Schönberger et al. 1988). Cyclodextrins are stable towards β-amylases, but γCD is degraded by salivary α-amylase (Szejtli 1987; Munro et al. 2004). αCD, βCD, and γCD, as well as their derivatives that are currently used in pharmaceutical products, undergo bacterial digestion in the gastrointestinal tract (Irie and Uekama 1997; Kurkov and Loftsson 2013). Formation of inclusion complexes increases the stability of cyclodextrins, both towards nonenzymatic and enzymatic degradation. There are no reports of transporter-mediated permeation of cyclodextrins across biological membranes, and in general, the oral bioavailability of cyclodextrins is well below 4 % (Kurkov and Loftsson 2013). After parenteral administration, cyclodextrins are, like low-molecular-weight dextrins, mainly excreted unchanged with urine. In humans, their biological half-life is about 1.9 h and volume of distribution about 0.2 L/kg (Kurkov and Loftsson 2013). The safety and toxicology of cyclodextrins have recently been reviewed (Stella and He 2008; Arima et al. 2011).

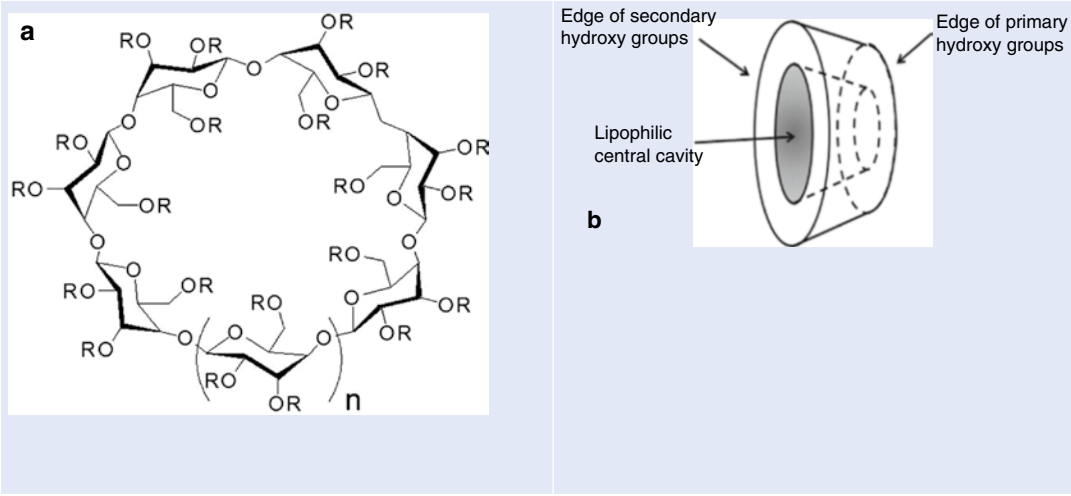
The regulatory status of cyclodextrins is slowly evolving as more and more cyclodextrin-containing products are being approved (Hincal et al. 2011). All three parent cyclodextrins and many of their derivatives can be found in US Pharmacopeia/National Formulary (USP/NF), the European Pharmacopoeia (Ph.EUR.), and the Japanese Pharmaceutical Codex (JPC). The parent cyclodextrins have been included in the "generally recognized as safe" (GRAS) list of the FDA, and they are commonly found in both food and toiletry products throughout the world. Worldwide cyclodextrins can be found in about 40 marketed pharmaceutical products (Loftsson and Brewster 2010; Hincal et al. 2011).

14.1.2 Cyclodextrin Complexes

Cyclodextrins are able to form drug-cyclodextrin inclusion complexes by taking up somewhat lipophilic drug moieties (or even small lipophilic molecules) into the central cavity (Fig. 14.1). No covalent bonds are formed or broken during the complex formation, and drug molecules bound in the complex are in very dynamic equilibrium with free drug molecules in solution. Thus, cyclodextrin complexes dissociate readily upon simple dilution, for example, upon injection into liquid chromatographic system or after parenteral administration.

The main purpose for adding cyclodextrins to percutaneous drug formulations is to enhance aqueous solubility of poorly soluble drugs and,

Table 14.1 Characteristics of the most common natural cyclodextrins and some of their derivatives that are of pharmaceutical interest



Cyclodextrin	*n*	R = H or	Abbreviation	Synonyms	MS[a]	MW (Da)	Solubility[b] (mg/ml)	Log$K_{o/w}$[c]
α-Cyclodextrin	0		αCD	Alfadex	–	972.8	130	−13
β-Cyclodextrin	1		βCD	Betadex	–	1,135	18.4	−14
2-Hydroxypropyl-β-cyclodextrin	1	$-CH_2CHOHCH_3$	HPβCD	Hydroxypropyl betadex	0.65	1,400	>600	−11
Sulfobutylether β-cyclodextrin sodium	1	$-(CH_2)_4SO_3^-\ Na^+$	SBEβCD	Betadex sulfobutyl ether sodium	0.9	2,163	>500	<−10
Randomly methylated β-cyclodextrin	1	$-CH_3$	RMβCD		1.8	1,312	>500	−6
γ-Cyclodextrin	2		γCD	Gammadex	–	1,297	249	−17
2-Hydroxypropyl-γ-cyclodextrin	2	$-CH_2CHOHCH_3$	HPγCD	Hydroxypropyl gammadex	0.6	1,576	>500	−13

[a]Molar substitution (MS) is defined as the average number of substituents per glucopyranose repeat unit
[b]From references (Loftsson and Brewster 2011; Sabadini et al. 2006)
[c]Calculated Log$K_{o/w}$ (octanol-water partition coefficient) at 25 °C (interactive LogK_{ow} Calculator, Syracuse Research Corporation: http://www.srcinc.com/what-we-do/free-demos.aspx). These are approximate values. The exact values for the cyclodextrin derivatives depend on their MS as well as the location of the substituents

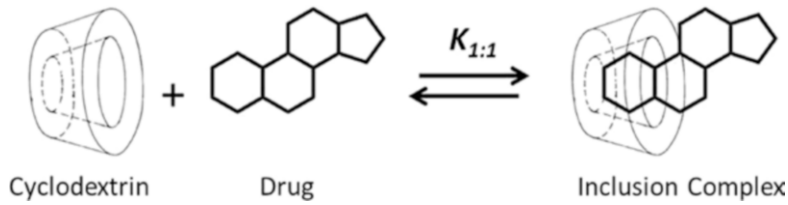


Fig. 14.1 Formation of one-to-one (i.e., 1:1) drug-cyclodextrin inclusion complex

thus, increase their topical bioavailability. Higuchi and Connors' phase-solubility method is used to study the effect of cyclodextrin concentrations on drug solubility (Fig. 14.2) (Higuchi and Connors 1965; Loftsson et al. 2005; Loftsson and Hreinsdóttir 2006). The complex formation is a reversible process:

$$m \cdot D + n \cdot \mathrm{CD} \underset{}{\overset{K_{m:n}}{\rightleftharpoons}} D_{\mathrm{m}}\,\mathrm{CD}_{\mathrm{n}} \qquad (14.1)$$

where m drug molecules (D) associate with n cyclodextrin (CD) molecules to form a complex of m:n stoichiometry. $K_{m:n}$ is the observed stability constant of the complex, also known as the binding constant, formation constant, or association constant. The stability constant can be written as follows:

$$K_{m:n} = \frac{\left[D_{\mathrm{m}}\mathrm{CD}_{\mathrm{n}}\right]}{\left[D\right]^{\mathrm{m}} \cdot \left[\mathrm{CD}\right]^{\mathrm{n}}} \qquad (14.2)$$

where the brackets denote the molar concentrations. Most commonly, one drug molecule forms a complex with one cyclodextrin molecule:

$$K_{1:1} = \frac{\left[D/\mathrm{CD}\right]}{\left[D\right] \cdot \left[\mathrm{CD}\right]} \qquad (14.3)$$

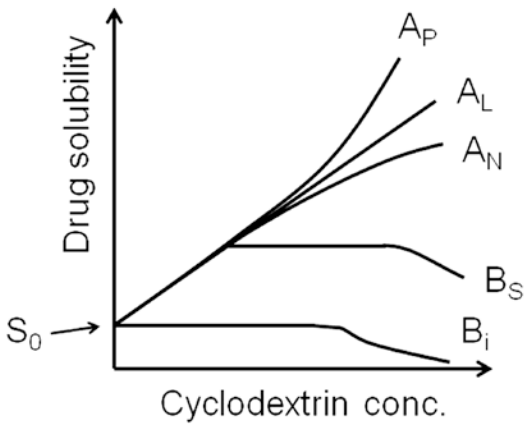


Fig. 14.2 Phase-solubility diagrams. A-type diagrams are due to formation of water-soluble complexes and are usually associated with the water-soluble cyclodextrin derivatives. B-type diagrams indicate formation of poorly soluble complexes that are usually associated with the poorly soluble parent cyclodextrins. S_0 is the intrinsic drug solubility, i.e., the solubility of the drug in the complexation media when no cyclodextrin is present

where, in saturated drug solutions, [D] is the intrinsic solubility of the drug (S_0), i.e., the solubility when no cyclodextrin is present in the aqueous complexation media. The total drug solubility ($[D]_T$) in a given media is then:

$$\left[D\right]_{\mathrm{T}} = S_0 + \left[D/\mathrm{CD}\right] \qquad (14.4)$$

assuming 1:1 D/CD complex formation according to Eq. 14.3. A plot of $[D]_T$ versus $[CD]_T$ for the formation of a 1:1 D/CD complex should give a straight line (i.e., A_L-type phase-solubility diagram, Fig. 14.2) with the y-intercept representing S_0 and $K_{1:1}$ defined as (Higuchi and Connors 1965):

$$K_{1:1} = \frac{\mathrm{Slope}}{S_0 \cdot \left(1 - \mathrm{Slope}\right)} \qquad (14.5)$$

where *Slope* is the slope of the linear A_L diagram. The slope is always less than unity when 1:1 complex is being formed. Complexes of other stoichiometry are less common (Brewster and Loftsson 2007; Loftsson and Brewster 2010). A_P-type profile can indicate formation of a complex that is second or higher order with respect to cyclodextrin or that cyclodextrin complex aggregates (nanoparticles) are being formed. The complexation efficiency (CE) is calculated from the slope of the phase-solubility diagram. It is independent of the intercept (or S_0) and frequently used when the influence of various pharmaceutical excipients on the solubilization is investigated (Loftsson and Brewster 2010, 2012). For 1:1 D/CD complexes, the CE is calculated as follows:

$$\mathrm{CE} = \frac{\left[D/\mathrm{CD}\right]}{\left[\mathrm{CD}\right]} = S_0 \cdot K_{1:1} = \frac{\mathrm{Slope}}{\left(1 - \mathrm{Slope}\right)} \qquad (14.6)$$

The drug:CD molar ratio in a particular complexation media saturated with the drug can thus be calculated from the CE:

$$D:\mathrm{CD\ molar\ ratio} = 1:\frac{\left(\mathrm{CE}+1\right)}{\mathrm{CE}} \qquad (14.7)$$

For a more detailed mathematical description of the complex formation, the reader is referred to

recent reviews (Brewster and Loftsson 2007; Loftsson and Brewster 2010) and the original publication by Higuchi and Connors (1965). Additionally, the effects of various pharmaceutical excipients on $K_{1:1}$ and CE and how they can enhance the solubilizing effects of cyclodextrins have been reviewed (Loftsson and Brewster 2012).

14.2 Cyclodextrins as Permeability Enhancers

In general, chemical penetration enhancers, such as sulfoxides, fatty acids, fatty acid esters, alcohols, amides, and surfactants, enhance drug permeation into and through the skin by permeating into the skin barrier where they temporarily decrease its barrier properties. These penetration enhancers enhance membrane permeation of both hydrophilic and lipophilic drugs and, in most cases, from both nonaqueous and aqueous vehicles. Studies have shown that the permeation-enhancing properties of cyclodextrins are quite different from these chemical permeation enhancers (Masson et al. 1999; Loftsson and Masson 2001; Loftsson et al. 2004; Dahan et al. 2010; Dahan and Miller 2012; Hymas et al. 2012). For example, only negligible amounts of cyclodextrins are able to permeate intact skin and, thus, they do not directly affect the skin barrier. In one study only 0.02 % of topically applied HPβCD was absorbed into intact hairless mouse skin over 24 h period, whereas 24 % was absorbed into stripped skin where stratum corneum had been removed (Tanaka et al. 1995). Another study showed that only 0.3 % of the more lipophilic dimethyl-β-cyclodextrin was absorbed into intact rat skin after topical application (Gerlóczy et al. 1988). In addition, cyclodextrins are only able to enhance drug permeation from aqueous vehicles and in most cases they are only able to enhance permeation of lipophilic poorly water-soluble drugs (Loftsson et al. 2007b, 2008; Loftsson and Brewster 2011; Loftsson 2012).

There are numerous reports on the effects of cyclodextrins on dermal and transdermal drug delivery (Table 14.2). Depending on the experimental conditions and vehicle composition, cyclodextrins either increase or decrease drug permeation through the skin. Still more studies can be found on the effects of cyclodextrins on drug absorption from the gastrointestinal tract and the buccal cavity through the nasal mucosa as well as through other mucosal membranes, all of which can give us some insight into how cyclodextrins act as penetration enhancers (Loftsson et al. 2007b, 2008; Loftsson and Brewster 2011; Loftsson 2012).

14.2.1 Theoretical Background

Drugs permeate the skin via passive diffusion. The driving force for passive diffusion through an aqueous vehicle into the skin and then through the skin is the gradient of chemical potential (μ) (Higuchi 1960; Idson 1971). Likewise, the partitioning of drug molecules from the skin exterior into the outermost skin layer is controlled by the chemical potential. High chemical potential of the drug in topical vehicle is a prerequisite for its good dermal bioavailability:

$$\mu_2 = \mu_2^\theta + \mathrm{RT}\ln a_2 = \mu_2^\theta + \mathrm{RT}\ln\left(\gamma_2 m_2\right) \tag{14.8}$$

and

$$a_2 = \gamma_2\, m_2 \tag{14.9}$$

where μ_2 is the chemical drug potential in the vehicle, μ_2^θ is the chemical potential in a given standard state, a_2 is the thermodynamic drug activity, R is the gas constant, T is the temperature in Kelvin, γ_2 is the activity coefficient, and m_2 is the molality of the drug. The thermodynamic definition of the partition coefficient ($K_{o/w}$) of a drug between organic (o) and aqueous (w) phases is:

$$\frac{\mu_w^\theta - \mu_o^\theta}{\mathrm{RT}} = \ln\frac{a_o}{a_w} \approx \ln\frac{\gamma_o \cdot C_o}{\gamma_w \cdot C_w} = \ln\frac{\gamma_o}{\gamma_w} + \ln K_{o/w} \tag{14.10}$$

Equation 14.10 states that equilibrium between the two phases is attained when the chemical potential of the drug in one phase (e.g., in water or the aqueous membrane exterior (μ_w)) is equal to

Table 14.2 Examples of cyclodextrin-containing dermal formulations and transdermal drug delivery studies

Drug	Cyclodextrin	Reference
Acitretin	RMβCD	Loftsson et al. (1995)
Alkannin	HPβCD	Chen et al. (1996)
Avobenzone	HPβCD	Yang et al. (2008)
Beclomethasone dipropionate	γCD	Uekama et al. (1985)
4-Biphenylylacetic acid	βCD, DMβCD, HPβCD	Arima et al. (1990, 1996)
Bupranolol	HPβCD, PMβCD	Babu and Pandit (2004)
Capsaicin	HPβCD	Zi et al. (2008)
Celecoxib	DMβCD	Ventura et al. (2006)
Curcumin	HPβCD, HPγCD	Hegge et al. (2008)
Dexamethasone acetate	βCD, HPβCD	Lopez et al. (2000)
17β-Estradiol	HPβCD	Loftsson et al. (1991)
Fludrocortisone acetate	γCD	Klang et al. (2012)
Human growth hormone	αCD, βCD, HPβCD	Shakory et al. (2010)
Hydrocortisone	βCD, CMβCD, HPβCD, MLβCD, RMβCD	Loftsson et al. (1991, 1994a, b), Loftsson and Sigurðardottir (1994), Sigurdardottir and Loftsson (1995), Preiss et al. (1995), Chang and Banga (1998), Masson et al. (1999), Kear et al. (2008)
Ibuprofen	HPβCD	Iervolino et al. (2000)
Indomethacin	βCD, DEβCD, DMβCD	Okamoto et al. (1986), Kawahara et al. (1992)
Ketoprofen	HPβCD	Batzdorf and Mullergoymann (1993)
Liarozole	HPβCD	Vollmer et al. (1993)
Lidocaine	DMβCD, HPβCD, SBEβCD	Dollo et al. (1998)
Loteprednol etabonate	DMβCD	Loftsson and Bodor (1994)
Melatonin	HPβCD	Lee et al. (1998)
Metopimazine	MβCD	Bounoure et al. (2007)
Methyl paraben	HPβCD	Tanaka et al. (1995)
Miconazole	αCD, HPβCD	Tenjarla et al. (1998)
Naproxen	βCD	Celebi et al. (1993)
Piribedil	RMβCD	Legendre et al. (1995)
Piroxicam	HPβCD	Doliwa et al. (2000), (2001)
Prednisolone	βCD, γCD	Uekama et al. (1987)
Progesterone	αCD, βCD, γCD	Klang et al. (2010)
Prostaglandin E1	αCD, βCD, CMEβCD,	Adachi et al. (1992, 1993), Uekama et al. (1992), Yuzuriha et al. (1999)
Shikonin	HPβCD	Chen et al. (1996)
Sulfanilic acid	βCD, DMβCD	Okamoto et al. (1986)
Testosterone	HPβCD	Loftsson et al. (1991)
Tolnaftate	βCD, βCD-polymer	Szeman et al. (1987)
Tretinoin	βCD, HPβCD, DMβCD	Amdidouche et al. (1994), Montassier et al. (1998), Ascenso et al. (2012)
Triamcinolone	HPβCD	Kear et al. (2008)

αCD α-cyclodextrin, *βCD* β-cyclodextrin, *CMβCD* carboxymethyl-β-cyclodextrin, *HPβCD* 2-hydroxypropyl-β-cyclodextrin, *RMβCD* randomly methylated β-cyclodextrin, *CMEβCD* carboxymethyl-ethyl-β-cyclodextrin, *DEβCD* diethyl-β-cyclodextrin, *DMβCD* dimethyl-β-cyclodextrin, *MLβCD* maltosyl-β-cyclodextrin, *PMβCD* partially methylated β-cyclodextrin, *SBEβCD* sulfobutylether β-cyclodextrin, *βCD-polymer* β-cyclodextrin polymer, *γCD* γ-cyclodextrin, *HPγCD* 2-hydroxypropyl-γ-cyclodextrin

the chemical potential in the other phase (e.g., the oil phase or the membrane itself (μ_o)). Thermodynamic activity is equal to unity in saturated solutions, and, thus, many ointments and creams consist of finely divided drug suspensions. Under such conditions, the vehicle is saturated with drug, and dissolved drug molecules are at their highest potential to leave the vehicle and partition into the skin. Addition of solubilizers, such as cyclodextrins, to an aqueous drug solution will lower the drug activity (i.e., lowers γ_w in Eq. 14.10), and, thus, under normal conditions, cyclodextrins lower the potential of the drug to exit the formulation (Másson et al. 2005). However, addition of cyclodextrin to aqueous drug suspension, increasing the amount of dissolved drug while keeping the solution saturated with drug, will not lower the drug activity as long as solid drug is present in the aqueous suspension. Under such condition, the thermodynamic activity (a_w in Eq. 14.10) will remain equal to unity, and, thus, dissolved drug molecules are at their highest "exiting" potential, while total amount of dissolved drug is increased. Adding too much cyclodextrin to an aqueous dermal formulation will, on the other hand, decrease the activity (a_w) below unity and, consequently, result in less than optimum topical bioavailability. Although passive diffusion is driven by the gradient of chemical potential, it is common to replace it by the concentration gradient. For example, according to Fick's first law, the driving force for steady-state drug diffusion between two points (i.e., from point 1 to point 2) in a solution is the concentration gradient:

$$J = \frac{D \cdot (C_1 - C_2)}{h} \tag{14.11}$$

where J is the drug flux, D is the drug diffusion constant, C_1 and C_2 are the drug concentrations at point 1 and point 2, respectively, and h is the distance between the two points.

Most biological membranes are multilayer membrane barriers, and most contain various diffusion pathways and transport systems. Higuchi described passive drug transport through multilayer barriers as series of additive resistances analogous to electric circuits (Higuchi 1960). Later drug permeation through biological membranes was described mathematically as drug permeation through a lipophilic membrane sandwiched between unstirred water layers (UWLs) emphasizing that the UWL must be treated as a part of the total membrane barrier (Zwolinski et al. 1949; Flynn et al. 1972; Flynn and Yalkowsky 1972; Loftsson et al. 2007b). Here a simple two-barrier model will be used to explain how cyclodextrins affect drug permeation from an aqueous vehicle into and through the skin or other biological membranes (Fig. 14.3) (Loftsson and Brewster 2011). In this model, the drug molecules encounter two barriers on their way from the vehicle through a lipophilic membrane. The first one is the aqueous boundary layer at the membrane surface, the UWL. The second one is the lipophilic membrane itself, frequently identified as the outermost layer of the skin, stratum corneum. The total skin barrier towards drug permeation consists of the UWL and the lipophilic membrane. Assuming independent and additive resistances of the two layers, the total drug permeation resistance (R_T) of this simple membrane can be defined as:

$$R_T = R_D + R_M \tag{14.12}$$

where R_D and R_M are the drug permeation resistances in the UWL at the exterior and within the lipophilic membrane, respectively. Since the permeability constants (P) are the reciprocals of the resistances, the following equation is obtained assuming sink conditions (i.e., $C_V - C_D \approx C_V$ and $C_1 - C_2 \approx C_1$ in Fig. 14.3):

$$J = P_T \cdot C_V = \left(R_D + R_M\right)^{-1} \cdot C_V = \left(\frac{1}{P_D} + \frac{1}{P_M}\right)^{-1} \cdot C_V \tag{14.13}$$

where J is the drug flux from the aqueous vehicle through the membrane, P_T is the overall permeability coefficient, C_V is the concentration of the compound in the aqueous vehicle, and P_D and P_M are the permeability coefficients in the UWL and within the membrane, respectively. Rearranging Eq. 14.13 gives:

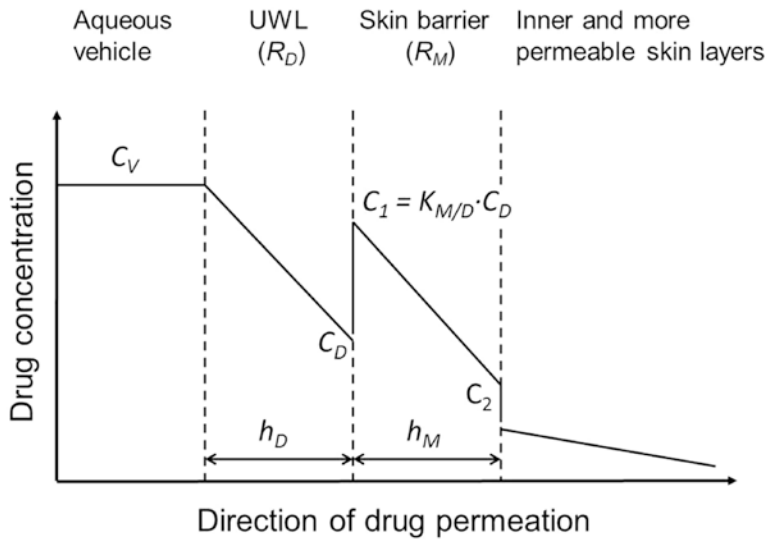


Fig. 14.3 Drug permeation through a simple two-layer barrier where an unstirred water layer (UWL) forms an aqueous diffusion barrier at the vehicle – skin surface and a skin barrier (stratum corneum) that is a lipophilic membrane barrier. The vehicle contains the dissolved drug; R_D, h_D, R_M, and h_M are the resistance and the thickness of the UWL (D) and the membrane (M), respectively. C_V is the drug concentration in the vehicle, C_D is the drug concentration in the UWL immediate to the membrane surface, C_1 and C_2 are the drug concentrations within the membrane, and $K_{M/D}$ is the drug partition coefficient between the membrane and the UWL

$$J = \left(\frac{P_D \cdot P_M}{P_D + P_M} \right) \cdot C_V \qquad (14.14)$$

If permeation is much slower through the membrane itself than the UWL (i.e., $P_D > P_M$), then:

$$J \approx \left(\frac{P_D \cdot P_M}{P_D} \right) \cdot C_V = P_M \cdot C_V \qquad (14.15)$$

In that case, stratum corneum is the main barrier, and the UWL has negligible effect on the drug permeation through the membrane and can be ignored (i.e., $R_M > R_D$). If, on the other hand, permeation through the lipophilic membrane, i.e., the skin itself, is much faster than permeation through the UWL (i.e., $P_M > P_D$), then:

$$J \approx \left(\frac{P_D \cdot P_M}{P_M} \right) \cdot C_V = P_D \cdot C_V \qquad (14.16)$$

In this case, the UWL is the main barrier (i.e., $R_D > R_M$), and drug permeation through the membrane becomes aqueous diffusion layer controlled. The relationship between the permeation coefficient (P) and the diffusion coefficient (D) is given by Eq. 14.17:

$$P = \frac{D \cdot K}{h} \qquad (14.17)$$

where h is the thickness of the UWL (h_D) or the lipophilic membrane (h_M) and K is either the partition coefficient between the membrane and the UWL ($K_{M/D}$) or equal to unity (i.e., $K = 1.00$) as in the case of the UWL. Finally, D can be estimated from the Stokes-Einstein equation:

$$D \approx \frac{R \cdot T}{6\pi \cdot \eta \cdot r \cdot N} \qquad (14.18)$$

where R is the molar gas constant, T is the absolute temperature, η is the apparent viscosity within the UWL or the lipophilic membrane, r is the radius of the permeating drug molecule, and N is Avogadro's number. Thus, the diffusion constant within the UWL (D_D) will decrease with increasing viscosity of the layer as well as with increasing molecular weight of the drug.

14.2.2 Cyclodextrins and Biological Membranes

The effects of cyclodextrins on drug permeation through the skin, mucus membranes, and various artificial and biological membranes have been thoroughly reviewed (Matsuda and Arima 1999; Loftsson and Masson 2001; Loftsson et al. 2007b; Cal and Centkowska 2008; Loftsson and Brewster 2011). Based on these studies, some general remarks can be made on how and when cyclodextrins enhance drug delivery into and through biological membranes.

14.2.2.1 The Drug Molecules Have to Be Released from the Complex

Hydrophilic cyclodextrins and their complexes do not, in general, permeate lipophilic biomembranes (i.e., their $K_{M/D} \approx 0$; Fig. 14.3 and Eq. 14.17). The $\text{Log}K_{o/w}$ of cyclodextrins that are currently used in pharmaceutical formulations is very low (≤ -6; Table 14.1 and Eq. 14.10), and, thus, these cyclodextrins and their complexes have virtually no tendency to partition from the aqueous exterior into lipophilic membrane. There are no reports of transporter-mediated permeation of cyclodextrins across biological membranes, and in general, the oral bioavailability of cyclodextrins is well below 4 % (Kurkov and Loftsson 2013). Only about 0.02 % of topically applied HPβCD (calculated $\text{Log}K_{o/w} \approx -11$) is absorbed into intact hairless mouse skin (Tanaka et al. 1995). Consequently, the drug molecules have to be released from the complexes before they can permeate biological membranes (Loftsson and Brewster 2011). Some lipophilic cyclodextrin derivatives are, however, able to penetrate into lipophilic membranes (e.g., the nasal mucosa) and act as conventional chemical penetration enhancers, increasing drug permeation by reducing the lipophilic membrane barrier.

14.2.2.2 Cyclodextrins Can Prevent Drug Permeation

Cyclodextrins can prevent drug permeation through biological membranes. For example, tablets containing large amounts of αCD (calculated $\text{Log}K_{o/w} \approx -13$) are used to complex triglycerides in the gastrointestinal tract and prevent their absorption (Comerford et al. 2011; Artiss et al. 2006). Hydrophilic cyclodextrins have been added to sunscreen formulations to reduce absorption of lipophilic sunscreen agents into the skin (Felton et al. 2002, 2004; Sarveiya et al. 2004; Yang et al. 2008). Cyclodextrins, like HPβCD and γCD (calculated $\text{Log}K_{o/w} \approx -17$), have been used to reduce absorption of the mosquito repellent *N*,*N*-diethyl-3-methylbenzamide (DEET) through the skin (Proniuk et al. 2002). Cyclodextrins can likewise be used to decrease dermal and transdermal uptake of sunscreen agents (Cal and Centkowska 2008; Berbicz et al. 2011). The key factor here is to use excess amounts of cyclodextrins in the aqueous vehicle, i.e., more than what is needed to solubilize the poorly soluble lipophilic agent. This is done to reduce the amount of free agent (i.e., drug, mosquito repellent, and sunscreen agent) present in the formulation, thus reducing its partition into the skin. In other words, addition of excess cyclodextrin to the vehicle will lower the potential of the drug to exit the formulation (Eq. 14.10).

14.2.2.3 Cyclodextrins Only Enhance Drug Permeation from Aqueous Vehicles

In general, cyclodextrins are unable to enhance drug delivery from nonaqueous vehicles through biomembranes but enhance delivery of lipophilic drugs when an aqueous phase is in contact with the lipophilic membrane surface. Hydrophilic cyclodextrins can enhance drug release from hydrophilic creams, i.e., oil-in-water emulsions, but frequently decrease drug release and permeation from lipophilic creams, i.e., water-in-oil emulsions (Preiss et al. 1994, 1995; Loftsson and Brewster 2011). Thus, cyclodextrins can be good permeation enhancers for dermal drug delivery from hydrophilic creams, hydrophilic ointments, hydrophilic gels, aqueous lotions, foams, shampoos, and solutions, but they will most likely have no effect when included in lipophilic creams, hydrophobic ointments, and lipophilic gels. For definition of these pharmaceutical vehicles, see the *European Pharmacopoeia*, 8th Edition, 2014.

There are few examples where cyclodextrins can enhance drug delivery to the skin from non-aqueous vehicles. Such effects are usually related to increased chemical (e.g., prevention of drug degradation) or physical (e.g., inhabitation of crystal growth) drug stability within the vehicles (Frömming and Szejtli 1994).

14.2.2.4 Cyclodextrins Do Not Enhance Delivery of Hydrophilic Drugs

In general, hydrophilic water-soluble drugs have little tendency to form hydrophilic cyclodextrin complexes, and, in general, cyclodextrins do not enhance transmembrane delivery of water-soluble drugs. However, cyclodextrins can form complexes with lipophilic moieties of water-soluble drugs, and, thus, in some cases cyclodextrin can reduce topical availability of water-soluble drugs. For example, cyclodextrins form complexes with water-soluble β-blockers (Gagyi et al. 2008), and HPβCD has been shown to reduce ocular bioavailability of the water-soluble β-blocker timolol maleate in aqueous eye drop formulation (Loftsson and Stefánsson 1997). The HPβCD complexation of timolol does increase the hydrophilicity of timolol (i.e., lowers $K_{M/D}$ in Fig. 14.3) and increases the hydrodynamic radius (i.e., r in Eq. 14.18) of the permeating species, both of which will result in lower membrane and transmembrane diffusion of timolol. Few studies have indicated that the somewhat lipophilic methylated cyclodextrins (like RMβCD in Table 14.1) are, under certain conditions, able to act as conventional chemical penetration enhancers, that is, by penetrating into the skin, and decrease its membrane barrier towards drug penetration (Babu and Pandit 2004; Babu et al. 2008).

14.2.2.5 Cyclodextrins Can Enhance Transmembrane Delivery of Drugs by Increasing Their Chemical Stability

Cyclodextrins are able to increase chemical stability of drugs in aqueous solutions and prevent enzymatic degradation of drugs at aqueous membrane exterior (Loftsson 1995; Loftsson and Brewster 1996, 2010). The enzymatic activity at some mucosal membranes can be quite high, and, thus, the observed permeation enhancement is sometimes due to enhanced drug stability through complexation, especially in the case of proteins and peptides (Irie and Uekama 1997; Loftsson and Brewster 2011).

14.2.2.6 In Combination with Conventional Penetration Enhancers Cyclodextrins Can Have Additive Effect

Cyclodextrins and conventional penetration enhancers, like fatty acids, or mechanical enhancers, like iontophoresis, can have additive or synergistic effect on drug delivery through biological membranes (Adachi et al. 1992, 1993; Uekama et al. 1992; Loftsson et al. 1998; Sinha et al. 2003; Karandea and Mitragotri 2009). Most often the cyclodextrins increase drug availability at the skin surface, while the other enhancers decrease the membrane barrier itself. In some cases, cyclodextrins increase delivery of a lipophilic penetration enhancer to the skin surface (Adachi et al. 1993). In other cases, cyclodextrin complexation of a penetration enhancer decreases its skin-irritating effect without decreasing its penetration-enhancing property (Martini et al. 1996).

14.3 Formulation Optimization

In general, stratum corneum is the main barrier towards drug permeation into and across the skin, and the UWL at the skin surface is very thin. Thus, drug permeation from topically applied drug formulations through intact skin most often follows Eq. 14.15. However, under certain conditions, cyclodextrins are able to enhance dermal and transdermal drug delivery. Furthermore, since cyclodextrin complexes tend to self-assemble in aqueous solutions to form nanoparticles, they are known to target drug delivery to the sweat ducts, hair follicles, and sebaceous glands (i.e., drug delivery via shunt route penetration) (Konrádsdóttir et al. 2009).

14.3.1 When Can Cyclodextrin Help?

Cyclodextrins only enhance drug delivery from aqueous vehicles and only when a UWL presents a barrier towards uptake of drug molecules into

the skin. Frequently, dermal formulations contain little or no water (e.g., hydrophobic ointments and lipophilic gels), and sometimes the water domains are not in contact with the skin surface (e.g., in lipophilic creams that consist of water-in-oil emulsions). Under such conditions, the UWL is very thin (h_D in Fig. 14.3) and, thus, does not present a barrier (i.e., the skin permeation follows Eq. 14.15). However, many aqueous dermal formulations, such as hydrophilic creams (i.e., oil-in-water emulsions) and hydrophilic gels, increase the thickness of the UWL in which case the resistance of the UWL (R_D in Eq. 14.12 and Fig. 14.3) can become comparable or greater than the resistance of stratum corneum (R_M in Eq. 14.12 and Fig. 14.3). Under such conditions, cyclodextrins can enhance drug permeation from the surface into the skin. Sweat can also increase the thickness of the UWL between a water-free drug donor, such as dermal patch, and the skin surface (i.e., increasing R_D).

Skin damage due to disease or injury can reduce its barrier function (i.e., R_M in Eq. 14.12) and increase drug permeation through the skin (i.e., increase P_M in Eqs. 14.13 and 14.14). Under such conditions, permeation through UWL might be the main barrier towards dermal and transdermal drug delivery (i.e., $P_M > P_D$) in which case the drug flux into and through the skin follows Eq. 14.16, creating conditions where cyclodextrins are known to act as penetration enhancers of lipophilic and poorly water-soluble drugs.

14.3.2 What Is the Desired Effect?

Most often the aim is to deliver drug molecules from the vehicle into and through the skin. In that case, it is important to include in the vehicle sufficient amount of cyclodextrin to enhance drug delivery to the skin surface and into the skin but to avoid excess amounts. For shunt delivery, the total cyclodextrin concentration, or rather the total concentration of drug-cyclodextrin complexes, has to be sufficient for formation of nanoparticles (Messner et al. 2011; Kurkov and Loftsson 2013). Still in other cases the target is the skin surface itself, and then cyclodextrins can be used to prevent drug partition into the skin adding excess amounts of cyclodextrins to the aqueous vehicle, more than what is needed to solubilize the drug (i.e., excess cyclodextrin lowers the value of $K_{M/D}$ in Eq. 14.17; Fig. 14.3).

14.3.3 How to Optimize the Formulation?

It is important to optimize cyclodextrin-containing vehicles with regard to the vehicle composition and the desired effect. Too little or too much cyclodextrin will result in less than optimum effect (Loftsson and Brewster 2011). Here we describe step by step the formulation of hydrophilic hydrocortisone gel. The hydrophilic gel (hydrogel) vehicle consists of water within a starch matrix (0.5–2 %) containing HPβCD as a solubilizer/penetration enhancer. Similar methods are used to optimize other aqueous skin preparations.

14.3.3.1 Phase-Solubility Study

One hydrocortisone molecule (MW 362.5 Da) forms an inclusion complex with one HPβCD molecule (MW 1400 Da). In aqueous solutions, the inclusion complexes are constantly being formed and dissociated at rates close to the diffusion-controlled limit, and, thus, the complexes are in dynamic equilibrium with free hydrocortisone and HPβCD molecules (Fig. 14.1) (Stella et al. 1999). The first step is to determine how much HPβCD is needed to dissolve given amount of hydrocortisone. This is done by determining the phase solubility of the drug in aqueous solution (Higuchi and Connors 1965; Loftsson et al. 2007a; Loftsson and Brewster 2010; Loftsson and Hreinsdóttir 2006). The aqueous solubility of hydrocortisone is determined as the function of HPβCD concentration. From the linear phase-solubility (i.e., A_L-type) diagram in Fig. 14.4, we see that we will need about 7 % (w/v) HPβCD to dissolve 10 mg/ml (i.e., 1 % w/v) of hydrocortisone, about 11 % to dissolve 15 mg/ml, and about 14 % to dissolve 20 mg/ml. To calculate the stability constant ($K_{1:1}$) and the complexation efficiency (CE), we need to determine the phase-solubility diagram using molar concentrations (Fig. 14.5). From the slope (0.5432), we can determine the CE according to Eq. 14.6 (CE = 1.19) and the hydrocortisone/HPβCD molar ratio in the aqueous HPβCD

solution saturated with hydrocortisone from Eq. 14.7 (about 1:2). Thus, in aqueous HPβCD solution at room temperature, at least two HPβCD molecules are needed to dissolve one molecule of hydrocortisone. Then according to Eq. 14.5, we can calculate $K_{1:1}$ from the slope and the hydrocortisone solubility in the aqueous complexation media when no HPβCD is present ($1.15 \cdot 10^{-3}$ M), the observed $K_{1:1} = 1{,}030\ M^{-1}$.

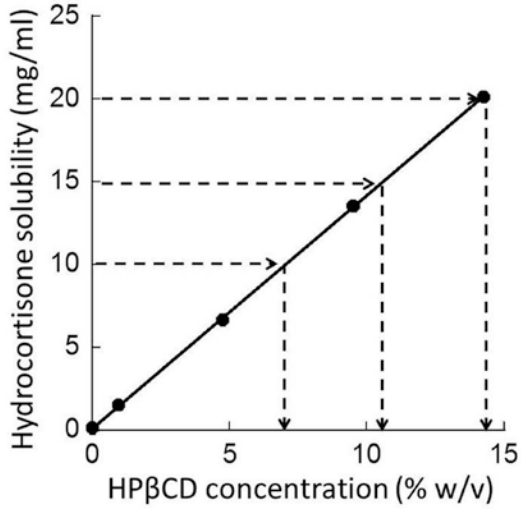


Fig. 14.4 The phase-solubility diagram of hydrocortisone in pure water-containing HPβCD at room temperature (22–23 °C)

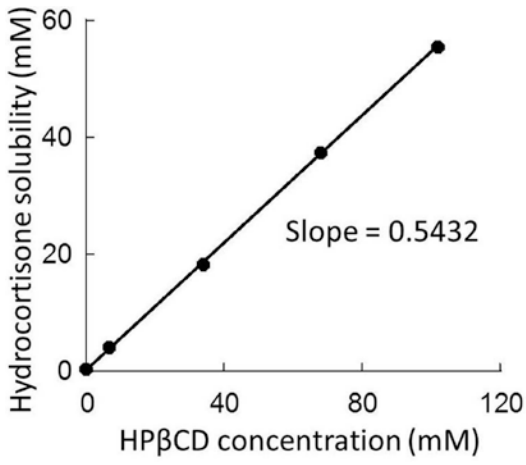


Fig. 14.5 The phase-solubility diagram of hydrocortisone in pure water-containing HPβCD at room temperature (22–23 °C)

14.3.3.2 The Amount of Cyclodextrin and Drug Availability

The starch (e.g., hydroxypropyl cellulose) used to form the matrix might decrease or increase the amount of HPβCD needed to solubilize hydrocortisone (Loftsson and Brewster 2012). However, it can be difficult to determine hydrocortisone solubility in a viscous gel. Alternatively, one can determine the effect of HPβCD concentration on hydrocortisone release. The phase-solubility study shows that about 11 % (w/v) HPβCD will be needed to dissolve 15 mg/ml (1.5 % w/v) hydrocortisone in the hydrophilic gel. To determine the exact amount of HPβCD needed, a series of gels are prepared, all of which contain the same amount of starch and hydrocortisone (1.5 % w/v) but different amounts (5–15 % w/v) of HPβCD, and the hydrocortisone permeation from the gels through an artificial membrane was determined (Fig. 14.6). The membrane consisted of semipermeable cellophane membrane with an octanol/nitrocellulose

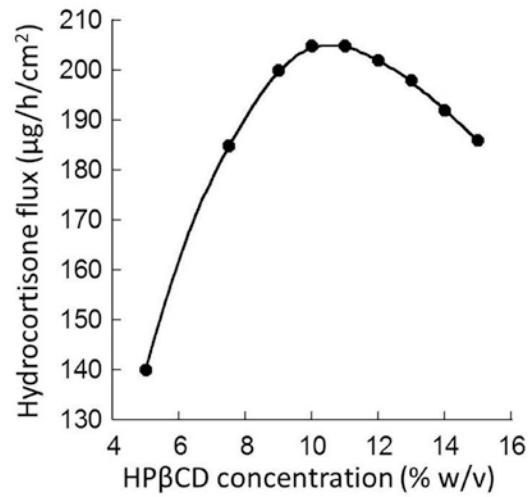


Fig. 14.6 Effect of HPβCD concentration on the hydrocortisone flux from a hydrophilic gel vehicle through an artificial biomembrane at room temperature (22–23 °C). The membrane consisted of semipermeable cellophane membrane (MWCO 12,000–14,000) with an octanol/nitrocellulose membrane fused to the receptor side. The gel contained fixed amount of hydrocortisone, 1.5 % (w/v). Both free hydrocortisone and the hydrocortisone/HPβCD complex were able to permeate the cellulose membrane, but only the drug was able to permeate the octanol/nitrocellulose membrane

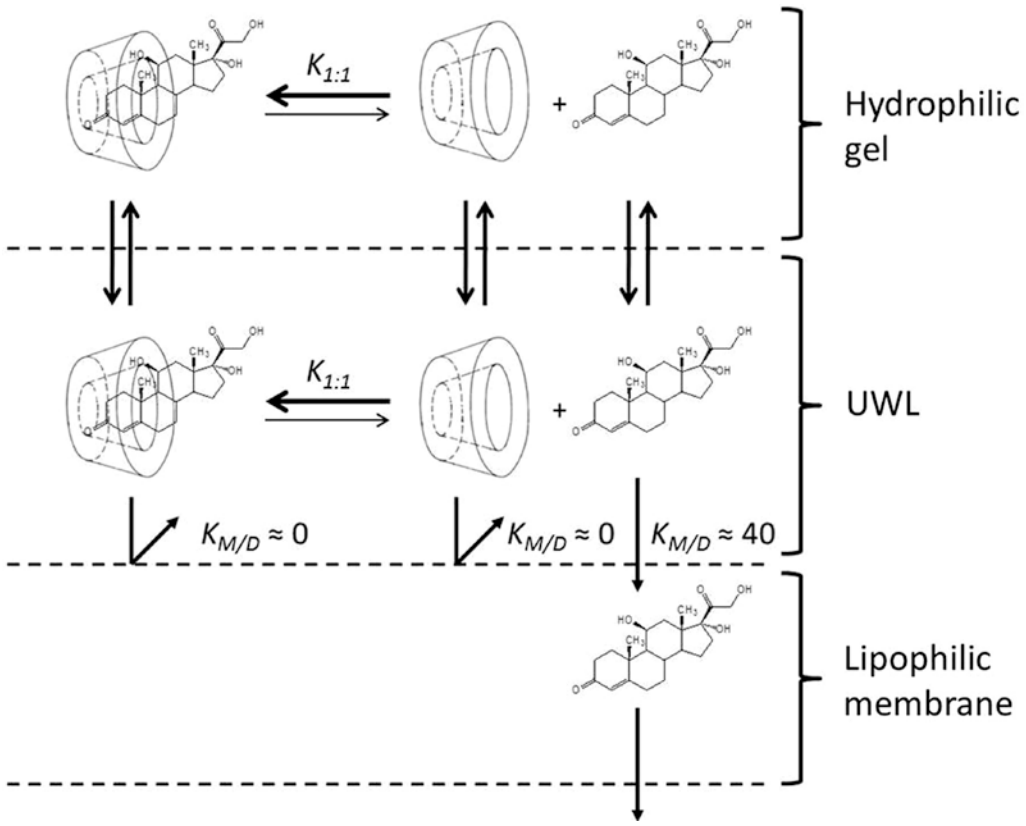


Fig. 14.7 Permeation of hydrocortisone from a hydrophilic gel containing hydrocortisone/HPβCD complex through a cellophane-octanol membrane. HPβCD is very hydrophilic ($K_{M/D} = K_{O/W} \approx 10^{-11}$; see Table 14.1) and, thus, is unable to permeate into the octanol membrane, while hydrocortisone is much more lipophilic ($K_{M/D} = K_{O/W} \approx 40$) (Másson et al. 2005) and is able to permeate the octanol membrane. The observed $K_{1:1} = 1{,}030\ M^{-1}$

membrane fused to the receptor side. Only the free drug is able to permeate the octanol layer (Fig. 14.7). Permeation of hydrocortisone molecules from the gel is at its maximum when just enough HPβCD is present to dissolve all hydrocortisone. At lower HPβCD concentration, the permeation is lower, and the gel is turbid due to undissolved hydrocortisone. At higher HPβCD concentrations, the gel is clear, but excess amounts of HPβCD will decrease the concentration of free hydrocortisone at the surface of the lipophilic membrane (Fig. 14.7) resulting in decreased hydrocortisone flux through the membrane. However, to avoid drug precipitation during storage, the gel should contain a small excess of HPβCD. Maximum flux (Fig. 14.6) is obtained at 10 % (w/v) HPβCD. Increasing the concentration to 12 % (w/v) (20 % excess HPβCD) only reduces the flux from 205 to 203 mg h^{-1} cm^{-2}. The final composition of the hydrophilic gel will then be 1.5 % (w/v) hydrocortisone and 12 % (w/v) HPβCD in a hydrophilic gel.

14.3.3.3 What Happens on the Skin?

The gel contains about 85 % water. The water content of the gel will decrease relatively rapidly after its application to the skin surface, due to both evaporation and water absorption into the skin. However, since hydrocortisone displays A_L-type phase-solubility diagram in aqueous HPβCD solutions, decreased amount of water will not result in hydrocortisone precipitation. The gel will become stiffer, and the increased viscosity (η) might decrease hydrocortisone permeation from the gel to the skin surface (see Eq. 14.18), but increased hydrocortisone concentration could,

on the other hand, result in increased hydrocortisone permeation. Hence, decreased water content might have less effect than expected.

Conclusions

Cyclodextrins can under certain conditions act as percutaneous penetration enhancers. In general, cyclodextrins can only enhance drug delivery through the skin from aqueous vehicles and only when an aqueous diffusion barrier at the skin exterior contributes to the overall skin permeation barrier. Cyclodextrins do not enhance drug penetration from lipophilic vehicles or when the skin barrier, i.e., stratum corneum, is the main permeation barrier. Cyclodextrins are able to prevent drug partition from an aqueous exterior into the skin. It is of uttermost importance to optimize composition of cyclodextrin-containing drug vehicles with regard to drug release and permeation.

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Part IV

Influence of Vehicle Effects in Penetration Enhancement

15 Dermal and Transdermal Formulations: How They Can Affect the Active Compound

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Contents

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15.1 Introduction

The development and distribution of dermal and transdermal therapeutics increased in the last decades. Their high popularity is based on the easy way of application, painless treatment, and reduced side effects. Topically applied formulations shall exhibit a high compatibility with low side effects, high patient acceptance, and adequate shelf life. Furthermore, they have to stay on the skin for a sufficient time in order to provide enough time for the active compound to diffuse through the formulation to the skin (Wiechers et al. 2004). Afterward, the active compound partitions into the skin (drug liberation) (Fig. 15.1). In dependence on the medical indication, the active compound has to be delivered to the site of action inside the epidermis (drug penetration) or dermis (drug permeation) and has to remain there for a sufficient time, in order to ensure a local treatment (Wiechers et al. 2004). On the other hand, a systemic effect can be aimed, when the active compound has to be delivered through the skin into systemic circulation (drug resorption) in enough high concentrations to provide sufficient plasma levels (Daniels and Knie 2007).

The ability of a drug to be liberated from the vehicle can be characterized by the ratio of the solubility of the active compound in the *stratum corneum* (C_{SC}) to the solubility of the active compound in the vehicle (C_V). This dependency can be described by the partition coefficient $K_{SC/V}$ according to Eq. 15.1 (Wiechers et al. 2004).

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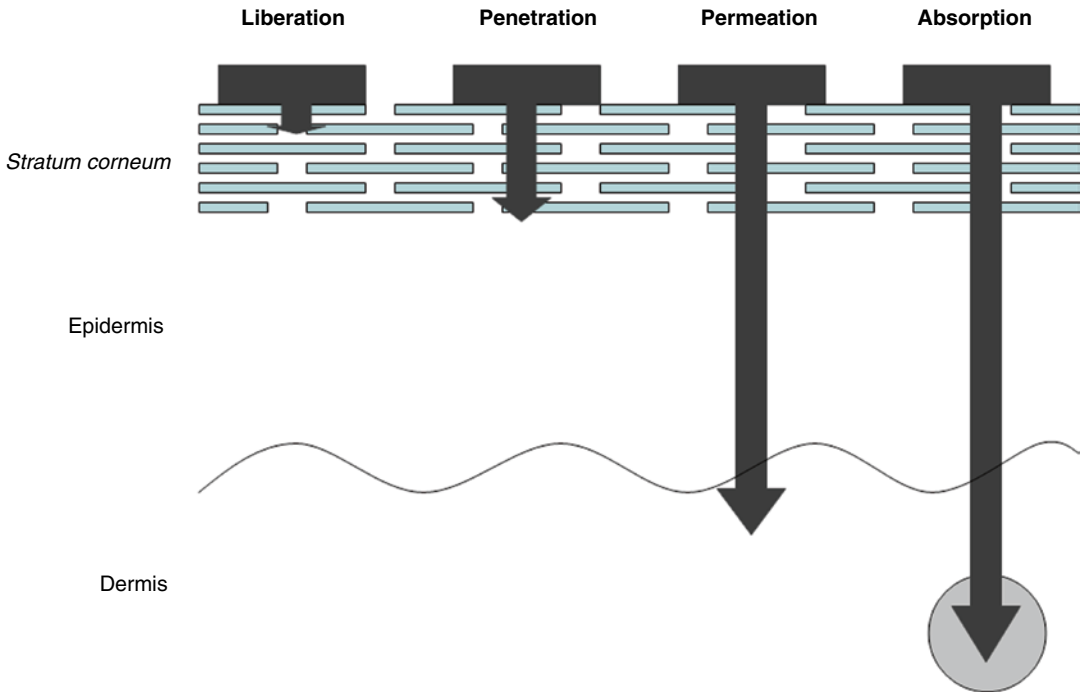

Fig. 15.1 Pathways of a compound from the topically applied product to the intended localization (Modified after Daniels and Knie (2007))

$$K_{SC/V} = C_{SC} / C_V \qquad (15.1)$$

Thus, the drug amount which is liberated from the formulation into the *stratum corneum* can be enhanced either by increasing the solubility of the compound in the *stratum corneum* or by decreasing the solubility of the compound in the formulation (Wiechers et al. 2004).

Topically applied compounds have to overcome the main skin barrier, the *stratum corneum*, which represents a complex mixture of various lipid and protein domains (Cross et al. 2001) in order to reach the underlying regions (Hadgraft and Pugh 1998). Thus, two processes, partition and diffusion, are important determinants for drug delivery into and through the skin (Wiechers et al. 2004), which in turn can be found in the flux of a compound according to Fick's law of diffusion (Eq. 15.2) (Scheuplein 1976; Potts and Guy 1992):

$$J = \left(D \cdot K_{ow} \cdot \Delta c\right) / x \left[\text{mol} \cdot \text{s}^{-1} \cdot \text{cm}^{-2}\right] \qquad (15.2)$$

where J is solute flux, D is diffusion coefficient, K_{ow} is octanol/water partition coefficient, Δc is drug concentration difference between the formulation and the deepest skin layer, and x is the length of the diffusion pathway of the active compound.

Furthermore, the permeability coefficient K_p is often used in order to simplify the permeation rate of an active compound through the skin (Eq. 15.3) (Hadgraft and Pugh 1998), since the actual diffusion pathway through the skin (x) is unknown. K_p is furthermore the parameter used for statistical analysis of results of skin diffusion studies.

$$K_p = \left(D \cdot K_{ow}\right) / x \left[\text{cm} \cdot \text{s}^{-1}\right] \qquad (15.3)$$

The number of transdermal applied compounds is still limited due to the lack of achieving therapeutic concentrations in the target tissue within the skin or in other body tissues after systemic circulation via blood or the lymphatic system (Bach and Lippold 1998), usually due to limitations in transdermal

permeation caused by physicochemical drug characteristics (Magnusson et al. 2004). Especially the lipophilicity, the molecular weight (MW), and the melting point have to be taken into account in order to deliver a compound through the skin (Hadgraft and Pugh 1998; Magnusson et al. 2004; Nielsen et al. 2004). Potts and Guy (1992) demonstrated that the permeation of a topically applied drug in an aqueous solution can be estimated by two physicochemical characteristics, which are combined in Eq. 15.4 with MW as molecular weight of the active compound:

$$\log k_p = -6.3 + 0.71 \log K_{ow} - 0.0061\,\mathrm{MW}\ \left[\mathrm{cm \cdot s^{-1}}\right] \qquad (15.4)$$

From Eq. 15.4 it can be concluded that small molecules (low MW) with adequate affinity to the horny layer (log K_{ow} from −3 to +6) can be expected to exhibit a sufficient drug penetration (Bach and Lippold 1998; Potts and Guy 1992; Wiechers et al. 2004). Compounds with good affinity to both water and oil will show a good penetration behavior. Such compounds tend to exhibit a low melting point (Hadgraft 2004). According to Potts and Guy (1992), Eq. 15.4 can be used for substances with MW from 18 to >750 and log K_{ow} from −3 to +6.

Furthermore, the rate of diffusion depends on the thermodynamic activity of the drug in the absence of penetration enhancing substances (Bach and Lippold 1998). It is characterized by the ratio of the drug concentration in the vehicle (C_V) to the maximum solubility in the vehicle (C_S) according to Eq. 15.5, with α as thermodynamic activity, C_V as concentration of the active compound in the vehicle, and C_S as saturation concentration of the active compound in the vehicle (Bach and Lippold 1998):

$$\alpha = \left(C_V / C_S\right) \qquad (15.5)$$

In this process the thermodynamic activity rises with the concentration of the compound up to the maximum concentration (saturation) in the vehicle (Hadgraft 2004). Thus, solutions show a decline of activity with the duration of incubation, while suspensions (having drug concentrations above their solubility) exhibit a constant activity (Bach and Lippold 1998).

Commercially available topical formulations present a complex mixture of several classes of substances (Wiechers et al. 2004). The effect of all added substances in the formulation and the thermodynamic activity of the active compound have to be considered when transdermal and dermal drug delivery is predicted (Bach and Lippold 1998).

According to that, not only the properties of the active compound but also the vehicle influence the diffusion of the active compound through the skin. Vehicle ingredients can interact with the skin or with the active compound, so that its diffusion is enhanced or decreased.

15.2 Vehicle Composition

Although there is diverse nature of ingredients contained in vehicles used to deliver drugs into/through the skin (Wiechers et al. 2004), the following classification of vehicle components has been published by Daniels and Knie (2007) with five major classes.

15.2.1 Bases

Bases for topical products, representing the main ingredient of the product, can be either of hydrophilic nature like water or ethanol or they show hydrophobic characteristics (e.g., petrolatum, triglycerides) (Daniels and Knie 2007). The choice of the base depends on various factors, all of which have to be considered in the development of the final product. The base should exhibit no irritating or sensitizing potential to the skin, the active compound has to be delivered in adequate amounts to the site of action, and the shelf life of the product has to be long enough (International Pharmacopoeia 2014). Furthermore, a desired consistency has

to be created (e.g., hydrophobic lipid bases can exhibit a special spreadable behavior over the skin without a greasy feeling) (Daniels and Knie 2007).

15.2.2 Gelling Agents

In order to enhance the viscosity of the product, which in turn improves storage and sensory attributes, macromolecules are added to form three-dimensional scaffolds. Popular thickeners are carbomer, hydroxypropyl cellulose, and carmellose sodium (Daniels and Knie 2007).

15.2.3 Preservatives (Antimicrobial Agents)

Preservatives against bacterial growth are necessary in order to enhance the shelf life of a product and for consumer protection against a contaminated product. Commonly used substances with properties of preservatives are ethanol or isopropanol, which have to be used in sufficient concentrations that means in concentrations about 20 %, which in turn are high enough to influence the penetration profile of a drug, as well. Furthermore, phenoxyethanol, benzoic acid, and sorbic acid are used in lower concentrations (0.1–0.2 % sorbic acid, 0.15–0.5 % benzoic acid, 0.5–1 % phenoxyethanol) (Daniels and Knie 2007). According to the International Pharmacopoeia (2014), antimicrobial agents are indicated unless the formulation itself has adequate preservative properties.

15.2.4 Antioxidants

These compounds are used in order to prevent oxidation reactions of lipids in exposition of light, air, and heat. Typical antioxidants are alpha-tocopherol in concentrations of 0.05–0.075 % and ascorbic acid esters in concentrations of 0.01–0.015 % (Daniels and Knie 2007).

Table 15.1 Classification of emulsifiers used in transdermal therapeutic systems according to Daniels and Knie (2007)

Compound classification	Examples
Anionic	Sodium stearate Sodium dodecyl sulfate
Cationic	Benzalkonium bromide Cetylpyridinium chloride
Amphoteric	Phosphatidylcholine Betaine monohydrate
Nonionic	Glycerol monostearate Polysorbate 20 (Tween® 20)

15.2.5 Emulsifier

Emulsifiers added to the vehicle enhance the thermodynamic stability between hydrophilic and hydrophobic compounds. They are classified as anionic, cationic, amphoteric, and nonionic surfactants and accumulate between a lipid-rich phase and a hydrophilic phase (Daniels and Knie 2007). Table 15.1 lists some emulsifiers according to Daniels and Knie (2007).

15.3 Formulation Types

According to the US Food and Drug Administration (FDA) (2013), topical dosage forms (Table 15.2) can be divided into semi-solid and liquid formulations, which are characterized by their physicochemical nature. Especially the localization of the topical treatment and the severity of the disorder determine the kind of formulation, which is characterized by the content of oil and water (Daniels and Knie 2007). Ointments, e.g., are traditionally preferred by clinicians for topical use on hairless skin or areas with short hair, while creams are preferentially applied onto genial or flexural areas (Huang et al. 2005). Moreover, skin conditions have to be considered not only in healthy skin, but also in diseased skin. Acute or subacute diseases are often treated with formulations based on liquid bases (shake lotions or emulsions), while subchronic to chronic

Table 15.2 Dosage forms according to the FDA (2013) with modifications

Semisolid and solid dosage forms	Liquid dosage forms	Specialties
Creams and ointments	Emulsion	Microemulsion
Gel	Lotion	Nanoemulsion
Paste	Shampoo	Patch
Powder	Solution	
	Spray	
	Suspension	

diseases are medicated with pastes, rich ointments, or gels. In brief, a more acute disease requires a high water and a low oil content (Daniels and Knie 2007). Thus, creams are preferentially used for wet conditions and ointments for dry conditions.

15.3.1 Semisolid Formulations

A semisolid preparation exhibits no pourable behavior and does not conform to its storage container at room temperature. It does not flow, but exhibits plastic flow behavior. Low shear stress does not induce a flow of semisolid preparations. Semisolid dosage forms for topical treatment can be classified into creams and ointments, gels, pastes, and powders (International Pharmacopoeia 2014; FDA 2013).

15.3.1.1 Cream and Ointment

Creams and ointments are generally used for topical application and are semisolid preparations, in which solid or liquid substances are dispersed (Pharmacopoeia Europaea 2011). The vehicle contains >20 % water and volatiles and/or <50 % hydrocarbons, polyols, or waxes (FDA 2013). The consistency and rheological characteristics of creams depend on the emulsion type (oil in water or water in oil) and on the physicochemical properties of the solids used (International Pharmacopoeia 2014). Ointments are preparations which are "immiscible, miscible, or emulsifiable with skin secretions." Therefore, bases of hydrophilic, hydrophobic, or water-emulsifying nature are used (International Pharmacopoeia 2014).

15.3.1.2 Gel

A gel is a clear preparation comprising a liquid phase (solution or colloidal dispersion) within a three-dimensional polymeric matrix caused by the addition of a gelling agent. It may contain suspended particles (International Pharmacopoeia 2014; FDA 2013). Gels can be divided into hydrophobic and hydrophilic gels.

15.3.1.3 Paste

Pastes are generally intended for topical application. They are preparations with high concentrations of insoluble solids (20–50 %) in a fatty vehicle (Pharmacopoeia Europaea 2011; International Pharmacopoeia 2014; FDA 2013).

15.3.1.4 Powder

Powders represent a mixture of drugs and/or chemicals, which are of dry and finely divided nature (FDA 2013).

15.3.2 Liquid Formulations

A liquid preparation is pourable and conforms to its storage container at room temperature. It flows and shows pseudoplastic flow and Newtonian behavior. The FDA (2013) distinguishes between emulsions, lotions, shampoos, solutions, sprays, and suspensions for external skin treatment.

15.3.2.1 Emulsion

Emulsions are characterized by at least two liquids that are immiscible, so that one component is dispersed in droplets within the other component. Thus, emulsions are special two-phase dosage forms. Stability is given by the addition of at least one emulsifier (FDA 2013).

15.3.2.2 Lotion

This dosage form is generally intended for topical application. A lotion represents an emulsion in a liquid dosage form (FDA 2013).

15.3.2.3 Shampoo

Shampoos represent liquid soaps or detergents, both of which are used to clean the hair and scalp or to apply special drugs to the skin (Pharmacopoeia Europaea 2011; FDA 2013).

15.3.2.4 Solution

This dosage form is clear and homogenous. It may contain more than one chemical dissolved in one solvent or a mixture of miscible solvents (FDA 2013).

15.3.2.5 Spray

A spray is characterized by a liquid, which is divided into small components by a jet of air or steam (FDA 2013).

15.3.2.6 Suspension

This dosage form contains a liquid vehicle as the basis, in which solid particles are dispersed in (FDA 2013).

15.3.3 Special Formulation Types

Beside the conventional dosage forms, several new formulation types have been developed. Such a new preparation is a patch, which represents a sophisticated drug delivery system with the intent to release the active compound in such a manner that the dosing frequency can be reduced in comparison to conventional dosage forms (FDA 2013). Patches are commercially available for a constant delivery of various substances like ethinyl estradiol (Sachdeva et al. 2013), fentanyl (Lehmann and Zech 1992), lignocaine (Kwon et al. 2012), nicotine (Benowitz et al. 1991), nitroglycerol (Minghetti et al. 2001), rivastigmine (Gauthier et al. 2013), rotigotine (Stiasny-Kolster et al. 2013), scopolamine (Gil et al. 2012), testosterone (James 1995), and others. The main advantages of patches are sustained and constant plasma levels and the bypass of the first-pass effect in the liver (Kapil et al. 2012). Due to drug storage within the patch, transdermal patches can be classified into three types: (1) drug directly dispersed in adhesive polymer, (2) drug reservoir between a rate controlling membrane and a backing membrane, and (3) drug reservoir within the center of a peripheral adhesive ring around the edges (Sachdeva et al. 2013).

Furthermore, special liquid preparations have been generated in the last decades, such as nanoemulsions (Uner et al. 2005; Alves et al. 2007; Baboota et al. 2007), or microemulsions (Kemken et al. 1991; Boltri et al. 1994; Pattarino et al. 1994), etc.

15.4 The Influence of Formulations on Drug Diffusion into and through the Skin

The impact of different dosage forms on transdermal drug delivery has been examined in several investigations. An extensively studied topically applied compound is ibuprofen. Hadgraft et al. (2003) demonstrated that various 5 % ibuprofen-containing products resulted in marked differences in the permeation rates of ibuprofen through excised human skin with the most efficient permeation observed for a spray, a gel, and a mousse, while two other gels, based on other ingredients like ethanol or benzyl alcohol, and a cream led to less efficient permeation rates. Comparable results were found by Herkenne et al. (2007) with different permeation rates observed in vitro and in vivo after topical administration of four 5–10 % ibuprofen-containing gels with different ingredients. In this study, the highest permeation through excised pig ear skin was for a 5 % ibuprofen gel containing propylene glycol and isopropanol, followed by the 5 % gel containing propylene glycol. Lower permeation rates were observed for the 10 % and the 5 % gel, both of which were enhancer-free. Interestingly, in vivo studies in man revealed similar results, with the highest permeation rate for the 5 % gel with both enhancers and the lowest permeation rate for ibuprofen from the 5 % gel without enhancers, while the addition of propylene glycol did not enhance permeation in vivo against the 10 % gel without enhancer as observed with pig ear skin (Herkenne et al. 2007).

Moreover, the fate of ibuprofen after topical administration depends on the formulation used. Comparative studies between different ibuprofen-containing formulations demonstrated the impact of the formulation on the amount of ibuprofen within the skin (skin absorption) (Stahl et al. 2011, 2012). The comparison of ibuprofen permeation through excised skin from a gel, a cream, and a solution, all of which were commercially available and contained 5 % ibuprofen, revealed different amounts of skin absorption depending on the formulation. The highest absorption (drug storage in the skin) was observed for the aqueous, enhancer-free solution and the gel containing the penetration enhancer 2-propanol, followed by the cream, which contained the permeation enhancer propylene glycol. Interestingly, skin diffusion showed a different ranking: cream > solution > gel (Stahl et al. 2011). Other commercially available 5 % ibuprofen-containing formulations (cream, gel, "microgel") were examined in a second study in vitro with excised bovine udder skin and ex vivo with the isolated perfused bovine udder (Stahl et al. 2012). Penetration enhancers in the formulations were propylene glycol in the cream and 2-propanol in the gel and the "microgel". In this study, skin absorption profiles with a general rank order, cream < "microgel" < gel, were obtained, as well as permeation rates of a differing general rank order, "microgel" < gel < cream.

Various other substances, like ketoprofen, betamethasone, or thiocolchicoside, show formulation-dependent skin diffusion rates, as well (Bonina et al. 2002; Guerol et al. 1996; Gallagher and Heard 2005; Gallagher et al. 2003; Huang et al. 2005; Kietzmann and Blume 1997; Mitriaikina and Mueller-Goymann 2009; Singh et al. 2009).

The comparison of the influence of four different ointment bases on ketoprofen release from the formulation in vitro revealed the following general rank order: white petrolatum < cold cream < hydrophilic ointment < carbomer gel, which correlated with the hydrophilicity of the formulations (Guerol et al. 1996). Moreover, comparison studies with gel formulations comprising different thickening agents (silica as Cab-O-Sil®, Cabot Carbon Ltd., UK, and hydroxypropyl cellulose (HPC)) on ketoprofen diffusion through synthetic membranes and excised pig skin were performed (Gallagher et al. 2003; Gallagher and Heard 2005). The content of the thickening agents was chosen in order to gain comparable viscosities of the formulations. Permeation studies with pig ear skin revealed that an increase in the content of the thickener was correlated with reduced diffusion rates of ketoprofen, while the diffusion rate from the HPC gel was almost doubled compared to that of silica gel.

The skin diffusion of the glucocorticoid betamethasone was studied ex vivo in the isolated perfused bovine udder, after topical administration of five formulations (cream, gel, ointment with and without propylene glycol, and solution). The ointments showed the highest potentials to promote betamethasone diffusion, followed by the cream, gel, and solution. Interestingly, there was no significant difference between the creams, although one formulation contained propylene glycol, which is often used as a penetration enhancer (Kietzmann and Blume 1997).

The effect of different formulations on the in vitro skin diffusion behavior of the muscle relaxant thiocolchicoside was studied by Bonina et al. (2002) in excised human skin. They examined the effect of a novel, noncommercially available foam based on the enhancers propylene glycol dipelargonate and propylene glycol in comparison to an ointment and a gel, both enhancer-free, and found a two- to threefold higher flux for the foam in comparison to the ointment and the gel.

The main problems of such comparative studies are that information about the ingredients provided to the consumers is only based on the kind of ingredients, but not on their content (except for the active compound), and that commercially available topical formulations are based on a high diversity of additives. Consequently, the main influencing additive of the vehicle can rarely be found out in such studies without systematic examinations about the effect of changes of single additives.

Table 15.3 Possibilities to enhance transdermal drug delivery considering Eqs. 15.1, 15.2, 15.3, 15.4 and 15.5

Parameter	Change	Possibilities to change the parameter
K_{ow}	↑	Enhance lipophilicity of the compound
D	↑	Decrease viscosity of the vehicle
		Use of penetration enhancers
Δc	↑	Enhance solubility of the compound in the vehicle
x	↓	Use of penetration enhancers
MW	↓	Change chemical structure of the compound
$K_{SC/V}$	↑/↓	Change the polarity or pH of the vehicle
α	↑	Enhance concentration of the compound in the vehicle (up to saturation)

Modified after Wiechers et al. (2004)

The dependency of the drug permeation on the drug formulation arises the question of how vehicles interact with the active compounds in order to exhibit an optimized permeation profile. Since formulations are a mixture of various compounds, many ingredients can influence transdermal drug delivery and have to be taken into account (Wiechers et al. 2004). Considering the Eqs. 15.1 15.2, 15.3, 15.4 and 15.5, several parameters of a topical formulation can be changed in order to influence transdermal drug delivery. The results are listed in Table 15.3 according to Wiechers et al. (2004).

However, the major problem is that the parameters listed in Table 15.3 are linked (Hadgraft and Pugh 1998): Since the alteration of a single parameter may affect several other parameters, it is necessary to have a closer look on the effects of variations of single parameters on transdermal drug diffusion.

An alteration of the octanol-water partition coefficient K_{ow}, e.g., can be performed by changing the chemical structure of the compound. Since that is not acceptable for various substances, alternatively a formulation with optimized polarity has to be chosen in order to enhance transdermal drug diffusion, in accordance with Wiechers et al. (2004). They described the optimized formulation as "the polarity of the formulation needs to be as far as possible from the polarity of the active ingredient in order to increase the driving force of the active ingredient into the skin, but at the same time as close as possible to that of the active ingredient to ensure that high concentrations can be reached to ensure that enough material penetrates the skin to reach effective tissue concentrations at the site of action in the skin." The effectiveness of their guideline "Formulating for Efficacy" has been demonstrated for the skin delivery of octadecene dioic acid (Wiechers et al. 2004) and should be noted in order to choose an appropriate formulation for topical skin delivery of drugs. The ionization state of a drug has to be considered, since unionized molecules exhibit a higher permeation rate than the ionized fraction (Hadgraft and Valenta 2000) due to the fact that unionized compounds exhibit a higher lipophilicity proportional to K_{ow} (Watkinson et al. 2009). Consequently, the pH of the formulation has to be optimized in correlation to the pKa value of the compound with respect to the physiological skin pH (4–7.4) in order to prevent skin damages (Hadgraft and Valenta 2000). Since the pKa value of a compound depends, furthermore, on the supplementation of cosolvents like ethanol (Watkinson et al. 2009), an addition of cosolvents can be used to optimize the solubility of the compound, which in turn affects both the thermodynamic activity α according to Eq. 15.5 and the concentration according to Eq. 15.2, as well.

The diffusion coefficient D can be altered by supplementation of suitable permeation enhancers (such as propylene glycol, ethanol, etc.) or by variations of the viscosity of the vehicle (Wiechers et al. 2004). Permeation enhancers can interact with the *stratum corneum* or with the active compound and are of such diversity that they are just briefly mentioned in this chapter. Viscosity changes are another elegant method to influence D, which is inversely correlated to the viscosity according to the Stokes-Einstein equation (Eq. 15.6). Thus, a high viscosity results in a diminished diffusion coefficient of the active compound:

$$D = k \cdot T / 6\pi \cdot \eta \cdot R \left[\mathrm{m} \cdot \mathrm{s}^{-1} \right] \qquad (15.6)$$

where k is the Boltzmann constant, T represents the absolute temperature, η is the viscosity, and R is the hydrodynamic radius of the diffusing particle (Raghavan et al. 2001). The difference in viscosity of a formulation has been subject of several studies. The supplementation of 3 and 10 % hydroxymethyl cellulose to aqueous solutions of flufenamic acid resulted in a viscosity-dependent permeation of flufenamic acid with higher viscosities correlating with lowered permeation rates (ENNEN 2009). Diminished permeation rates depending on the degree of viscosity have also been described for other substances.

The diffusion coefficient of theophylline, e.g., declined exponentially with the HPC concentration (0–2 %) of five HPC varieties (molecular weight between 5×10^5 and 1.2×10^6). In case of the lowest-molecular-weight HPC, a constant diffusion coefficient was observed up to 0.8 %, which was explained by a high entanglement concentration of the HPC (Alvarez-Lorenzo et al. 1999). The influence of different contents of polymers on ketoprofen release was studied in vitro, as well as with the result that an enhancement of the polymer concentration was correlated with a higher release of ketoprofen from the gels (Chi and Jun 1991).

Moreover, the release and permeation rate of several model anesthetics decreased with increasing macroviscosity of the formulation through guinea pig skin and a silicone membrane (Welin-Berger et al. 2001).

However, viscosity-independent permeation behavior was reported for some active compounds, like caffeine or naproxen (Suh and Jun 1996; Ennen 2009). Therefore, the viscosity had to be characterized in a better way: the apparent viscosity is represented by the macroviscosity (macroscopic flow properties of a system), which does necessarily not reflect the matrix through which the drug diffuses (Alvarez-Lorenzo et al. 1999; Raghavan et al. 2001). On the other hand, microviscosity (Alvarez-Lorenzo et al. 1999; Raghavan et al. 2001) is characterized by molecular drug movements of the active compound "at the microscopic scale" (Alvarez-Lorenzo et al. 1999). Thus, formulations with the same macroviscosity may provide different permeation rates for the active compound. This could be demonstrated for ketoprofen permeation after application of two formulations sharing the same macroviscosity due to the addition of 1 % Cab-O-Sil® or 0.5 % HPC (Gallagher and Heard 2005). After 24 h, the amount of ketoprofen, which permeated from the 1 % Cab-O-Sil® gel through pig ear skin, was nearly twofold higher than that from the 0.5 % HPC gel that leads to the conclusion that complexation (binding interactions between compound and thickening agent) of some compounds has a greater influence on transdermal permeation rate than changes in the viscosity (Gallagher and Heard 2005). Thus, macroviscosity can influence the solubility of the active compound in such a way that absorption of the active compound to the thickening agent enhances solubility of the active compound, which in turn changes the thermodynamic activity and the saturation of the compound. In the end, the diffusion rate of the compound is modified.

The concentration gradient Δc can easily be increased by enhancing the total amount of the drug in the formulation. Furthermore, the solubility of the compound in the vehicle can be elevated by adding cosolvents (Watkinson et al. 2009), by using microemulsions (Sabale and Vora 2012), by optimizing the polarity of the vehicle, or by drying effects on the skin surface. Immediately after application of a topical formulation on the skin, drying effects may occur, which have a significant impact on the efficacy of the applied compound (Bowen and Heard 2006). This could be demonstrated for simple gel formulations containing ketoprofen with different concentrations of propylene glycol as solvent and HPC as gelling agent (Bowen and Heard 2006). In this study, a lower content of the solvent propylene glycol correlated with a higher permeation rate of ketoprofen, which was connected with the increase of the content of ketoprofen and HPC in the formulation, in order to simulate the concentration effects of ingredients during the drying process of a solvent. A rapid crystallization process of formulations may also diminish the percutaneous

permeation of a substance, since crystallized drug particles may be unable to pass the *stratum corneum* due to steric hindrance (Kietzmann and Blume 1997). Thus, formulations drying on the skin surface after application show altered permeation behaviors of the active compound. Consequently, drying effects will behave in different ways depending on the composition of the formulation, since fast evaporating solvents may result in rapid crystallization processes (Bowen and Heard 2006), while a moderate evaporation process enhances the concentration of the active compound, which in turn increases skin diffusion according to Eq. 15.2.

The diffusion path length x of a compound is quite longer than the thickness of the *stratum corneum*, since a topical applied compound primarily diffuses through the intercellular lipid-rich spaces (Hadgraft 2004). Possibilities of shortening the diffusion pathway are independent of the formulation and comprise various methodologies like abrasion of the skin via tape-stripping (Tokumura et al. 2006), laser or microdermabrasion (Lee et al. 2003), or the creation of shunt ways via microneedles (Henry et al. 1998).

Conclusion

Topical products are of great diversity concerning their ingredients. The complex connection between all diffusion parameters highlights the importance of their knowledge in order to develop an optimized formulation for transdermal drug delivery. Each additive even for sensory purposes or a long shelf life can interact with either the active compound or the skin and has to be chosen with caution to produce a suitable formulation.

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Part V

Emulsions as Vehicles for Skin Delivery

16 The Effects of Emulsifiers and Emulsion Formulation Types on Dermal and Transdermal Drug Delivery

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16.1 Introduction

Emulsions are heterogeneous systems consisting of at least two immiscible liquid phases, in which the one liquid is dispersed as globules (dispersed phase) in the other (continuous phase). The two immiscible liquids are, in general, an oil phase and an aqueous phase. However, an emulsion can also consist of other immiscible phases, e.g. polar and nonpolar oil phases (Tadros 2009). The droplet size of macroemulsions (or conventional emulsions) is in the micrometre range and usually comprises a radius between 0.15 and 100 μm (Friberg 1990). Nanoemulsions, on the other hand, are emulsions that contain very small droplets (r <100 nm) and are also known as submicron emulsions, ultrafine emulsions or mini-emulsions (McClements 2012). However, macro- and nanoemulsions cannot be distinguished solely on the basis of the droplet size, as the physicochemical or thermodynamic properties do not clearly change when the droplet size is reduced from the micrometre to the nanometre range, and therefore no definite cut-off value for

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the droplet size exists. But there are other emulsion properties that can be used to differentiate between macro- and nanoemulsions. For example, nanoemulsions can become translucent or transparent and exhibit a much higher stability against creaming or sedimentation (McClements 2012).

In contrast to microemulsions, which are in a thermodynamic equilibrium and can hence form spontaneously, macro- and nanoemulsions are thermodynamically unstable and necessitate external energy for formation. Macro- and nanoemulsions are fundamentally different from microemulsions. However, there are similarities in composition, dimensions, structures and fabrication methods between micro- and nanoemulsions, e.g. both can contain droplets in a size below 100 nm and be translucent (McClements 2012). These similarities have led to confusion about the correct use of the terms in literature, and recent articles have reviewed similarities and differences between micro- and nanoemulsions in order to clarify the terminology (McClements 2012; Anton and Vandamme 2011).

This chapter focuses on macroemulsions, here referred to as emulsions, and their effect on dermal and transdermal delivery. Nano- and microemulsions as topical drug delivery systems are discussed in different chapters of this book.

16.2 Emulsions as Topical Delivery Systems

Emulsions are widely used in the cosmetic and pharmaceutical fields/industries for the topical administration of both hydrophilic and lipophilic active ingredients, owing to their pleasant skin sensations and their good solubilising effects on these substances (Förster and Von Rybinski 1998). The consistency of topical emulsions ranges from liquid lotions to semisolid ointments and creams (Eccleston 1997a). Since topical emulsions should be designed to feature adequate physical and chemical stability, aesthetic acceptability as well as optimal delivery characteristics, they generally are not simply two-phase formulations, but rather complex formulations (Eccleston 1997b). For example, emulsions can also contain solid particles, vesicles, liquid crystals or a third liquid (Friberg and Ma 2006). Additionally, they undergo considerable structural changes after the application onto the skin, as water and other volatile substances evaporate within a short period of time. The modified formulation structure left on the skin after evaporation of the volatile compounds is important for the topical performance of emulsions (Friberg and Ma 2006). Therefore, not only an understanding of the microstructure of the initial emulsion but also of the film that is left on the surface of the skin is important for optimisation of topical emulsions.

Despite of the complexity of emulsions, Bernardo and Saraiva (2008) presented a theoretical model for transdermal drug delivery from emulsions. The model incorporated the formulation heterogeneity and was conceived for the prediction of transdermal delivery of drugs as a function of emulsion composition. It allows the investigation of the effect of excipients on the drug activity in the two emulsion phases as well as on the drug diffusivity in the continuous phase and the influence of surfactants forming interfacial layers with different resistance on drug transfer. A simulated case study, for example, indicated that the estimated interfacial resistance by the surfactant layer may not be negligible with regard to its effect on drug delivery rates. Furthermore, the model could also be used to predict the effect of the dosing condition (e.g. applied emulsion volume) on drug absorption. Different modes of emulsion application could significantly affect drug delivery time profiles. At the same time, Grégoire et al. (2009) developed a model to predict the transport of actives into and through the skin from a cosmetic or dermatological formulation, addressing in particular simple oil-in-water emulsions. The model assumed, inter alia, that only the fraction of the active ingredient in the continuous phase of the emulsion was available for partitioning into the skin and good correlation was obtained with experimental data.

16.3 Emulsifiers

Since emulsions are thermodynamically unstable, emulsifiers are required for the formation and stabilisation of emulsions. Generally, an emulsifier is defined as a substance that stabilises emulsions. However, no absolute classification exists, as

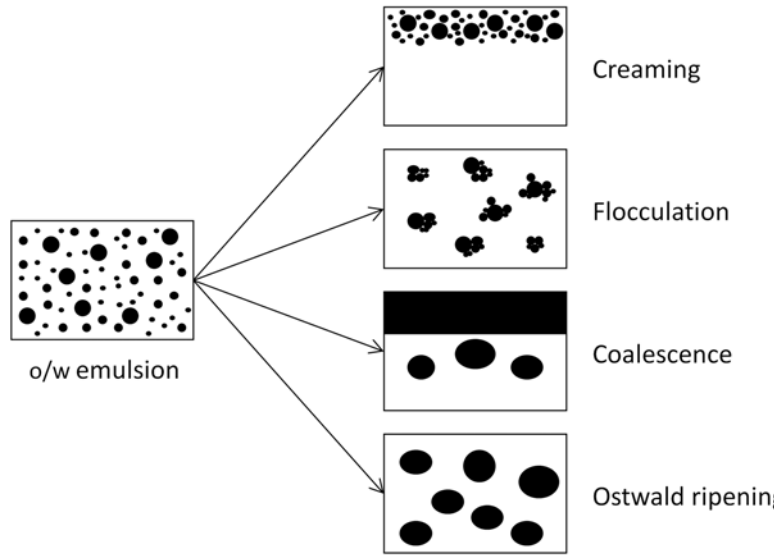

Fig. 16.1 Schematic illustration of breakdown processes of emulsions

constituents may perform different functions, e.g. a fatty alcohol can be incorporated as an emulsifier, thickener or emollient (Eccleston 1997b).

Different types of emulsifying agents exist, e.g. ionic and nonionic surfactants, polymers and solid particles. In the absence of any emulsifiers, the emulsions tend to break down, e.g. by coalescence (fusion of droplets into larger droplets by thinning and disruption of the emulsifier layer), flocculation (aggregation of droplets without disrupting the emulsifier layer), creaming/sedimentation (gravity-induced separation) and/or Ostwald ripening (Fig. 16.1) (Tadros 2009).

Emulsifiers are required for the formation of emulsions as they form a film around the newly formed drops and consequently prevent coalescence during emulsification and storage. In addition, surfactants reduce the interfacial tension and are important for the deformation and break-up of droplets during the emulsification process (Tadros 2009). Furthermore, the properties of the interface are significant for the rate and extent of coalescence (Friberg and Ma 2006). To prevent or retard flocculation and consequently also coalescence, it is important to keep a minimum distance between the droplets to overcome the van der Waals attraction. This could be achieved, for example, by electrostatic repulsion in the presence of a surface charge (ionic surfactants or charged particles) or by steric hindrance (nonionic surfactants or polymers) (Tadros 2009). Creaming or sedimentation can be prevented or retarded by increasing the viscosity of the continuous phase, e.g. by the development of a three-dimensional network of particles or polymers (Eccleston 1997a; Aveyard et al. 2003).

Cosmetic and pharmaceutical emulsions commonly comprise of blends of emulsifiers, instead of a single emulsifying agent. Most of these mixed emulsifiers consist of ionic or nonionic surfactants, in combination with fatty amphiphiles, which may be added separately during the emulsification process, or as a pre-manufactured blend (emulsifying wax). In addition to promoting the stability of emulsions, mixed emulsifiers and emulsifying waxes have the further advantages of improving emulsification by stabilising the oil droplets during formation and by controlling the rheological properties of the emulsion (Eccleston 1997b). Another example is the simultaneous inclusion of surfactants and solid particles that could yield synergistic stabilisation of the emulsion against coalescence and creaming (Binks and Whitby 2005; Lan et al. 2007).

16.4 Different Types of Emulsions

Several types of emulsions can be distinguished, for instance:

- Simple emulsions: oil-in-water (o/w) and water-in-oil (w/o)

- Multiple emulsions: oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w)

The type of emulsion that is formed mainly depends on the property of the emulsifier, e.g. the hydrophilic-lipophilic balance (HLB) value of the surfactants. The HLB is an arbitrary scale (e.g. from 1 to 20 for nonionic surfactants), and the higher the HLB, the more hydrophilic the surfactant. According to the *Bancroft* rule, the phase in which the emulsifier is more soluble constitutes the continuous phase (Bancroft 1913). For example, lipophilic surfactants with a low HLB (HLB <7) tend to act as w/o emulsifiers, whilst hydrophilic surfactants with a high HLB (HLB >7) tend to form o/w emulsions. However, Harusawa et al. (1980) suggested a change to the *Bancroft* rule by proposing that the phase in which the surfactant forms micelles constitutes the external phase, independent of the solubility of the surfactant monomers in the oil and aqueous phases. For particle-stabilised emulsions (also known as Pickering emulsions), it was demonstrated that the wettability of the solid particles, which is determined by the contact angle, defines which type of Pickering emulsion will be formed. For example, particles with a contact angle at the oil-water interface θ_{ow} <90° tend to form o/w emulsions, whereas particles with a contact angle θ_{ow} >90° prefer to stabilise w/o emulsions (Fig. 16.2) (Aveyard et al. 2003).

16.5 The Effects of Emulsion Types on Dermal and Transdermal Delivery

16.5.1 Overview

Various studies have been performed to compare different types of emulsions (Dal Pozzo and Pastori 1996; Förster et al. 1997; Wiechers 2005). However, not only the type of emulsion was different, the formulation ingredients also varied, and their interactions with the active ingredients (e.g. solubilisation in micelles, supramolecular complex formation) may therefore have impacted on the dermal and transdermal delivery of the active ingredients (Dal Pozzo and Pastori 1996). Consequently, due to the complexity of topical emulsions, the investigation of the emulsion type effects on dermal and transdermal delivery requires a systematic approach.

A study by Lalor et al. (1995), for example, showed the effects of the incorporation of surfactants in o/w and w/o emulsions (polysorbate 60 (Tween® 60) in o/w emulsion and sorbitan

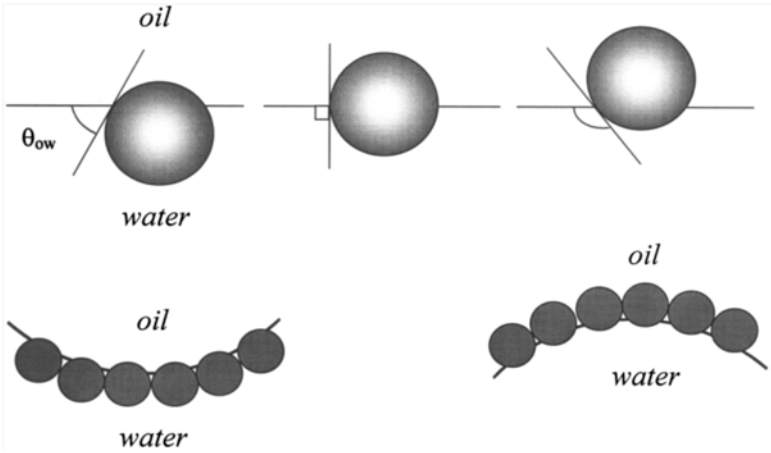

Fig. 16.2 (*Upper*) Position of a small spherical particle at a planar oil-water interface for a contact angle (measured through the aqueous phase) less than 90° (*left*), equal to 90° (*centre*) and greater than 90° (*right*). (*Lower*) Corresponding probable positioning of particles at a curved interface. For θ <90°, solid-stabilised o/w emulsions may form (*left*). For θ >90°, solid-stabilised w/o emulsions may form (*right*) (Reprinted from Aveyard et al. (2003), with permission from Elsevier)

sesquioleate (Arlacel® 83) in w/o emulsion) on the partitioning and permeation of three model compounds (methyl-, ethyl- and butyl-p-aminobenzoate), due to the solubilisation capabilities of the emulsifiers. It was demonstrated that the emulsifier (surfactant) and its distribution between the oil and water phase played an important role in the solubility and therefore thermodynamic activity of the permeants in the vehicle, i.e. the thermodynamic activity of the compounds in the external phase of the emulsions was found to be the driving force for permeation through the polydimethylsiloxane membranes. Polysorbate 60 (Tween® 60), the surfactant used in the o/w emulsion, was mainly available in the external aqueous phase of the emulsion, where it formed micelles and solubilised the three test permeants, methyl-, ethyl- and butyl-*p*-aminobenzoate, thereby reducing their thermodynamic activity and the permeability coefficient. The permeability coefficient between the o/w emulsion and its corresponding, externally isolated aqueous phase was equal, signifying the importance of the thermodynamic activity of the permeants in the external phase for promoting the permeation process. However, the solubility of the three compounds in the oil phase of the same o/w emulsion was similar to the solubility in the oil without surfactant, indicating no solubilising effect of polysorbate 60 (Tween® 60) in the oil phase of the o/w emulsion, hence resulting in similar permeability coefficients between the internal oil phase and the pure oil. Analogous results were obtained with the w/o emulsion in which the emulsifier, sorbitan sesquioleate (Arlacel® 83), was nearly entirely distributed in the oil phase of the emulsion, whereas the aqueous phase was in effect free of sorbitan sesquioleate (Arlacel® 83). This yielded no solubility increase in the internal aqueous phase and thus no reduction in permeability, when compared with water. However, the solubility of each compound increased in the oil phase, because of the formation of inverse micelles. Consequently, the permeability of the three test permeants was reduced from both the w/o emulsion and from the corresponding isolated external oil phase, when compared with the pure oil.

Several studies have been reported, involving different emulsion types with identical composition (e.g. o/w, w/o and w/o/w), hence allowing for the systematic investigation of the effect of the type of emulsion on dermal and transdermal delivery only. These study outcomes are summarised in Table 16.1.

The results in Table 16.1 demonstrate that the type of emulsion significantly influenced both skin penetration and skin permeation of the active ingredients. The effect of the type of emulsion on dermal and transdermal delivery was furthermore dependent on the dosing condition (finite non-occluded vs. infinite occluded). With finite dosing under non-occluded conditions, the physicochemical and thermodynamic properties of the formulation modified rapidly after application onto the skin, whereas at infinite dosing under occluded conditions, the thermodynamic properties did not alter significantly (Laugel et al. 1998b).

16.5.2 Hydrophilic Active Ingredients

In summary, it can be concluded that the percutaneous absorption, as well as the skin penetration of hydrophilic drugs (e.g. glucose, metronidazole and lactic acid), is generally superior for o/w emulsions, compared to w/o/w and w/o emulsions (Fig. 16.3). Various suggestions were made for the differences in performance between the diverse emulsion types. For example, the higher skin uptake from o/w emulsions could have been due to a higher concentration of free hydrophilic actives in the external aqueous phase of the emulsions being directly in contact with the skin, whilst the actives were encapsulated in the internal phase of the w/o/w and w/o emulsions and as a result not readily available to the stratum corneum (Ferreira et al. 1995b; Youenang Piemi et al. 1998; Sah et al. 1998). It was hence suggested that w/o/w and w/o emulsions could be utilised for the controlled release of water-soluble actives (Sah et al. 1998). As shown by Ferreira et al. (1994, 1995b), the release of glucose and metronidazole through cellulose membranes and silicone membranes was in the following order: o/w > w/o/w > w/o. The release from the w/o/w

Table 16.1 Influence of emulsion type on release, dermal and transdermal delivery – list of examples for different model drugs

Model drug	Emulsifier system	Dosing F vs. IF[a]	Effect on release	Effect on dermal delivery	Effect on transdermal delivery	Reference
Glucose	Hypermer™ A60, Synperonic™ PE/F127[b]	IF	o/w > w/o/w > w/o	o/w > w/o/w > w/o	o/w > w/o/w > w/o	Ferreira et al. (1995b)
		F	–	o/w = w/o/w = w/o (dermis: o/w > w/o/w)	o/w > w/o/w = w/o	Ferreira et al. (1995a)
	Lecithin (Emulmetik ™ 100/300)	F	–	Epidermis: o/w = w/o/w = w/o	o/w > w/o/w = w/o	Youenang Piemi et al. (1998)
				Dermis: o/w > w/o/w = w/o		
		IF	–	Epidermis: o/w = w/o/w = w/o	o/w > w/o/w = w/o	
				Dermis: o/w = w/o/w > w/o		
Metronidazole	Hypermer™ A60, Synperonic™ PE/F127[b]	IF	o/w > w/o/w > w/o	–	o/w = w/o/w > w/o	Ferreira et al. (1994)
		F	–	–	o/w = w/o/w = w/o (o/w < w/o)	Ferreira et al. (1995a)
Lactic acid	Hypermer™ A60, Synperonic™ PE/F127[b]	F	–	o/w > w/o/w > w/o	o/w < w/o/w = w/o	Sah et al. (1998)
Hydrocortisone	Nonionic surfactants[c]	F	–	w/o < o/w/o	w/o > o/w/o	Laugel et al. (1998b)
		IF	w/o > o/w/o	–	w/o = o/w/o	
Terpenes (asiatic acid, madecassic acid and asiaticoside)	Mixture of nonionic surfactants	F	–	w/o < o/w/o	w/o > o/w/o	Laugel et al. (1998a)

[a]Finite (F), infinite (IF)
[b]Hypermer™ A60 (a modified polyester)/Synperonic™ PE/F127 (poloxamer 407)
[c]Glycerol sorbitan fatty acid ester, hydroxyoctacosanyl hydroxystearate and copolymer of ethylene and propylene oxides

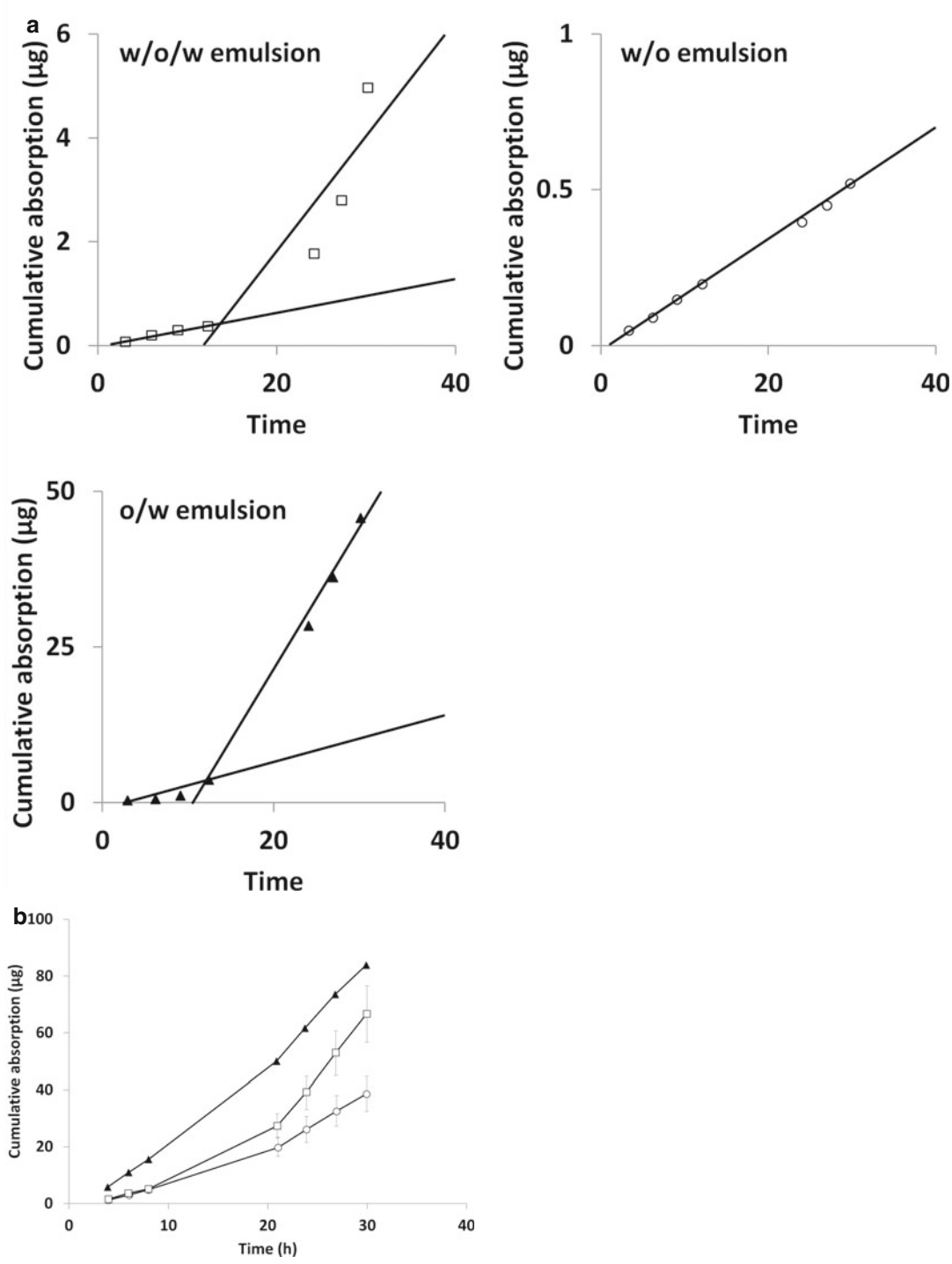

Fig. 16.3 (**a**) Percutaneous absorption profiles of glucose from w/o/w, o/w and w/o emulsions through hairless rat skin; typical plots of the cumulative amount of glucose as a function of time. Values are means ($n=6$) (Reproduced from Ferreira et al. (1995b) with permission from Elsevier). (**b**) Percutaneous absorption profiles of metronidazole from w/o/w (□), o/w (▲) and w/o (○) emulsions. Values are the means ($n=5$) ± SD. The SD values for the o/w emulsions are not represented for purposes of clarity (Reproduced from Ferreira et al. (1994), with permission from Elsevier)

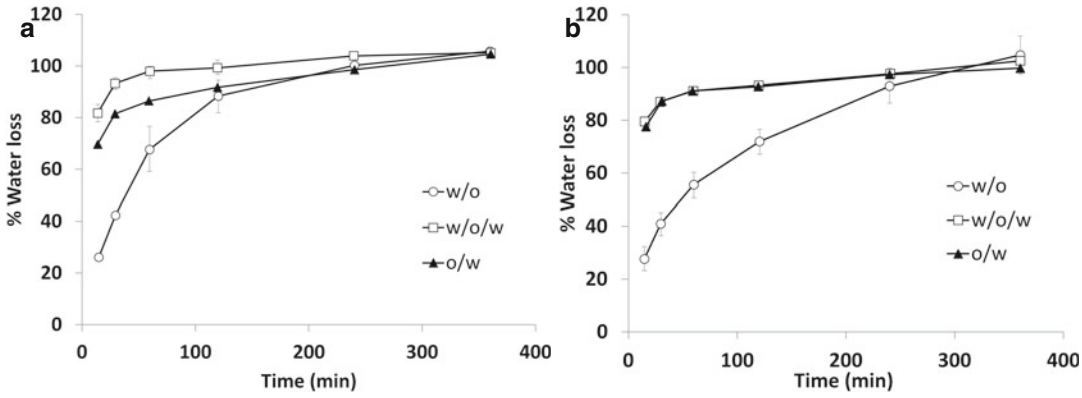

Fig. 16.4 Evaporation of water from emulsions containing (**a**) metronidazole and (**b**) glucose as a function of time. The data are expressed as percentage of water loss of the applied amount (Ferreira et al. (1995a), with permission from Elsevier)

emulsion was higher than from the w/o emulsion, due to the leakage of glucose and metronidazole into the external aqueous phase, resulting in a higher effective concentration of the hydrophilic actives in the external aqueous phase of w/o/w emulsions than in the external oil phase of w/o emulsions (Ferreira et al. 1995a, b). It should also be noted that the differences in polarity of the active ingredients further impacted on the performances of the various emulsions. So the difference between the various emulsions was more pronounced for glucose (high polarity) than for metronidazole (intermediate polarity), due to a smaller oil-water partitioning coefficient of glucose, indicating a higher concentration of glucose in the aqueous phase, when compared with metronidazole (Ferreira et al. 1995b). Furthermore, the partitioning between the oil and water phases in the emulsion was better for metronidazole, which in turn yielded a decrease in the internal release barrier and therefore a less pronounced difference in performance between the various metronidazole emulsions (Ferreira et al. 1995a).

Differences in dermal and transdermal delivery could also have occurred as a result of different partitioning coefficients between the stratum corneum and the various emulsions, e.g. a higher partitioning between the stratum corneum and external aqueous phase (for o/w and w/o/w emulsions) than between the stratum corneum and external oil phase (for w/o emulsions). In addition, the external aqueous phase may have contributed to the hydration of the stratum corneum, which in turn may have enhanced the permeability of hydrophilic compounds (Ferreira et al. 1995b). This effect could be more pronounced for an infinite, occluded dosing condition than for a finite, non-occluded dosing (Sah et al. 1998).

It is also important to consider the fate of the emulsion after application onto the skin and its effect on the delivery of the actives. Following the application onto the skin, volatile components (e.g. water) can evaporate, and therefore phase transitions, inversion, flocculation and coalescence may occur (Friberg and Langlois 1992). In addition, the drug concentration in the residual film could increase, due to water evaporation from a finite dose (Sah et al. 1998). Consequently, consideration of the evaporation of volatile components, as well as the vehicle structure of the remaining film after the evaporation of volatile components, is of importance when investigating skin penetration of actives. Ferreira et al. (1995a) investigated water evaporation from three different emulsions (o/w, w/o/w and w/o) and found that the rate thereof was higher from emulsions with an aqueous continuous phase (o/w and w/o/w) than from emulsions with an oily continuous phase (w/o) (Fig. 16.4), which may partially explain the differences in dermal and transdermal delivery. They also investigated the structure of the residual film after evaporation was completed and its

effect on the lipid organisation of the stratum corneum. No differences were detected among the three emulsions (o/w, w/o/w and w/o) though. Youenang Piemi et al. (1998) reasoned in their article that the similar performances of w/o/w and w/o emulsions in the dermal and transdermal delivery of glucose could be due to a similar vehicle structure of the w/o/w and w/o emulsions after application onto the skin, because of the evaporation of the external water phase of the w/o/w emulsion.

It should be noted that most of the studies on hydrophilic compounds, as listed in Table 16.1, were performed with the same synthetic emulsifier system Hypermer™ A60 (a modified polyester)/poloxamer 407 (Synperonic™ PE/F127). However, the one study by Youenang Piemi et al. (1998) incorporated natural soybean phospholipids (lecithin, Emulmetik™ 100/300) as emulsifier in order to obtain different types of emulsions. Overall, the same trend was observed with the soybean phospholipids as with the synthetic emulsifiers. This was indicative of the importance of the nature of the continuous phase of an emulsion on the dermal and transdermal delivery of hydrophilic drugs (Youenang Piemi et al. 1998). Furthermore, the results of the various studies with hydrophilic active ingredients indicated that the type of emulsion (o/w, w/o/w, w/o) may not significantly affect the distribution of the actives between dermal and transdermal delivery, as the order of emulsions was similar for dermal and transdermal delivery (see Table 16.1).

16.5.3 Lipophilic Active Ingredients

As with the hydrophilic drugs, the encapsulation of the lipophilic actives (hydrocortisone and three different triterpenic derivatives) in the internal oily phase of multiple o/w/o emulsions reduced the percutaneous absorption of these lipophilic actives, compared to that of simple w/o emulsions, when applied as a finite dose. However, the uptake of the lipophilic actives into the epidermis and dermis was higher from the multiple o/w/o emulsion than from the simple w/o emulsion (Laugel et al. 1998a, b). Similarly, a release study showed that the release of hydrocortisone was slower from a multiple o/w/o emulsion than from a simple w/o emulsion, as the active needed to diffuse from the internal phase, across the aqueous phase and into the external phase (Laugel et al. 1998b). Both studies hence confirmed that multiple o/w/o emulsions could be used as prolonged, topical delivery systems for lipophilic drugs, when incorporated in the internal oily phase and applied as a finite dose. Furthermore, these multiple emulsions exhibited the advantages of reducing the transdermal delivery and therefore the systemic effects of the lipophilic drugs, whereas the dermal delivery was increased, thus showing a controlled release of the drugs to the site of action (Laugel et al. 1998b). However, it should be noted that no significant differences in transdermal delivery were observed between w/o and o/w/o emulsions, when an infinite dose of hydroquinone containing emulsions was applied onto the skin (Laugel et al. 1998b).

16.5.4 Stabilisation Effects of the Formulation

When investigating the dermal and transdermal delivery of active ingredients from emulsions, consideration of the stabilisation effects of a formulation on delivering degradation-sensitive active ingredients intact into and/or across the skin can prove beneficial. Schmidts et al. (2011) investigated the stabilisation effects of various emulsion systems against enzymatic degradation of topically applied oligonucleotides. They found that the enzymatic degradation of water-soluble DNAzymes, encapsulated in the inner aqueous phase of w/o/w and w/o emulsions, was significantly reduced, compared to DNAzymes, incorporated in the outer aqueous phase of a microemulsion and a submicron emulsion. The outcomes of their study suggested that w/o and w/o/w emulsions are promising formulations for effective encapsulation of DNAzymes with concurrent protection against enzymatic degradation.

16.6 The Effects of Various Emulsifiers on Dermal and Transdermal Delivery

16.6.1 Overview

As was mentioned above, various substances exist that can be utilised as emulsifiers (e.g. surfactants, polymers, solid particles), and often the stabilisation effect of emulsifiers can be attributed to more than one method. For instance, some surfactants may form a monolayer at the oil-water interface, whilst an excess thereof may also arrange in liquid crystalline structures in the aqueous phase, which could improve emulsion stability by preventing coalescence (Friberg and Solans 1986). Hydrophobically modified water-soluble polymers can stabilise o/w emulsions by adsorbing at the oil-water interface (hydrophobic part of the polymer), as well as by gelation of the aqueous continuous phase (hydrophilic part of the polymer) (Eccleston 1997a). In case of Pickering emulsions, the particles can, in addition to the adsorption at the oil-water interface, also form a three-dimensional network in the continuous phase surrounding the droplets. The resulting increased viscosity of the emulsions can reduce the rate and extent of creaming (Aveyard et al. 2003). Furthermore, the complexity of emulsions makes it more difficult to study the exclusive effect of emulsifiers on dermal and transdermal delivery, as other emulsion ingredients also contribute to interactions with the active ingredient in the vehicle, as well as with the stratum corneum. Therefore, this chapter aimed at focusing mainly on studies in which a more systematic approach to investigating the effects of emulsifiers on dermal and transdermal delivery was followed, such as using emulsions with the same oil and aqueous phases and hence reducing the influences of varying formulation ingredients on the active's transport into and across the skin.

Montenegro et al. (2004) investigated the effects of various silicone emulsifiers using the same oil and aqueous phase ingredients for the preparation of the emulsions. The study illustrated that the type of silicone emulsifier could significantly affect the permeation of octyl methoxycinnamate (Uvinul MC 80®) across human skin, whereas the permeation of butyl methoxydibenzoylmethane (Uvinul BMBM®) was insignificantly affected. It was assumed that changes in the thermodynamic activity in the emulsion and modification of the interaction between permeant and emulsion components could account for the different effects of the emulsifiers on skin permeation. Though the inclusion of different silicone emulsifiers altered the viscosity of the emulsions and the release of the active ingredients, these factors could not be related to the modification in permeation.

Wiechers et al. (2004) suggested that the emulsifier system may influence the distribution of the active ingredient between dermal and transdermal delivery, whilst emollients may significantly affect the total skin absorption (dermal + transdermal delivery) thereof. For example, the emulsion with the emulsifier system, sorbitan stearate/sucrose cocoate (Arlatone® 2121), exhibited a higher transdermal, but lower dermal, delivery of octadecenedioic acid (Arlatone™ DIOIC DCA), in comparison with the emulsion containing steareth-2/steareth-21 (Brij® 72/721). No explanations could be given as more investigations were required to understand the influence of the emulsifier system on skin delivery.

These studies, using the same oil and aqueous phases for the emulsions, demonstrated that emulsifiers significantly affected dermal and transdermal delivery. However, the emulsifiers being compared varied in structure and physicochemical properties, making it difficult to explain their particular effects on skin penetration and permeation. The following examples focus more on studies that compare emulsifiers with particular differences (e.g. variation in hydrophilic chain length of nonionic surfactants, HLB values, emulsion droplet charge).

16.6.2 Effects of Hydrophilic Chain Length of Nonionic Surfactants

Oborska et al. (2004) investigated the effect of three different polyoxyethylene cetostearyl ethers

of various oxyethylene chain lengths (ceteareth-12 (Eumulgin® B1), ceteareth-20 (Eumulgin® B2) and ceteareth-30 (Eumulgin® B3)) in o/w emulsions on the permeation of two flavonoids, quercetin and rutin, through a liposome model membrane. The study revealed that the permeability coefficient of both permeants decreased with increasing length of the oxyethylene chain and their effects were more pronounced for rutin, the more water-soluble flavonoid. No explanation was given. However, a similar trend was observed in a study by Dalvi and Zatz (1981), when investigating the effects of the polyoxyethylene chain length of nonionic surfactants on benzocaine flux from aqueous solutions. The reduction in benzocaine flux from non-saturated solutions with increasing polyoxyethylene chain length was explained by a higher micellar entrapment of benzocaine and therefore a lower concentration of free benzocaine giving rise to a lower driving force for permeation. Although this study was performed on aqueous solutions, the solubilisation effects of the nonionic surfactants with increasing length of the oxyethylene chain could have also occurred with quercetin and rutin in the o/w emulsions used by Oborska et al. (2004).

A study by Förster et al. (2011) tested o/w emulsions with different surfactants of the polyethylene glycol ester type, which varied in the length of the alkyl- and polyethylene glycol chains (PEG6C18:1, PEG20C12 and PEG20C18:1), for skin penetration of the lipophilic active, retinol. The results confirmed that surfactants with a short polar head group had an enhancement effect on penetration into the skin, as the penetration into the epidermis and dermis from emulsions with PEG6C18:1 was higher than from emulsions with PEG20C12 and PEG20C18:1. Confocal Raman microspectroscopy revealed that all three tested emulsions did not differ in the lateral interaction (ratio of I_{2880}/I_{2850}), an indicator for the lateral packing of lipids. Furthermore, the ratio I_{2880}/I_{2850} for all three emulsions did not significantly differ from the ratio for untreated skin. As there was no indication of disruption of the lipid structure of the stratum corneum by any of the emulsions, the increased dermal delivery with PEG6C18:1 was linked to a change in partitioning behaviour of retinol between the skin and formulation. These results were congruent with those from studies by Oborska et al. (2004) and Dalvi and Zatz (1981), demonstrating that drug permeation was inversely related to the hydrophilic chain length of the surfactants, due to a change in solubilisation capacity of the surfactant micelles, without an apparent interaction between the surfactants and the stratum corneum lipids (Dalvi and Zatz 1981).

16.6.3 Effects of Emulsifier Ratios and HLB Value

As mentioned, often emulsifier combinations are used to manufacture and stabilise topical emulsions. The studies discussed next illustrate the influence that an emulsifier mixture may exhibit on dermal and transdermal delivery. These examples also include nanoemulsions, although a separate book chapter deals with nanoemulsions as skin delivery systems in more detail.

Nam et al. (2012) showed that the incorporation of a second emulsifier (Phospholipon® 90 G, mostly soybean phosphatidylcholine with max. 4.0 % lysophosphatidylcholine) with skin permeation-enhancing capabilities could increase skin absorption of the active ingredient, tocopheryl acetate. Mixtures of Phospholipon® 90 G and a polymer (poly(ethylene oxide)-*block*-*poly*(ε-caprolactone) (PEO-*b*-PCL)) in various ratios were used to stabilise o/w nanoemulsions being tested for their skin absorption effects. The permeation-enhancing effects of the unsaturated lipid yielded improved skin absorption of tocopheryl acetate from nanoemulsions, with increasing lipid-to-polymer ratios (Fig. 16.5). In comparison, the nanoemulsion, solely being stabilised with the polymer, PEO-*b*-PCL, exhibited a much lower skin penetration. It was furthermore indicated that not only the permeation-enhancing effect of the unsaturated lipid but also the smaller size of the emulsion droplets could have yielded an improved skin delivery of tocopheryl acetate.

Another study by Cho et al. (2012) utilised a triblock copolymer (poly(ethylene oxide)-*block*-*poly*(ε-caprolactone)-*block*-poly(ethylene oxide) (PEO-PCL-PEO)) to co-stabilise a retinol

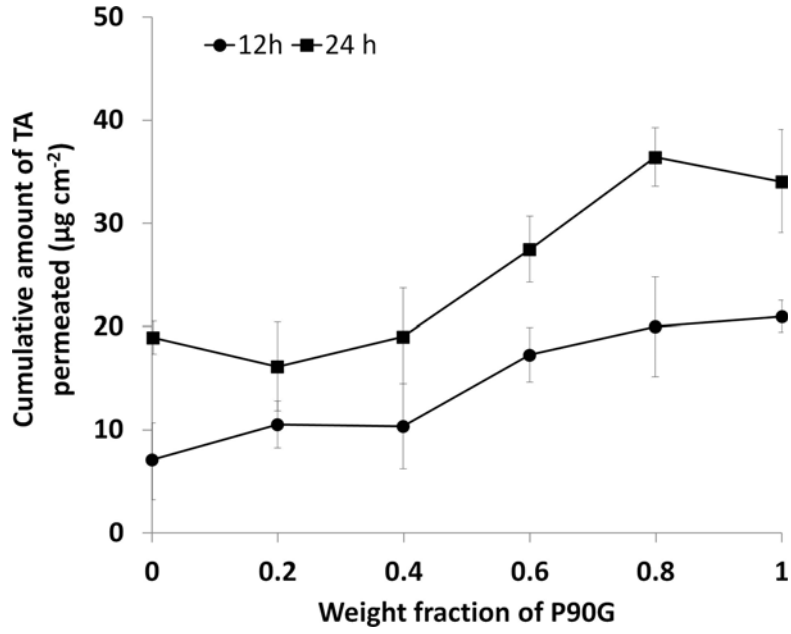

Fig. 16.5 Cumulative amount of tocopheryl acetate absorbed by the hairless guinea pig skin form nanoemulsions stabilised using a mixture of a lipid (P90G) and a polymer (PEO_{45}-*b*-PCL_{42}) as a function of the lipid-polymer ratio for 12 h (●) and 24 h (■) (Reproduced from Nam et al. (2012), with permission from Elsevier)

emulsion that was primarily stabilised using polysorbate 20 (Tween® 20). Their work included an investigation of the effect of the PCL block length of the triblock copolymer on the topical delivery of retinol, and it was ascertained that the accumulation of retinol in the artificial skin was enhanced by employing the triblock copolymer, as well as by increasing the PCL block length. It was, furthermore, found that with increasing length of the PCL block, the hydrophilic-lipophilic balance (HLB) value, as well as the size of the emulsion droplets decreased. The outcomes from this study suggested that the PCL block length and HLB value are important considerations for the topical delivery of actives from emulsions being co-stabilised with a triblock copolymer.

Wu et al. (2001) found that the rate and extent of inulin permeation from water-in-oil nanoemulsions were highly dependent on the HLB values of the incorporated surfactant mixtures (different ratios of sorbitan monooleate (Span® 80) and polysorbate 80 (Tween® 80)). Surfactant mixtures with a low HLB resulted in significantly higher permeation of inulin than surfactant mixtures with a high HLB (Fig. 16.6). It was suggested that inulin, encapsulated into the aqueous phase of the w/o nanoemulsions, was mainly transported via the transfollicular route and that its transport was facilitated by nanoemulsions in which the HLB of the oil phase was compatible with the sebum environment of the hair follicle.

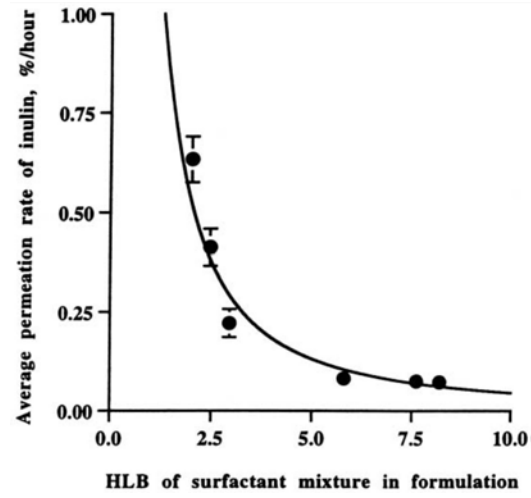

Fig. 16.6 Correlation between permeation rate of inulin across hairy mouse skin (expressed as per cent of applied formulation per h ± SE) and HLB of surfactant mixture in nanoemulsion or micellar formulation. HLB of surfactant mixture = ((volume per cent Span® 80 in formulation × 4.3) + (volume per cent Tween® 80 in formulation × 15.0))/100 (Reprinted from Wu et al. (2001), with permission from Elsevier)

These examples illustrate that the type of emulsifier/emulsifier system, together with the

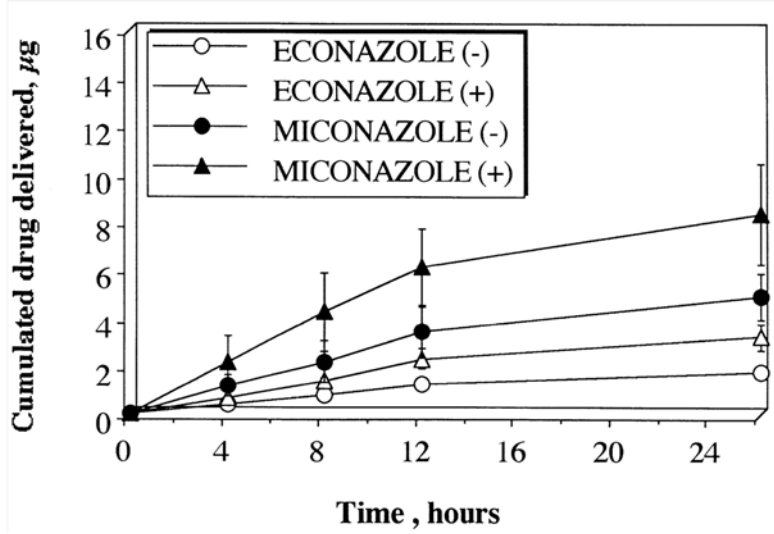

Fig. 16.7 Permeation profiles of econazole and miconazole nitrate incorporated in negatively (−) and positively (+) charged submicron emulsion across hairless rat skin determined by HPLC. Approximately 37 µg of drug were applied per cell with an effective diffusional area of 0.635 cm^2; $n = 6$; mean ± S.D (Reprinted from Youenang Piemi et al. (1999), with permission from Elsevier)

ratio of the emulsifier mixture, is not only essential to the emulsification and stabilisation effects but potentially also for modifying skin penetration significantly. Moreover, the HLB value may considerably influence topical performance.

Some studies reported that the various emulsifiers and/or ratios of emulsifier mixtures also altered the droplet size and viscosity of the emulsions, which may have affected dermal and transdermal delivery. The study by Klang et al. (2011), however, revealed that neither skin permeation nor penetration was influenced by the droplet size and viscosity. Klang et al. (2011) used sucrose stearate S-970 (Ryoto® Sugar Ester) as emulsifier to formulate stable o/w emulsions by forming a hydrophilic network around the oil droplets. Through a slight modification of the manufacturing process, they were able to produce a highly viscous macroemulsion and a less viscous, fluid nanoemulsion with exactly the same composition, thus enabling the investigation of the influence of droplet size and viscosity on dermal and transdermal delivery. The macro- and nanoemulsions showed neither significant difference in flux, penetration depth nor in accumulation of the three model drugs used (curcumin, flufenamic acid, diclofenac acid) in the stratum corneum. The results regarding droplet size are congruent with the outcome of the study by Izquierdo et al. (2007), where also no correlation could be found between the droplet size and the dermal and transdermal delivery. On the contrary, Nam et al. (2012) showed that the size of the emulsion droplets could affect skin absorption. They prepared nanoemulsions with the same chemical composition but different droplet sizes by applying different pressure in the microfluid process and found that smaller droplet sizes enhanced the skin absorption of tocopheryl acetate. However, transdermal delivery was not determined in this study.

16.6.4 Effects of Emulsion Droplet Charge

Positively and negatively charged submicron emulsions were compared by Youenang Piemi et al. (1999) for their effects on the dermal and transdermal delivery of econazole and miconazole nitrate. The composition of the emulsions was the same and only differed in the emulsifier component giving the charge to the emulsion droplets, i.e. stearylamine in the case of positively charged emulsions and deoxycholic acid in case of negatively charged emulsions. In overall, the positively charged o/w emulsion containing stearylamine was more effective in dermal and transdermal delivery of econazole and miconazole nitrate than the negatively charged o/w emulsion containing deoxycholic acid (Fig. 16.7). It was suggested that positively charged submicron emulsion droplets could facilitate the transport of

the permeants into and through the skin, most probably owing to a superior binding of the positively charged droplets to the skin, which is negatively charged at neutral pH.

Two studies by Ghouchi Eskandar et al. (2009a, 2010), investigating oil-in-water submicron emulsions, also showed the possible dependency of dermal delivery on the emulsifier charge. The skin retention of the tested lipophilic compounds, all-*trans*-retinol and acridine orange 10-nonyl bromide, was significantly higher for those emulsions being stabilised by the positively charged oleylamine, compared to emulsions, stabilised by the negatively charged lecithin. Two explanations were given. Firstly, the higher skin accumulation of the lipophilic compounds being released from the oleylamine-stabilised emulsions could be attributed to the electrostatic attraction between the positively charged oleylamine droplets and negatively charged skin surface. Secondly, it could also have been due to reduced skin barrier properties, as a result of the disrupting effect of oleylamine on the stratum corneum lipid organisation.

16.6.5 Effects of Surfactant Association Structures

When viewing the effects of surfactants, one should not solely consider the surfactant but also the association structures that could form in emulsions. In addition to the formation of a surfactant monolayer at the oil-water interface, some surfactants, when in excess, may arrange in liquid crystalline structures in the aqueous phase, which may aid the stabilisation of emulsions (Friberg and Solans 1986) and could affect skin permeation. Only two studies are mentioned below to illustrate the effect of the emulsifier association structures on skin permeation. For more information on liquid crystalline structures, the reader is referred to a separate chapter in the book.

A study by Brinon et al. (1998) illustrated that the flux of benzophenone-4 (Uvinul® MS40) was increased by emulsions containing lamellar liquid crystals (triethanolamine stearate, sorbitan stearate/sucrose cocoate (Arlatone® 2121) and steareth-2/-21 (Brij® 72/721) in comparison to emulsions without liquid crystals (polysorbate 60 (Tween® 60), poloxamer 407 (Synperonic™ PE/F127) and acrylates/C_{10-30} alkyl acrylate crosspolymer (Pemulen® TR1)). The differences in permeation could be due to modified interactions between surfactants and permeant that may have influenced the interactions between the surfactants and stratum corneum. Furthermore, the partitioning between the formulation and the skin could have been altered, e.g. partitioning between the skin and the aqueous phase (emulsions without liquid crystals) and partitioning between the skin and the liquid crystal phase (emulsions with liquid crystals).

The effect of liquid crystalline structures in o/w emulsions on dermal and transdermal delivery was also investigated by Otto et al. (2010). Five o/w emulsions were tested:

- 'Hydrosome' emulsion, stabilised by sorbitan stearate and sucrose cocoate (Arlatone® 2121), with lamellar gel structuring of the water phase (Tadros et al. 2006)
- 'Phosphosome EFA' (essential fatty acid) emulsion, stabilised by linoleamidopropyl PG-dimonium chloride phosphate (Arlasilk™ Phospholipid EFA), with structuring of the water phase and lamellar liquid crystalline phases around the oil droplets
- 'Phosphosome PTC' (phosphatidylcholine) emulsion, stabilised by cocamidopropyl PG-dimonium chloride phosphate (Arlasilk™ Phospholipid PTC), with structuring of the water phase and lamellar liquid crystalline phases around the oil droplets
- 'Oleosome' emulsion, stabilised by steareth-2/steareth-21 (Brij® 72/721) incl. cetearyl alcohol (Laurex CS™) and PPG-15 stearyl ether (Arlamol™ E), with surfactant bilayers around the oil droplets (Tadros et al. 2006)
- 'Conventional' emulsion, stabilised by steareth-2/steareth-21 (Brij® 72/721), without liquid crystalline phases

The study revealed that the hydrosome and phosphosome emulsions, with lamellar gel structuring of the water phase, enhanced the dermal and transdermal delivery of hydroquinone and octadecenedioic acid (Arlatone™ DIOIC DCA), when compared with the conventional o/w

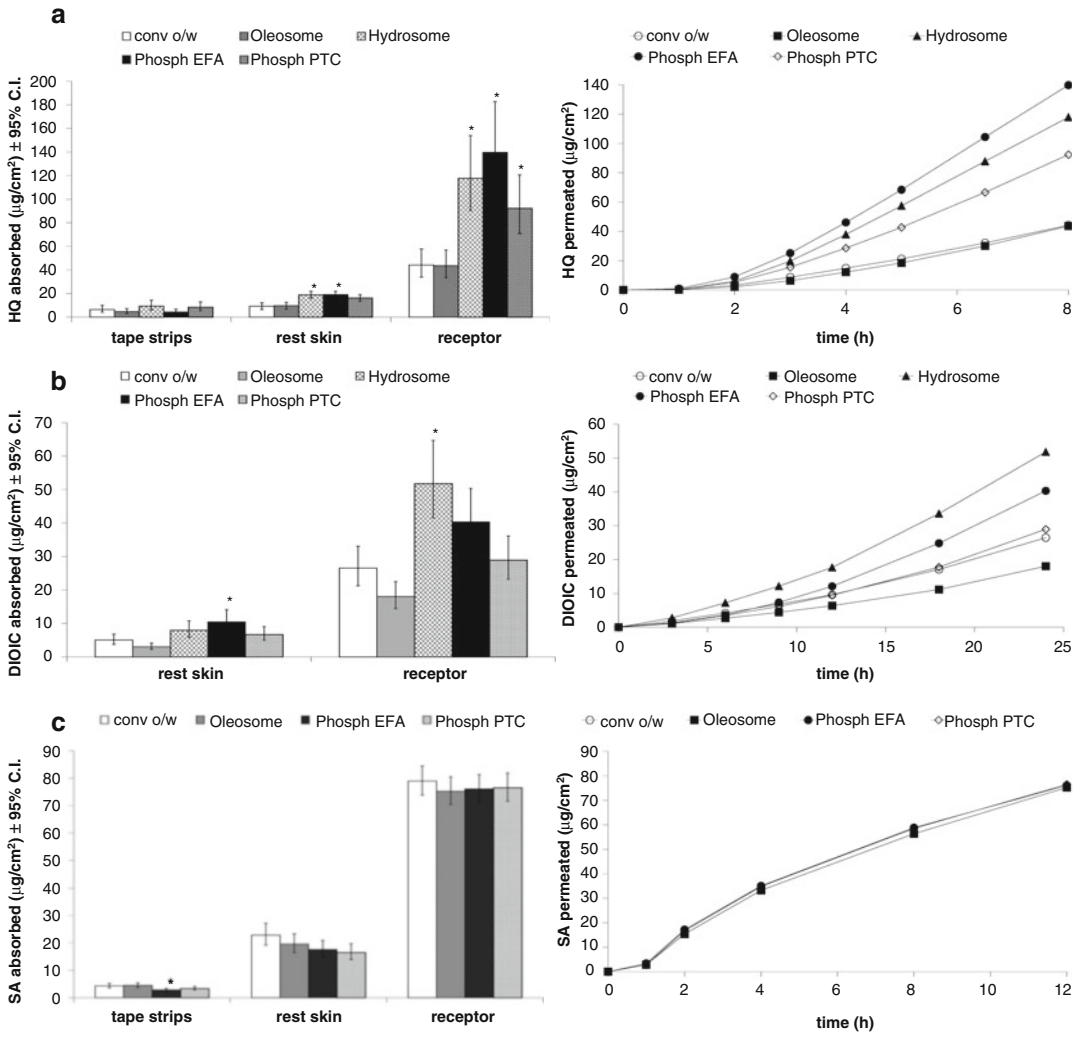

Fig. 16.8 (**a**) Skin permeation data on hydroquinone (*HQ*) expressed as total amount delivered over 8 h of skin penetration as least square means (formulation effect) ± 95 % confidence interval (*CI*) obtained after two-way ANOVA. *Conv.* conventional, *Phosph.* phosphosome. *Statistically significant compared with the conventional o/w emulsion (Reprinted from Otto et al. (2010), with permission from Karger). (**b**) Skin permeation data on octadecenedioic acid (DIOIC) expressed as total amount delivered over 24 h of skin penetration as least square means (formulation effect) ± 95 % confidence interval (*CI*) obtained after two-way ANOVA. *Conv.* conventional, *Phosph.* phosphosome. *Statistically significant compared with the conventional o/w emulsion (Reprinted from Otto et al. (2010), with permission from Karger). (**c**) Skin permeation data on salicylic acid (SA) expressed as total amount delivered over 12 h of skin penetration as least square means (formulation effect) ± 95 % confidence interval (*CI*) obtained after two-way ANOVA. *Conv.* conventional, *Phosph.* phosphosome. *Statistically significant compared with the conventional o/w emulsion (Reprinted from Otto et al. (2010), with permission from Karger)

emulsion without liquid crystalline phases and the oleosome emulsion with lamellar liquid crystalline phases around the oil droplets (Fig. 16.8a, b). The increase in skin penetration was ascribed to an improved partitioning of both permeants into the skin. However, a different pattern was observed for salicylic acid, where no differences in skin penetration occurred between the o/w emulsions with and without liquid crystalline phases (Fig. 16.8c). It was assumed that the interactions between the different emulsifiers and active ingredients varied and therefore the solubilisation

capacities of the various emulsifiers and their association structures, which could have resulted in changes in the thermodynamic activity of the permeants in the emulsions.

16.6.6 Effects of Solid Particles as Emulsifiers

More recently, new topical emulsion systems have been introduced that are surfactant free and being stabilised by solid particles. These particle-stabilised emulsions are also known as Pickering emulsions. Another chapter in this book discusses Pickering emulsions as topical delivery systems; however, a few examples are mentioned below to illustrate the differences in topical performance due to different emulsifiers.

Frelichowska et al. (2009b) investigated the skin absorption of the hydrophilic active caffeine from silica particle-stabilised w/o emulsions (Pickering emulsions) and compared it to the absorption from surfactant-stabilised w/o emulsions (conventional emulsions). These emulsions had the same physicochemical properties (droplet size and viscosity), as well as chemical composition and only varied in the type of emulsifier. The study revealed that the caffeine flux was threefold higher from the Pickering emulsion than from the conventional emulsion, although the release was slower from the Pickering emulsion. The increased skin absorption could be explained by the higher adhesion of the silica particle-stabilised water droplets onto the skin surface. Furthermore, it was found that silica particles penetrated into the upper layers of the stratum corneum and it was hypothesised that caffeine was transported into the skin by means of adsorption onto the silica particles.

Another study by Frelichowska et al. (2009a), investigating oil-in-water emulsions, revealed that the total skin uptake of the lipophilic drug, all-*trans*-retinol, was similar for the conventional emulsion and the Pickering emulsion and no transdermal delivery was observed. However, the distribution of all-*trans*-retinol within the skin varied. The Pickering emulsion showed an enhanced accumulation of all-*trans*-retinol in the stratum corneum, whereas the conventional emulsion increased the transport through the stratum corneum into the viable epidermis and dermis. It was hypothesised that the enhanced accumulation of all-*trans*-retinol in the stratum corneum could be due to a lack of flexibility of the Pickering emulsion droplets.

The former studies compared Pickering emulsions being stabilised solely by solid particles with conventional emulsions being stabilised solely by surfactants. However, emulsions with mixed interfacial layers, for instance, including both surfactants and solid particles, were also tested for their topical performances, as discussed next.

Silica nanoparticle coatings of lecithin- and oleylamine-stabilised oil-in-water submicron emulsions exhibited an enhanced dermal delivery of all-*trans*-retinol and acridine orange 10-nonyl bromide, compared to non-silica-coated emulsions (Ghouchi Eskandar et al. 2009a, 2010). As the silica nanoparticles significantly increased the emulsification efficiency, as well as the physical stability of the emulsions (Ghouchi Eskandar et al. 2007), they could be considered as emulsifiers. In both studies it was observed that the extent and the depth of penetration of lipophilic all-*trans*-retinol and acridine orange 10-nonyl bromide into full-thickness porcine skin were increased by nanoparticle-coated emulsions and they both were affected by the initial loading phase of the silica nanoparticles during the preparation of the emulsions (e.g. incorporation into the water phase vs. oil phase). However, the silica coating did not influence the transdermal delivery of the two compounds, which was considered negligible. It was further shown that silica nanoparticles could also penetrate the skin up to the viable epidermis and dermis, with negligible permeation across the skin. There was a correlation found between the skin accumulation and distribution of acridine orange 10-nonyl bromide and silica nanoparticles, and it was hypothesised that the transport of acridine orange 10-nonyl bromide could have been facilitated by electrostatic complexation with silica nanoparticles (Ghouchi Eskandar et al. 2010). Furthermore, the improved physical stability of silica-coated

emulsions and enhanced chemical stability of all-trans-retinol (Ghouchi Eskandar et al. 2009b) are other possible mechanisms for the increased skin retention. Additionally, the formation of a thick film of silica nanoparticles and emulsion oil droplets on the skin surface could have had an occlusive effect that may have increased the hydration of the stratum corneum, which in turn could have enhanced skin penetration (Ghouchi Eskandar et al. 2009a).

Conclusion

This review has emphasised that different parameters/variables require consideration when deciding on an appropriate emulsion type and emulsifier system. For example, the skin uptake into the skin as well as the permeation through the skin of a hydrophilic active could be enhanced, when the active is incorporated into the continuous phase of the emulsion. Furthermore, multiple o/w/o emulsions, in comparison to simple w/o emulsions, reduced the transdermal delivery of lipophilic active ingredients, whereas the dermal delivery was increased. Therefore, multiple emulsions could be considered if prolonged topical delivery or the protection of the active ingredient against external influences (e.g. enzymatic degradation, oxidation) is desired. It was demonstrated that the effects of surfactants, as incorporated in emulsions, on dermal and transdermal delivery could vary, depending on their structure and physicochemical properties, e.g. HLB value, hydrophilic chain length of nonionic surfactants, emulsifier charge or their association structures in the emulsion. However, the emulsifier could also influence the droplet size and viscosity of emulsions, which in turn may additionally affect topical performance. Pickering emulsions, a rather new topical delivery system type, are not only interesting in terms of improving the physical stability of emulsions but also show the potential for dermal skin targeting and controlled release.

In summary, it can be concluded that the choice of the emulsifier is not only critical for the emulsification process and for the stability of the resulting emulsion but it is also important for the dermal and transdermal performance that additionally is influenced by the type of emulsion.

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Skin Permeation: Enhancing Ability of Liquid Crystal Formulations

17

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Contents

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17.1 Introduction

The primary dosage forms used in drug therapy are oral formulations and injections; however, oral formulations have disadvantages, such as the side effect on the gastrointestinal tract and the first-pass effect, while injections cause pain by needle puncture and may cause infection; transdermal drug delivery systems (TDDS) can avoid these disadvantages. Interestingly, more TDDS were developed at the beginning of this century than oral DDS. The outermost layer of the skin, the stratum corneum (SC), has a role as the primary barrier against water evaporation from the body and skin permeation of most drugs into the body. Thus, overcoming the SC barrier is important to advance the development of TDDS.

Chemical approaches, such as penetration enhancers (Purdon et al. 2004), and physical approaches, such as iontophoresis (Banga and Kasha 2008), phonophoresis (Ogura et al. 2008), and electroporation (Tokudome and Sugibayashi 2004; Tokumoto et al. 2005), have been evaluated to increase the skin permeation of several drugs. Formulation approaches have also been investigated to increase the skin permeation of drugs. Liposomes and niosomes are examples for new topical formulations having high penetration-enhancing activity of the entrapped drugs (Abraham and Downing 1989; Fang et al. 2001; Kirjavainen et al. 1999); in general, however, such nano-sized materials have poor stability.

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Recently, solid lipid nanoparticles (Dingler and Gohla 2002; Müller et al. 2000) and liquid crystal (LC) dispersions have been developed for topical formulations, and the latter is highly bioadhesive (Geraghty et al. 1997) and physically stable. The LC structure is very similar to the intracellular lipid structure in the SC (Norlén 2001). Very stable LC dispersions made of monoolein/oleic acid (Garg et al. 2007; Gustafsson et al. 1996) and phytol (Barauskas and Landh 2003) have been applied to the skin and mucosa. LC nanodispersions containing monoolein/oleic acid were used to modify the skin permeation of indomethacin (Esposito et al. 2005), cyclosporine (Lopes et al. 2006a, b), vitamin K (Lopes et al. 2007), and propranolol (Namdeo and Jain 2002). These LCs have also been investigated for skin care products (Brinon et al. 1999; Esposito et al. 2007) and cubic lipid structures have already been used as lotions and creams in cosmetics (Yamaguchi et al. 2006).

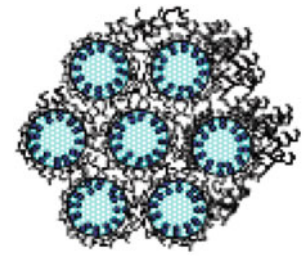
17.2 Liquid Crystals (LC)

LCs are self-assembled mesophases, which are intermediates between isotropic liquids and crystalline solids (Gin et al. 2008b). In LC phases, long-range periodicity exists, although the molecules exhibit a dynamical disorder at atomic distances, as is the case of liquids. Therefore, these materials can also be considered ordered fluids (Larsson 1989). On the other hand, lyotropic LCs are materials that are composed from at least two molecules: an amphiphilic molecule and its solvent. A hydrophilic solvent, such as water, hydrates the polar moieties of the amphiphiles via hydrogen bonding, while the flexible aliphatic tails of the amphiphiles aggregate into fused hydrophobic regions, based on van der Waals interactions between aliphatic lipids and hydrophobic regions. In addition to morphologic dependence on the chemical composition, lyotropic LCs are also sensitive to external parameters, such as temperature and pressure (Gin et al. 2008b; Larsson 1989; Yuli-Amar 2008). Therefore, the shape of the amphiphilic molecule, critical packing parameters, and interfacial curvature energy are important parameters for the structure and nature of LCs (Silver 1985).

An important feature of lyotropics is the self-assembly of the amphiphilic molecules as supermolecular structures, which are the basic units of these mesophases.

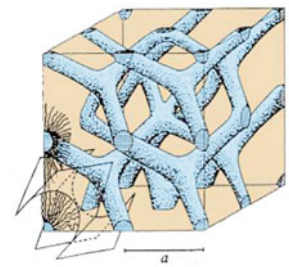
The major LC structures are lamella, hexagonal, reverse hexagonal, bicontinuous cubic, and micellar cubic. Among them, hexagonal liquid crystals, reverse-hexagonal liquid crystals, and cubic liquid crystals are known as non-lamella liquid crystals (non-lamella LC).

Hexagonal liquid crystal and reverse-hexagonal liquid crystals are formed by some amphiphilic molecules when they are mixed with water or another polar solvent. In this phase, the amphiphilic molecules are aggregated into cylindrical structures of indefinite length and these cylindrical aggregates are disposed on a hexagonal lattice, giving the phase long-range orientational order (Scheme 17.1).



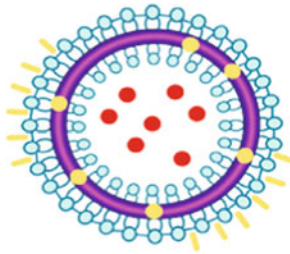
Scheme 17.1 A schematic representation of hexagonal phase

Whereas cubic liquid crystals are formed when the concentration of micelles dispersed in a solvent is sufficiently high that they are forced to pack into a structure having long-ranged positional order (Scheme 17.2).



Scheme 17.2 A schematic representation of cubic phase

On the other hand, liposomes as lamella LC have an entrapped, discontinuous aqueous phase separated by bilayered lamellae from the continuous aqueous phase (Scheme 17.3).



Scheme 17.3 A schematic representation of a liposome

Thus, the evaluation of lamella LC as a drug carrier might be difficult because entrapped drug could easily transfer from the internal phase to the dispersion medium.

17.3 Current Problems in LC for TDDS

Phytantriol and glyceryl monooleate (GMO) are well-known compounds as non-lamella LC-forming lipoids (Phan et al. 2011). However, not all of drugs can be entrapped in non-lamella LC because hydrophilic/lipophilic balance (HLB) and molecular size of drugs (Charlotte and Drummond 2013) might affect the self-assemble of non-lamella LC-forming lipids. Furthermore, conventional non-lamella LC-forming lipids have so high viscosity that it would be tough to handle them in drug and cosmetic formulations and these lipids can form non-lamella LC in a narrow temperature range. These are problems that need to be overcome for the development of transdermal formulations using non-lamella LCs.

17.4 Structure of Liquid Crystal Dispersion

Figure 17.1 illustrates a scheme of the formation of lamella LC and non-lamella LC from amphiphiles in water (Gin et al. 2008a). Lamellar, hexagonal, bicontinuous cubic, and discontinuous cubic phases are well known and there are many research studies in that field. The structure of self-assembled mesophases is affected by amphiphile's concentration. On increasing the concentration of amphiphiles in water, liposomes (lamellar), bicontinuous cubic lamella phases (Q2), and hexagonal phases (H2) are formed. There have been numerous descriptions of the liquid crystalline phase behavior (Hyde 1990). The dimensionless shape parameter known as critical packing parameter (CPP) has provided useful information regarding the choice of type of rational design of amphiphile-water phase behavior (e.g., micelle structure is when CPP <1, lamellar phase is when CPP=1, and inverse cubic structure is when CPP> 1) (Israelachvili 1976). In this chapter, we focus on cubic lamella phase and hexagonal phase that show skin permeation enhancement effects.

17.5 Preparation of a Mixture of Mono-, Di-, and Triesters (1) and Monoesters (2) Composed of Erythritol and Phytanylacetic Acid

Erythritol (2.50 kg) was dissolved in dimethyl sulfoxide (10.8 kg) at 100 °C under nitrogen purging, before the addition of anhydrous calcium carbonate (37.8 g) at 80 °C. Methyl phytanylacetate (5,9,13,17-tetramethyloctadecanoate) (4.83 kg) was added dropwise to the solution under reduced pressure over 2.5 h. The reaction mixture was refluxed under reduced pressure overnight, while the methanol produced was gradually distilled. After cooling, the mixture was neutralized by the addition of formic acid (29 g) and concentrated in vacuo. The residue (6.1 kg) was diluted with t-butyl methyl ether (18.3 kg) and filtered to remove the remaining erythritol. The filtrate was diluted again with t-butyl methyl ether (24 kg), washed twice with aqueous sodium bicarbonate, and concentrated in vacuo at 100 °C. The product obtained (4.7 kg, mixture 1) consisted of

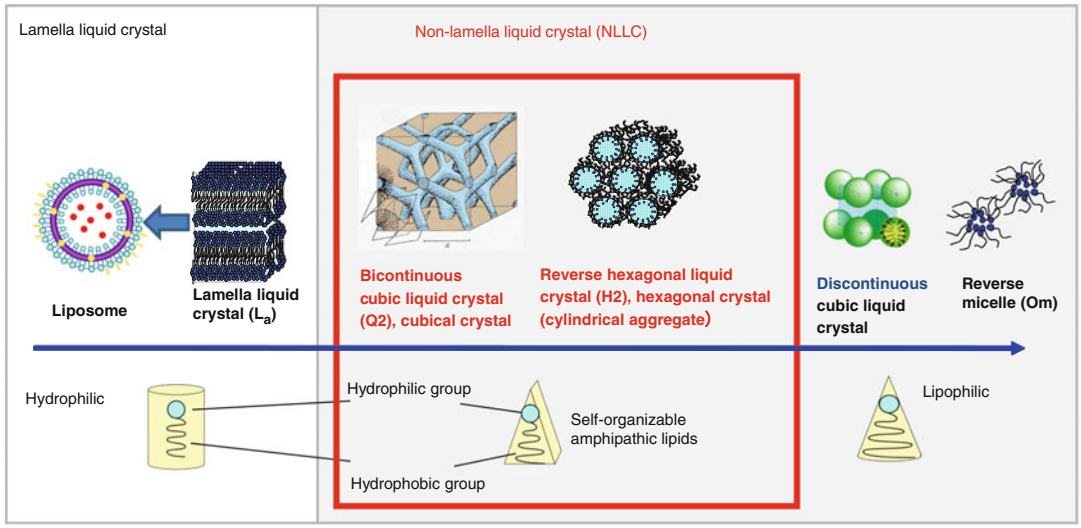


Fig. 17.1 Change of self-assembled structure of LC and non-lamella LC-forming lipids. The states of self-assembled structure depend on balance of polar/nonpolar groups (CPP value) and existence of salt or pH values in water phases. The *arrow* shows transitions of liquid crystals regarding lipophilicity of solvent

Table 17.1 Composition of liquid crystal dispersions

Ingredients	30 mM calcein-entrapped LC-A (%)	Blank (%)
1-o-(5,9,13,17-tetramethyloctadecanoyl) erythritol (crude, 1)	10.0	10.0
Sodium calcein	2.0	–
Pluronic® F127 (10 %)	10.0	10.0
Methyl *p*-hydroxybenzoate	0.1	0.1
Purified water	77.9	79.9
Total	100	100.0
Ingredients	30 mM calcein-entrapped LC-B (%)	Blank (%)
1-o-(5,9,13,17-tetramethyloctadecanoyl) erythritol (pure, 2)	10.0	10.0
Calcein (sodium)	0.2	–
Pluronic® F127 (20 %)	10.0	10.0
Methyl *p*-hydroxybenzoate	0.1	0.1
Purified water	79.7	79.9
Total	100	100.0

monoesters (36 %), diesters (12 %), and triesters (52 %) of erythritol and phytanylacetic acid (the ratio was determined by gas chromatography analysis). Mixture 1 was purified by column chromatography using silica gel (Wakogel C-300, Wako Pure Chemicals Industries, Ltd., Osaka, Japan) to afford monoesters 2 of 1-O- and 2-O-phytanylacetyl-erythritol.

17.6 Preparation of Liquid Crystal Dispersion

Table 17.1 shows the composition of liquid crystal A (LC-A) and liquid crystal B (LC-B) nanodispersions. Ten grams of crude ester 1 or pure ester 2 was used to prepare LC-A and LC-B, respectively. In this step, LC-A and LC-B were

semisolid and were nano-dispersed using a high-pressure emulsifier (NM2-L200AR, Yoshida Kikai Co., Ltd, Nagoya, Japan) or an ultrasonic homogenizer (USP-50, Shimadzu Corp., Kyoto, Japan), respectively, in aqueous solution (90.0 g) containing sodium calcein, Pluronic® F127 and methyl p-hydroxybenzoate. Calcein concentrations in LC-A and LC-B were different (see Table 17.1).

17.7 Structure of Liquid Crystal Dispersions Observed by Cryo-TEM Microscope

Skin permeation enhancement effects of non-lamella LCs, which consist of crude or pure non-lamella LC-forming lipids, such as 1-O-(5,9,13,17-tetramethyloctadecanoyl) erythritol (Fig. 17.2), are discussed in this chapter. The crude and pure esters were used to prepare non-lamella LC-A and non-lamella LC-B, respectively. Non-lamella LC-A- and non-lamella LC-B-forming lipids were dispersed in aqueous solution containing sodium calcein to prepare non-lamella LCs. Sodium calcein (*M.W.*; 623) is a good indicator used as hydrophilic fluorescent marker for skin permeation experiment. Thus, penetration-enhancing ability of non-lamella LC was investigated by evaluating the permeation of calcein through skin.

Figure 17.3 shows cryo-transmission electron microscope (TEM) micrographs of non-lamella LC-A and non-lamella LC-B. Similar to the cryo-TEM micrographs of cubic non-lamella LC prepared by monoolein and oleic acid (Garg et al. 2007; Gustafsson et al. 1996), non-lamella LC structures were observed in non-lamella LC-A and non-lamella LC-B (in white circles in

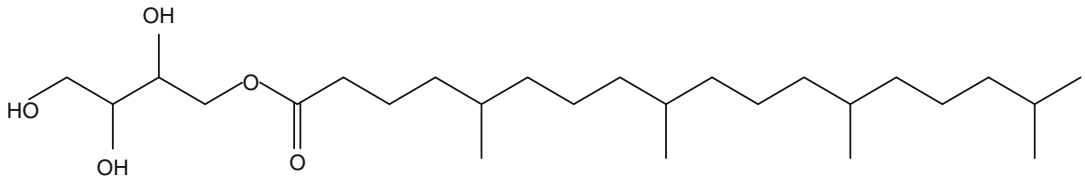


Fig. 17.2 Chemical structure of 1-o-(5, 9, 13, 17-tetramethyloctadecanoyl) erythritol

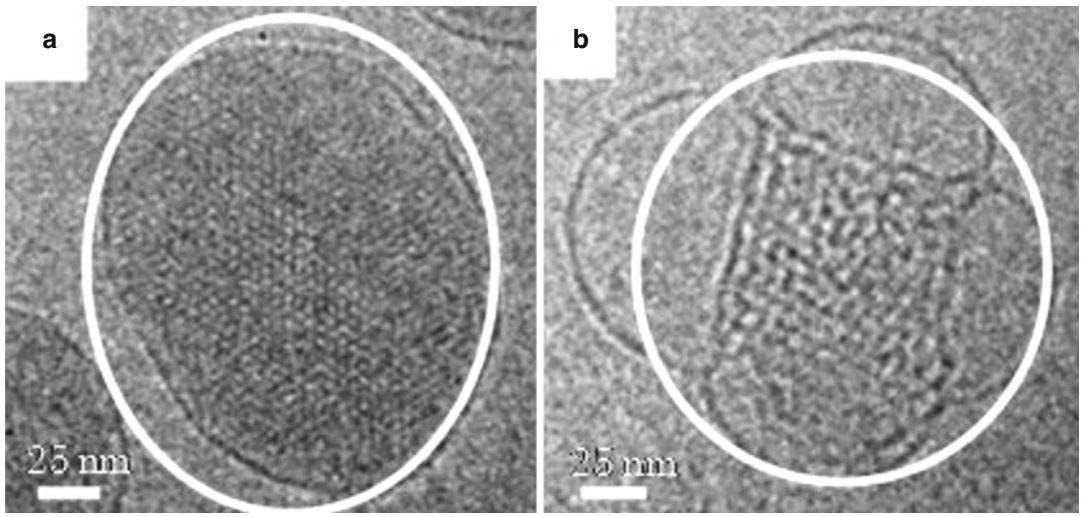


Fig. 17.3 Cryo-TEM microscope images of liquid crystal dispersions. Images (**a**, **b**) are non-lamella LC-A and non-lamella LC-B, respectively. Non-lamella LC structures were found in both (**a**, **b**) (see *circles* in images). Each *white bar* indicates 25 nm length

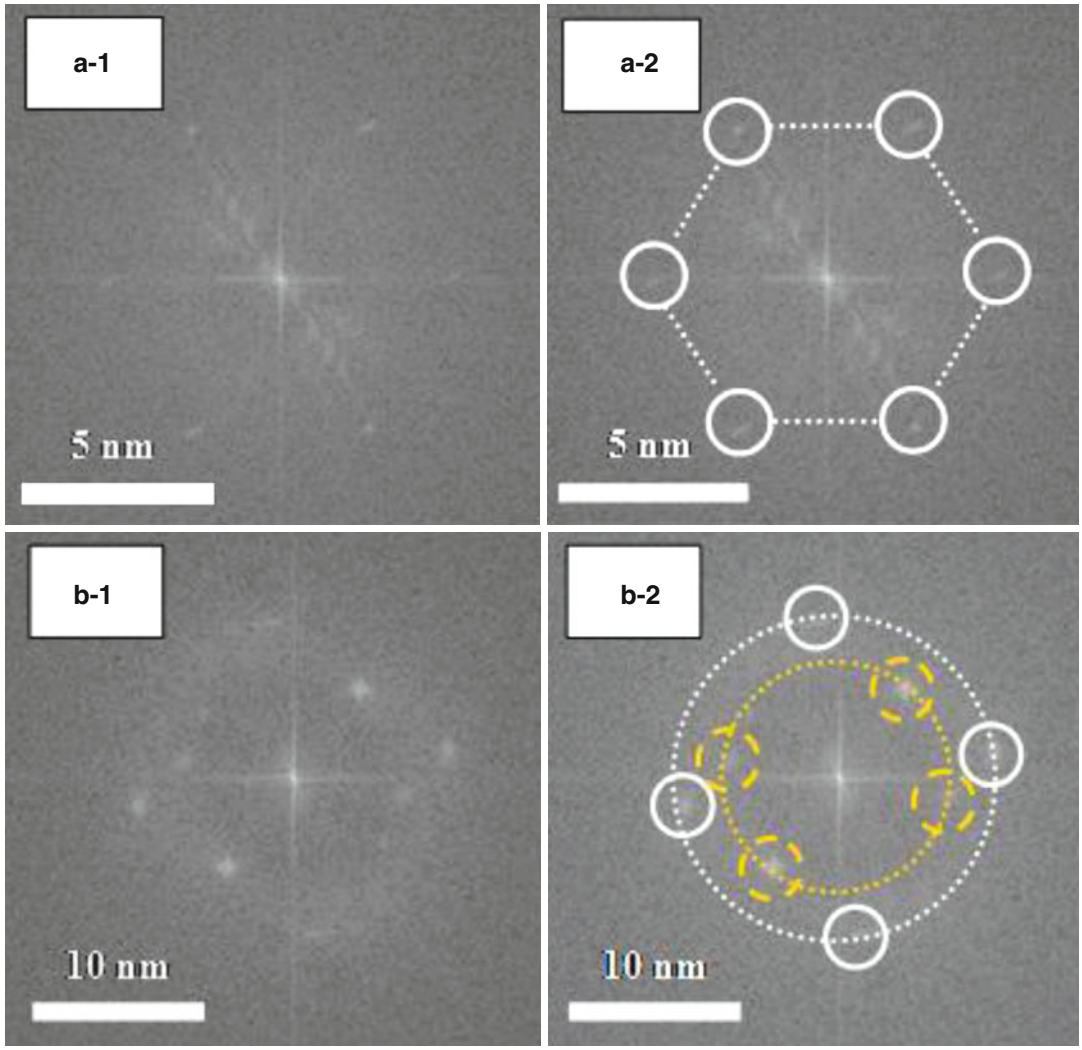


Fig. 17.4 Electron diffraction pattern of liquid crystal dispersions. (**a**-1) and (**a**-2) and (**b**-1) and (**b**-2) show the same images of LC-A and LC-B, respectively, without and with auxiliary lines and marks. (**a**-2) shows hexagonal LC, having 4.6 nm periodic structure. (**b**-2) illustrates cubic LC, having two periodic structures of 6.0 and 9.0 nm cycles. *White bars* indicate 5 or 10 nm length

Fig. 17.3). Figure 17.4 shows electronic diffraction patterns of non-lamella LC determined by cryo-TEM photographs. It was found from these diffraction patterns that non-lamella LC-A was a hexagonal non-lamella LC, having 4.6 nm periodic structure (A-2 dashed lines in Fig. 17.4), and non-lamella LC-B was a cubic non-lamella LC, having two periodic structures of 6.0 and 9.0 nm (B-2 dashed lines in Fig. 17.4).

17.8 Skin Penetration-Enhancing Effect of Liquid Crystals

The penetration-enhancing effectiveness of non-lamella LC in topical formulations was evaluated by measuring permeation of a mal-absorbable compound, calcein, through excised hairless rat skin and through the three-dimensional cultured human skin model (LSE-high).

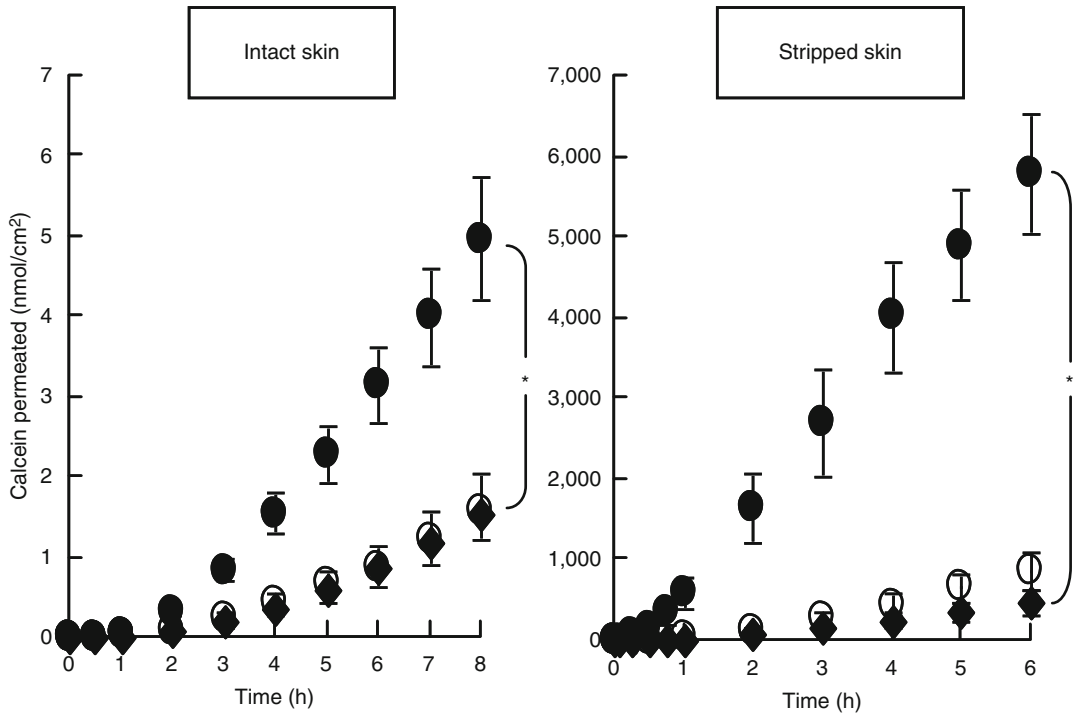


Fig. 17.5 Effect of non-lamella LC-A formulation on the time course of the cumulative amount of calcein that permeated intact and stripped hairless rat skin. Symbols are as follows: ◆ free calcein, ○ free calcein plus non-lamella LC-A, ● calcein entrapped in non-lamella LC-A. *Asterisks* mean significant difference between calcein entrapped in non-lamella LC-A and free calcein or free calcein plus non-lamella LC-A (p <0.05). Each point represents the mean ± S.E. of at least three to seven experiments

Figure 17.5 shows the time course of calcein permeation through excised hairless rat skin from the reverse-hexagonal liquid crystal (non-lamella LC-A) formulations. In both intact and stripped skin, the skin permeations of calcein from calcein entrapped in non-lamella LC-A formulation provided a 3 and 10 times higher, respectively, than the permeation of calcein from free calcein solution. In addition, the mixture of blank non-lamella LC-A dispersion and free calcein showed similar skin permeation to that of free calcein solution. Thus, the blank non-lamella LC formulation itself did not show any penetration-enhancing effect. Permeation parameters were determined from the time course of the cumulative amount of the permeated drug. The calculated parameters are shown in Table 17.2. Partition coefficient, K, of calcein was markedly increased by application of the calcein entrapped in non-lamella LC-A formulation, suggesting that non-lamella LC-A could improve the calcein distribution into the skin by providing a high affinity to intercellular lipid structure in the skin (stratum corneum). Next, the time course of the skin permeation of calcein from the cubic liquid crystal dispersion (non-lamella LC-B) was evaluated; the results are shown in Fig. 17.6. Non-lamella LC-B as well as non-lamella LC-A enhanced the permeation of calcein through intact hairless rat skin; Table 17.3 summarizes the permeation parameters for non-lamella LC-B. Increased partition of calcein was observed by non-lamella LC-B, as by non-lamella LC-A. In contrast, no increase in drug partition was observed in stripped skin from non-lamella LC-B or from non-lamella LC-A. Non-lamella LC-A has a high affinity for the stratum corneum as well as the viable epidermis, whereas non-lamella LC-B shows high affinity for the stratum corneum, but not for the viable epidermis.

Table 17.2 Partition coefficient (K), diffusion coefficient (D), and permeability coefficient (P) of calcein through hairless rat skin after application of different formulations of non-lamella LC-A

	30 mM calcein	Calcein entrapped in non-lamella LC-A	Free calcein and non-lamella LC-A
Intact skin			
K	0.10 ± 0.02	0.22 ± 0.04	0.09 ± 0.03
D ($\times 10^{-11}$ cm^2/s)	6.05 ± 0.52	7.54 ± 0.18	6.75 ± 0.68
P ($\times 10^{-9}$ cm/s)	2.91 ± 0.55	8.32 ± 1.33	2.80 ± 0.91
Stripped skin			
K	0.52 ± 0.13	2.51 ± 0.87	1.19 ± 0.20
D ($\times 10^{-7}$ cm^2/s)	1.04 ± 0.07	3.22 ± 1.14	0.89 ± 0.07
P ($\times 10^{-7}$ cm/s)	9.41 ± 2.86	110 ± 12.5	18.9 ± 4.51

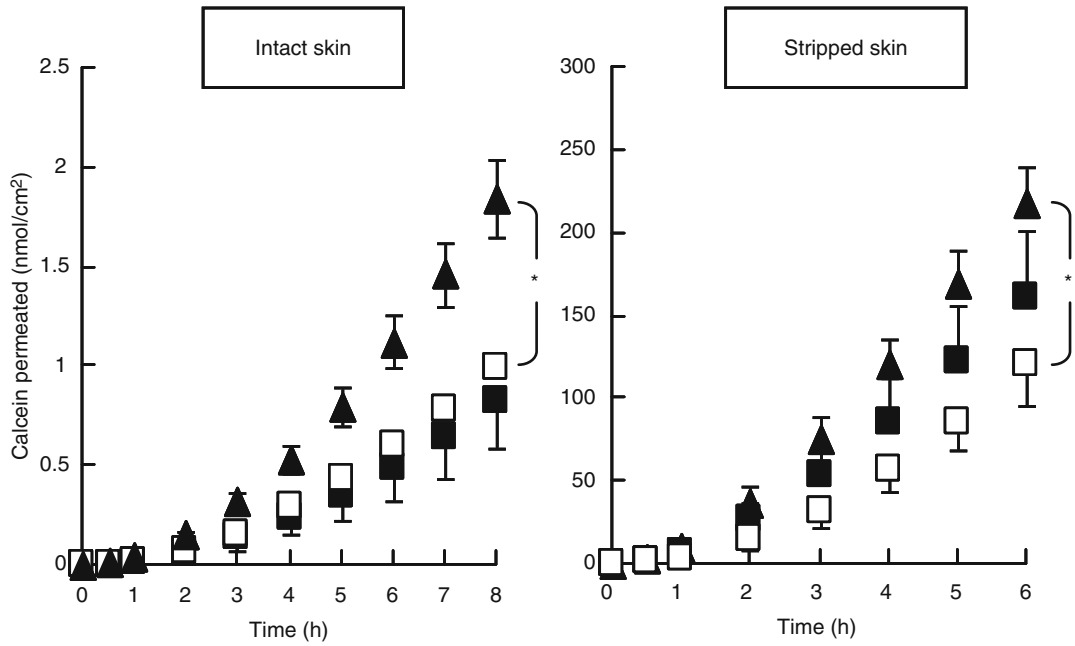


Fig. 17.6 Effect of LC-B formulation on the time course of the cumulative amount of calcein that permeated through intact and stripped hairless rat skin. Symbols are as follows: ■ free calcein, □ free calcein plus non-lamella LC-B, ▲ calcein entrapped in non-lamella LC-B. Asterisks mean significant difference between calcein entrapped in LC-A and free calcein or free calcein plus non-lamella LC-B (p <0.05). Each point represents the mean ± S.E. of at least 3–12 experiments

Table 17.3 Partition coefficient (K), diffusion coefficient (D), and permeability coefficient (P) of calcein through hairless rat skin after application of different formulations of non-lamella LC-B

	3 mM calcein	3 mM calcein in 2 % Pluronic sol.	Free calcein and non-lamella LC-B
Intact skin			
K	0.53 ± 0.12	0.59 ± 0.11	0.95 ± 0.10
D ($\times 10^{-11}$ cm^2/s)	5.95 ± 0.78	6.47 ± 0.74	6.94 ± 0. 39
P ($\times 10^{-8}$ cm/s)	1.56 ± 0.38	1.84 ± 0.13	3.23 ± 0.32
Stripped skin			
K	2.18 ± 0.08	2.44 ± 0.39	2.53 ± 0.26
D ($\times 10^{-8}$ cm^2/s)	9.04 ± 1.33	6.92 ± 2.83	10.7 ± 1.45
P ($\times 10^{-6}$ cm/s)	3.46 ± 0.65	2.95 ± 0.55	4.48 ± 0.32

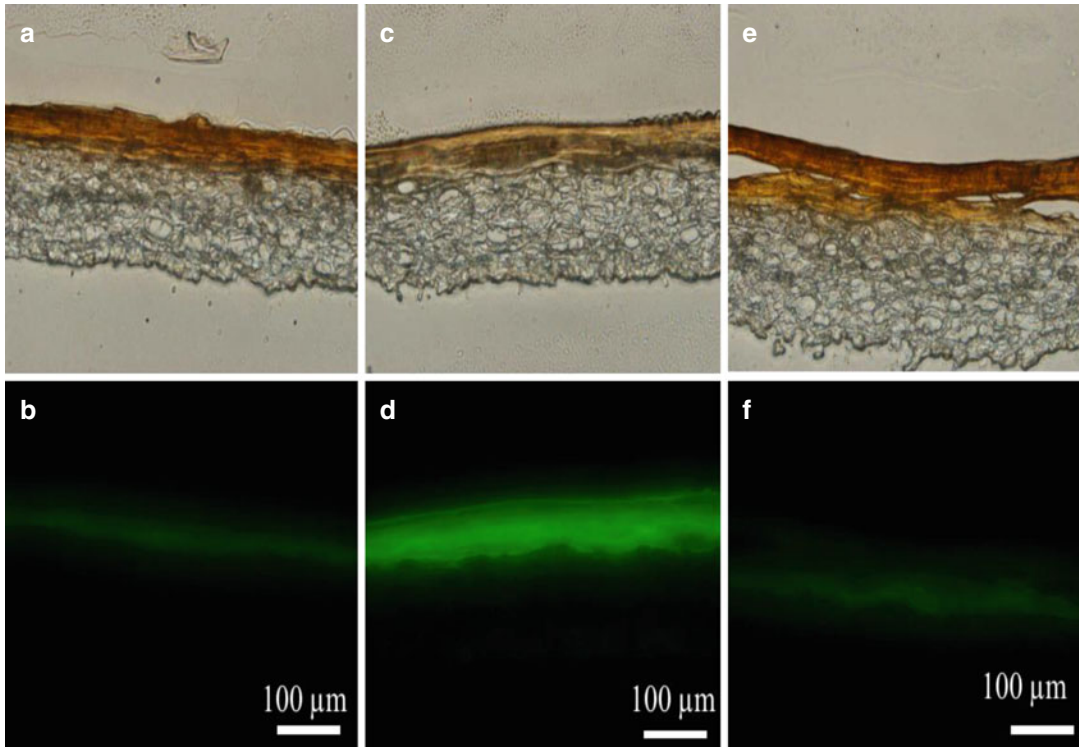


Fig. 17.7 Histological observation of LSE-high, 8 h after application of non-lamella LC-A dispersion with entrapped calcein. Images (**a**, **b**) are for free calcein, (**c**, **d**) for calcein entrapped in non-lamella LC-A dispersion, and (**e**, **f**) for physical mixture of free calcein and non-lamella LC-A dispersion. Images (**a**, **c**, **e**) show light micrograph images and (**b**, **d**, **f**) show corresponding fluorescent images. Bars indicate 100 mm (*vertical slice*)

17.9 Drug Distribution in Skin After Topically Applied Non-lamella LCs

Similar permeation experiments were carried out using a three-dimensional cultured human skin model (LSE-high). Since no appendages, such as hair follicles and sweat ducts, are present in LSE-high, the penetration-enhancing effect by non-lamella LC formulations must be due to the enhanced drug penetration through the stratum corneum barrier, i.e., through the intercellular route. Figures 17.7a–f show the cross section of LSE-high, 8 h after application of free calcein and calcein entrapped in non-lamella LC-A and simultaneous application of free calcein and non-lamella LC-A, respectively. Figures 17.7a, c, and e show hematoxylin and eosin (HE)-stained micrographs and Fig. 17.7b, d, and f show corresponding fluorescent micrographs. Similar fluorescent level was observed for groups of free calcein (Fig. 17.7b) and free calcein plus non-lamella LC-A dispersion (Fig. 17.7f). In contrast, a much higher fluorescent level was found for the calcein entrapped in non-lamella LC-A (Fig. 17.7d). These results were also obtained by the skin permeation study using non-lamella LC-A dispersion with entrapped or free calcein and the calcein solution (Fig. 17.5).

Conclusion

It was found from cryo-TEM observation and electron diffraction pattern analysis that these LC-A and LC-B nano-dispersions have reverse-hexagonal LC and cubic LC dispersions, respectively. These LC dispersions were very stable, since no aggregation (diameter growth) was observed. The entrapping ratio of a hydrophilic model compound, calcein, in LC-B was higher than in LC-A, suggesting that cubic LC has great entrapping potency for hydrophilic materials. Other drugs with different lipophilicity and molecular weights must

be determined in the future. The present skin permeation experiments showed that LC-A and LC-B nano-dispersions increased the skin permeation of the entrapped drug, probably due to increased partition of the LC in the skin barrier. Thus, the presently prepared LC formulations can be utilized as new topical formulations for therapeutic drugs and cosmetic ingredients, especially for hydrophilic compounds. The skin content of the entrapped drugs and detail mechanistic analysis must be determined in the near future.

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Nanoemulsions in Dermal Drug Delivery

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Contents

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18.1 Introduction

18.1.1 Definition and Characterisation of Nanoemulsions

Classical emulsions are dispersions of a liquid phase in another immiscible liquid phase. They have been employed for the dermal application of active substances for a long time since they can easily be produced through mild energy input and can be kinetically stabilised with the help of suitable surfactants.

During the last decades, technological and methodological progress has led to the development of oil-in-water emulsions with increasingly small droplet sizes. The first systems known as *submicron emulsions* emerged during the 1950s and were originally employed for parenteral nutrition (Wabel 1998; Calder et al. 2010). They were composed of natural oils such as soybean oil dispersed within an aqueous phase with the help of natural lecithin mixtures. The average droplet sizes of these systems produced through high-energy emulsification usually ranged between 100 and 500 nm. During the next decades, different high-energy production methods were established, including homogenisation with high-pressure homogenisers or microfluidisers as well as ultrasonication. Depending on the production technique and the employed compounds, droplet

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sizes around 100 nm could be reached, and the prevailing terminology changed from *submicron emulsions* to *nanoemulsions*.

Nanoemulsions of the oil-in-water type have been adapted for various routes of drug delivery, including sensitive routes such as intravenous, ocular or dermal application (Benita and Levy 1993; Yang and Benita 2000; Klang and Valenta 2011) while water-in-oil nanoemulsions have been investigated for technical applications as well as for theoretical surfactant studies (Chiesa et al. 2008; Porras et al. 2004). In addition to classical lecithin-based nanoemulsions produced by high-energy emulsification, new low-energy production methods have been developed, such as the phase inversion temperature method or the solvent evaporation method (Fernandez et al. 2004; Tadros et al. 2004; Sole et al. 2010). These methods require the use of specific surfactants or additional solvents to produce nano-sized oil droplets from a microemulsion matrix due to changes in the spontaneous surfactant curvature. Conveniently, these methods can be conducted rapidly without the need for specific equipment but may involve strong heating of the pre-emulsion system. Discussions about the exact nature of the produced systems are still ongoing. Important parameters such as mean droplet size and droplet size distribution again depend strongly on the employed compounds and exact processing conditions. Progress in the field of nanoemulsion production is constantly being reported for both high- and low-energy emulsification methods (Cortés-Muñoz et al. 2009; Anton et al. 2007).

In summary, the term *nanoemulsion* is employed to describe conventional emulsions with droplet sizes in the lower submicron range. It should be noted that droplet sizes of classical nanoemulsions, i.e. nanoemulsions stabilised by lecithin-type surfactants that are produced through high-energy emulsification, rarely reach mean droplet sizes below 100 nm. The general recommendation of accepted guidelines today is that the prefix *nano* is to be employed for systems with size ranges between 1 and 100 nm (Mason et al. 2006). Accordingly, the term *nanoemulsion* would only be appropriate for submicron emulsions with droplet sizes below 100 nm. However, the term *nanoemulsion* is widely – albeit inaccurately – employed as a synonym for the term *submicron emulsion* today. We would like to point out this slight discrepancy from nanoscale conventions: nanoemulsions do not necessarily exhibit droplet sizes below 100 nm.

To further complicate the terminology issue, increasing numbers of publications about 'nanoemulsions' are in fact dealing with microemulsions. As well known, microemulsions are thermodynamically stable isotropic systems that contain large amounts of surfactants and solvents and form spontaneously. Microemulsions may exhibit complex internal structures including bicontinuous sponge phases or droplet-shaped phases. Nevertheless, they bear no similarities to real emulsions such as nanoemulsions; the well-established term *microemulsion* is of historical nature. Several groups have taken up the task of clarifying these terminology issues that render literature research increasingly difficult (Klang and Valenta 2011; Mason et al. 2006; Anton and Vandamme 2011; McClements 2012).

To avoid confusion, we adhere to the nowadays established term *nanoemulsion* for emulsions with droplets in the lower submicron range. The majority of publications are dealing with oil-in-water nanoemulsions since they are more relevant for most applications than water-in-oil systems. Recent developments include adaptations such as multiple nanoemulsions for the delivery of drugs of different logP values (Anton et al. 2010; Schwarz et al. 2012).

With decreasing droplet size, the general properties of nanoemulsions start to differ significantly from those of conventional emulsions. Although nanoemulsions exhibit basic emulsion properties such as an inherent metastability, they differ from macroscale emulsions in regard to optical appearance, physicochemical properties and prevailing destabilisation processes (Klang and Valenta 2011; Mason et al. 2006). Nanoemulsions may exhibit a transparent to translucent appearance if the droplet size is small enough while emulsions are generally creamy white due to multiple scattering of light. In addition, nanoemulsions are of a fluid nature and exhibit near-Newtonian flow behaviour, while

classical emulsions are usually of higher viscosity and shear-thinning flow behaviour. Most importantly, nanoemulsions may exhibit significantly improved physical stability during storage when compared to conventional emulsions. Classical emulsions are sooner or later destabilised by gravity-induced alterations, coalescence of oil droplets and eventual phase separation. Nanoemulsions are hardly affected by coalescence due to their small droplet size. If the dispersed droplets have a relatively high solubility within the continuous phase, Ostwald ripening may occur: individual molecules of the dispersed phase diffuse from smaller to larger droplets due to differences in Laplace pressure, thus leading to a continuous increase in droplet size. By considerate choice of excipients, however, this destabilisation mechanism occurring in nanoemulsions may be largely eliminated (Wabel 1998; Mason et al. 2006). Enhanced electrochemical stabilisation through modification of the droplet surface charge and sterical stabilisation with spacious surface-active agents are successful strategies to further optimise nanoemulsion stability (van Nieuwenhuyzen and Szuhaj 1998).

Characterisation of the above-mentioned properties of nanoemulsions can usually be performed by common analytical techniques for aqueous colloidal systems. Dynamic light scattering as a population-based technique of droplet size characterisation is frequently employed, ideally in combination with static laser diffraction to exclude the presence of individual large droplets. In addition, microscopic techniques such as cryo-transmission electron microscopy are recommended to obtain information about the exact morphology of the system including droplet shape and the presence of vesicular structures (Klang et al. 2012a). Examples of different techniques of analysis including electron microscopy and atomic force microscopy are presented in Fig. 18.1.

Apart from analysing droplet size distributions, the droplet surface charge as an indicator for electrochemical stabilisation can be determined by

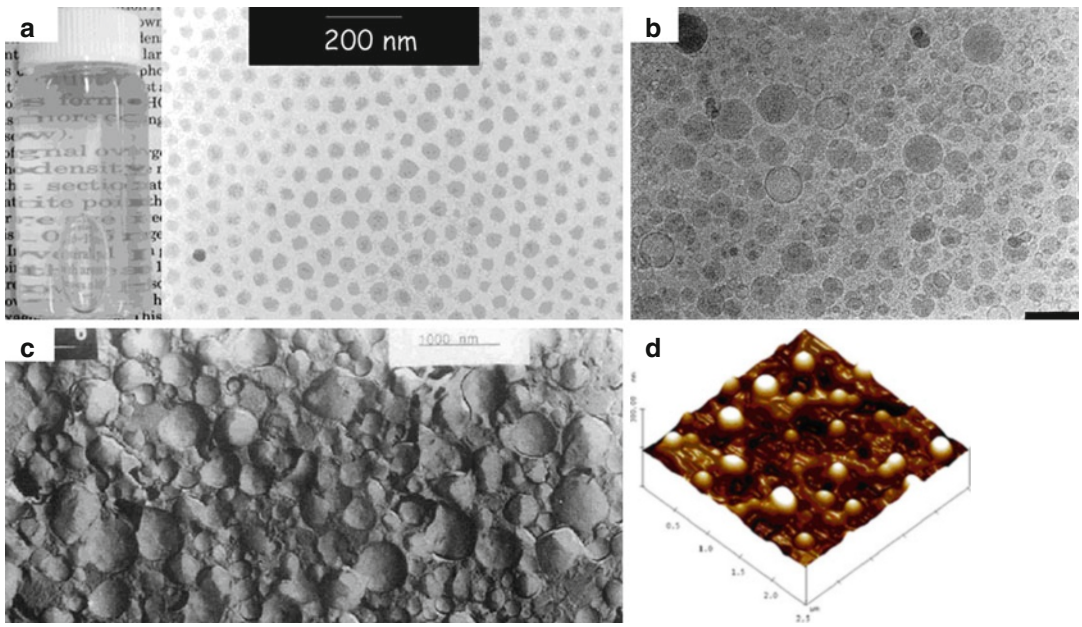

Fig. 18.1 (**a**) Optical appearance and cryo-TEM image of a translucent nanoemulsion (Reprinted from Sonneville-Aubrun et al. (2004), p. 146, with permission of Elsevier). (**b**) Cryo-TEM image of nano-sized oil droplets (*homogeneously filled circles*) and vesicles (*unfilled circles*); the scale bar represents 200 nm (Reprinted from Norden et al. (2001), p. 400, with permission of Elsevier). (**c**) Freeze-fracture TEM micrograph of an amphotericin B nanoemulsion (Reprinted from Benita and Levy (1993), p. 1078, with permission of Wiley). (**d**) Nanoemulsion morphology visualised by atomic force microscopy (Reprinted from Marxer et al. (2011), p. 433, with permission of Elsevier)

laser Doppler electrophoresis (Mueller 1996). Stability investigations are usually performed by monitoring parameters such as the mean droplet size, the polydispersity index, droplet surface charge and pH value of the nanoemulsions in question. In our experience, the physical stability of classical nanoemulsions produced by high-pressure homogenisation can be expected to be around 2 years or longer at slightly refrigerated storage. However, the chemical stability of individual compounds has to be taken into account as well. Oil, surfactants and incorporated actives may be subjected to chemical degradation by oxidation or hydrolysis, potentially leading to an unpleasant optical and olfactory appearance despite unchanged droplet size (Wabel 1998; Baker and Naguib 2005). Optical monitoring of the formulations, assessment of drug stability and monitoring of both zeta potential and pH are useful tools in detecting such changes (Klang et al. 2011a).

18.1.2 Dermal Application: Advantages and Limitations

Most marketed nanoemulsion products can be found in the realms of intravenous nutrition and drug delivery as well as in dermal drug delivery and cosmetics. In terms of dermal drug delivery, nanoemulsions offer distinct advantages: high physical stability compared to conventional emulsions, high skin friendliness due to the low amount and the mild nature of the employed surfactants and last but not least the ease of preparation and scale-up. They avoid the limitations of other colloidal drug delivery systems, such as the limited drug loading and stability issues of liposomes and the potentially irritating compounds required for the production of nanoparticles or microemulsions. Depending on composition and the nature of the employed drug, nanoemulsions may achieve higher rates of skin penetration and drug accumulation within the skin than lipid nanoparticles (Calderilla-Fajardo et al. 2006). In a recent study investigating the dermal delivery of lutein, a more rapid release in case of nanoemulsions was found, which achieved higher skin permeation rates than nano-structured lipid carriers and solid lipid nanoparticles (Mitri et al. 2011).

Classical nanoemulsions prepared by high-pressure homogenisation are usually stabilised by skin-friendly surfactants such as lecithins and do not require synthetic surfactants. Thus, skin lipids are not washed out during cleaning. Lipophilic drugs can be incorporated into the oil phase according to their solubility. The release of the drugs from the system is generally acknowledged to be quite rapid; however, attempts towards retarded or controlled release as well as site-specific targeting have been reported as well (Yang and Benita 2000; Eskandar et al. 2009). Nanoemulsions support the penetration of incorporated actives into the skin and may thus promote their accumulation in the skin. In addition, the cosmetic effect of the basic vehicles is of further interest. In recent approaches, the possibility to incorporate hydrophilic drugs into nanoemulsion systems is being investigated with the aim of promoting the incorporation efficiency, stability and penetration of water-soluble actives (Anton et al. 2010; Schwarz et al. 2012).

Retarded drug delivery from nanoemulsions is difficult to achieve because of the low viscosity of the systems. When compared to aqueous solutions or dispersions, retarded drug release from nanoemulsions may of course be observed. In general, a retarded release may be achieved for very lipophilic drugs with high affinity to the oil phase.

From another viewpoint, the low viscosity of nanoemulsions is an advantageous feature: it renders them attractive systems for development of aerosol sprays such as sunscreens that show no phase separation during storage. If a higher viscosity is required, modification of the nanoemulsion is possible by incorporation of gelling agents. Successful reports on the development of thickened nanoemulsions can be found in the literature (Alves et al. 2005; Mou et al. 2008).

Regarding transdermal penetration, there is a certain amount of recent literature proposing nanoemulsions for this task. However, a closer look at these articles reveals that the systems in question are exclusively dealing with thermodynamically stable microemulsion phases. Nanoemulsions in the sense of skin-friendly submicron-sized emulsion systems are not specifically designed for the purpose of delivering

drugs transdermally. To allow for the passage of intact nanovectors across pores of the skin, much smaller and deformable carriers are required since the skin is a strong and complex barrier (Cevc and Vierl 2010). Small drug amounts may of course be found in deeper skin layers after application of a nanoemulsion system due to mechanisms such as penetration across hair follicles (Lademann et al. 2001) or a general penetration enhancement caused by the involved excipients, in particular the involved lecithin phospholipids (Yu et al. 2009). In general, however, nanoemulsions are not employed for transdermal drug delivery, but rather for targeting the outermost skin layers, for instance, in case of fungal infections or inflammation. The versatile nature of nanoemulsions is highlighted by another recent study by Spagnul et al., who designed a calixarene nanoemulsion aimed at chelating uranium molecules at the skin surface (Spagnul et al. 2011). The patented formulation is a promising approach towards treating cutaneous uranium contamination if the formulation is applied quickly. This study shows that nanoemulsions exhibit advantageous properties for different applications on skin and can be tailor-made according to the envisioned task.

In another recent study, a lecithin-based nanoemulsion containing 5-aminolevulinic acid (5-ALA) was developed for dermal application in photodynamic therapy (Maisch et al. 2010). Significantly deeper skin penetration was found for the nanoemulsion when compared to a conventional creamy emulsion (Fig. 18.2). As intended in localised therapy, no penetration beyond the basal cell membrane was observed. The increased drug transport into the epidermis may be ascribed to a stabilising effect of the nanoemulsion on 5-ALA, thus preventing dimerisation. Furthermore, the nanoemulsion formulation seemed to support the cellular uptake of 5-ALA. Recently, the pivotal phase III studies were completed for the developed system. The results support the claims of improved stability and skin penetration when tested against a conventional cream and placebo (Dirschka et al. 2012). The corresponding product has successfully been marketed (Ameluz®, Biofrontera Pharma GmbH).

Regarding the mechanism of nanoemulsion penetration, it can be summarised that no evidence exists that conventional colloidal systems such as nanoemulsions may penetrate into the skin as intact structures. It may thus be assumed that their skin penetration is related to penetration of the system into shunt pathways such as hair follicles, accumulation of the system between corneocyte clusters or in furrows to interact with skin lipids or merging of the system into extended lipidic structures on the skin surface. In this context, penetration enhancement of drugs may be caused by the occlusion of the skin surface (Eskandar et al. 2009; Zhou et al. 2010) as well as the interaction of nanoemulsion excipients such as phospholipids with skin compounds. The systems may alter the thermodynamic activity of incorporated drugs on the skin surface as well (Cevc and Vierl 2010). According to their composition, nanoemulsions may thus be employed to enhance the penetration of incorporated actives into the skin. Different aspects may influence the success of this strategy, such as the exact formulation morphology, the droplet surface charge and the nature of the involved compounds. The role of these factors will be discussed in the following sections.

18.2 Penetration Enhancement Strategies

18.2.1 Role of Droplet Size

The large surface area and low surface tension of small nanoemulsion oil droplets has been described to improve skin interaction and thus dermal drug delivery (Benita 1999; Klang et al. 1998). An enhanced local therapeutic effect of incorporated drugs due to prolonged residence time in the uppermost skin layers may thus be expected. One of the earliest studies dealing with the effect of nanoscale droplet size in topical emulsions was conducted by Friedman et al. (1995). Both steroidal and nonsteroidal antiinflammatory drugs including betamethasone valerate and dipropionate, naproxen, diclofenac, indomethacin and piroxicam were incorporated

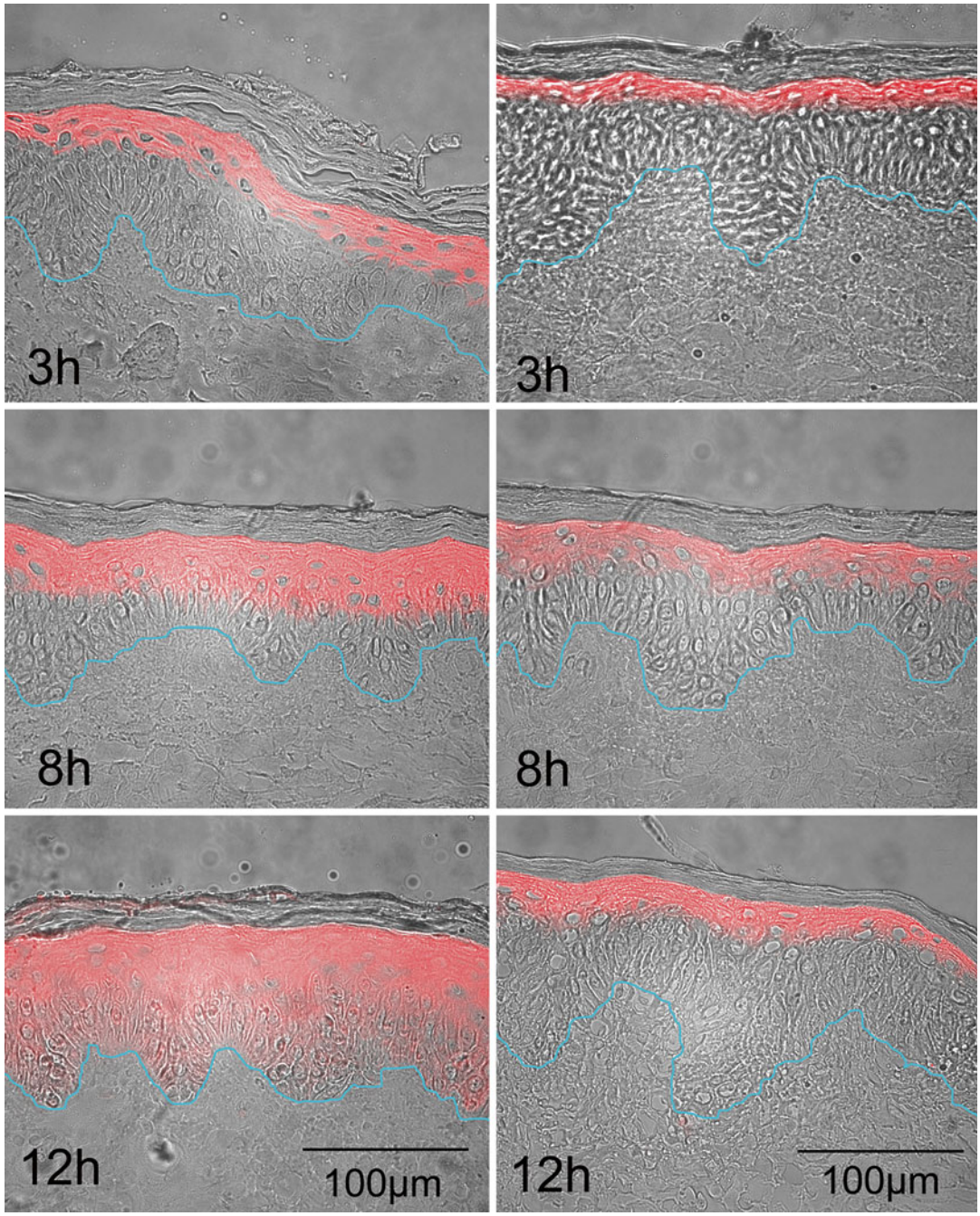

Fig. 18.2 Distribution of protoporphyrin IX after application of a nanoemulsion-based formulation containing 10 % 5-ALA hydrochloride (*left column*) or a commercial cream containing 16 % w/w aminolevulinate methyl ester hydrochloride (*right column*). Scale bars represent 100 µm. The images were taken after incubation of the skin with the respective formulation. The blue line represents the basal membrane, i.e. the border between epidermis and dermis. Samples were removed after 3, 8 and 12 h, and the induction of protoporphyrin IX was determined (*coloured areas*) (Image reprinted from Maisch et al. (2010), p. e304, with permission of Wiley)

into nanoemulsions and regular creamy emulsions of identical composition. The antiinflammatory efficacy of the systems was compared against each other and to established marketed formulations by using the carrageenan-induced paw edema rat model. Significantly improved antiinflammatory efficacy was found for the nanoemulsion systems, especially in case of indomethacin, diclofenac and betamethasone esters. Noticeable systemic activity of antiinflammatory drugs formulated in nanoemulsions was observed as well. Preliminary in vivo studies on human volunteers showed good acceptability and comparable properties to marketed products. The authors hypothesised that lipid disruption within the stratum corneum caused by the nanoemulsion phospholipids, the formation of gaps and the increased swelling of the skin may favour the penetration of lipid droplets with diameters below 100 nm. Under these circumstances, shunt pathways such as hair follicles or sebaceous channels may lead to enhanced penetration as well. It should be kept in mind, however, that these conditions may be particularly encountered in the employed edema model. In a more recent study, Kotyla and co-workers compared the bioavailability of tocopherol from a nanoemulsion and a micrometre-sized emulsion of the same composition. After dermal application of the nanoemulsion in vivo on golden hamster skin, a 2.5-fold increase in plasma tocopherol levels was determined (Kotyla et al. 2008).

These studies held aside, literature about the role of droplet size on the skin penetration of otherwise identical emulsions is scarce. However, by experimenting with certain sucrose ester surfactants with peculiar gelling behaviour, we were able to create both fluid nanoemulsions and semisolid emulsions of identical composition. When evaluating the skin penetration potential of these systems in vitro (Klang et al. 2011b) and in vivo (Klang et al. 2012b), we found a highly similar penetration behaviour irrespective of the respective droplet size distribution. This may however be related to the specific nature of the developed emulsions, in particular the hydrophilic gel network of the semisolid emulsions. For aqueous dispersions based on the same network, the microviscosity and thus the drug transport were found to be comparable to that of corresponding fluid nano-sized systems (Ullrich et al. 2008). For the developed sucrose stearate emulsions, droplet sizes around 120–150 nm did not significantly affect the skin penetration of incorporated drugs when compared to corresponding emulsions with droplet sizes in the micrometre range. Further comparative studies in this direction using nanoemulsions with droplet sizes below 100 nm would be of interest to gain further information on this matter.

In another recent study, lecithin nanoemulsions for topical delivery were developed using snake oil, soybean lecithin, glycerol and purified water (Zhou et al. 2010). When applied in an O/W cream, the nano-sized droplets were shown to enhance skin hydration and skin penetration of the incorporated model dye nile red into the dermis when compared to a control O/W cream. The authors observed increased skin adhesion of the nanoemulsion formulation to the skin surface and subsequent formation of an occlusive film, thus increasing skin hydration and consequently skin penetration of the model dye. The increased skin penetration can therefore be ascribed to physical effects caused by the presence of small oil droplets. In addition, the authors assumed that an increased partitioning of lecithin molecules into the stratum corneum might be responsible for subsequent changes in the barrier properties, i.e. that the observed effects could be ascribed to the employed nanoemulsion compounds.

Another study reported increased in vitro skin permeation rates of camphor, menthol and methyl salicylate through rat skin when the substances were applied in form of a hydrogel-thickened nanoemulsion instead of a control gel (Mou et al. 2008). The authors speculated that different factors, e.g. high concentration gradients, formation of drug reservoirs or embedding of the small oil droplets within the stratum corneum lipids, might account for the observed enhancement effect.

In another recent study, penetration enhancement of glycyrrhetic acid incorporated into a nanoemulsion was observed in vitro when compared to a conventional oil-in-water emulsion of different composition (Puglia et al. 2010). In

addition, an increased antiinflammatory activity in vivo was observed for the nanoemulsion. However, the authors stated that the nature of the employed excipients for the different nanoemulsion and emulsion systems may have influenced the obtained results. This again confirms that the beneficial effects obtained with nanoemulsion depend not only on the droplet size but on the nature of the developed systems as well as the employed compounds.

18.2.2 Role of Droplet Surface Charge

The surface charge of nanoemulsion oil droplets in dispersion, often described by the zeta potential, is an important aspect that may strongly affect the interaction between formulation and skin and drug penetration. A positive droplet surface charge has been observed to enhance penetration of drugs into the nasal and ocular mucosae as well as the stratum corneum of the skin (Yang and Benita 2000; Mou et al. 2008). A high absolute surface charge of the nanoemulsion droplets is also a crucial prerequisite to ensure physical stability upon storage. From this viewpoint, a high positive surface charge may fulfil both the tasks of enhancing stability and promoting skin penetration.

A positive zeta potential can be induced by using cationic excipients such as lipids, polymers and surfactants, which can result in an improved interaction with negatively charged membranes such as the skin or corneal membrane. Negatively charged protein or fatty acid residues of the skin as well as the presence of selective active ion pumps render the skin surface selective to positively charged substances (Yang and Benita 2000; Piemi et al. 1999). It has been established by Benita and co-workers that positively charged nanoemulsions exhibited better wettability on the cornea compared to saline or negatively charged systems (Yang and Benita 2000). In dermal drug delivery, the same strategy was followed in several studies. Piemi et al. found that the in vitro penetration of econazole and miconazole nitrate into rat skin was higher from positively charged nanoemulsions containing stearylamine than from negatively charged formulations containing deoxycholic acid (Piemi et al. 1999). The binding of surfactants to the skin can be attributed to non-specific hydrophobic interactions with keratin as well as to specific electrostatic interactions in case of charged surfactants; the latter phenomenon is strongly dependent on the pH of formulation and skin. The obtained results suggest that the surface charge of nanoemulsion droplets may affect skin penetration of drugs due to increased interaction with the target site. Similar results were reported in subsequent studies for stearylamine (Fang et al. 2004) and phytosphingosine (Hoeller et al. 2009; Klang et al. 2010), although the observed enhancement effects were not as pronounced in case of the latter. Baspinar and co-workers likewise employed phytosphingosine to produce a positively charged nanoemulsion for dermal application of prednicarbate. Higher drug release was observed for negatively charged nanoemulsions in vitro when using diffusion cells and a synthetic model membrane. However, significantly higher amounts of the active ingredient were found to penetrate into the skin from the positively charged nanoemulsion when using excised human skin (Baspinar and Borchert 2012). These results suggest that a positive surface charge in nanoemulsions may indeed be of value for in vivo skin penetration.

18.2.3 Role of Compounds

18.2.3.1 Role of Surfactant Type

A wide range of permeation enhancers are known to promote drug penetration into the skin or mucous membranes. Classical nanoemulsion surfactants such as phospholipids and lysophospholipids act by interfering with the structure of skin lipids, thereby enhancing the transport of co-applied substances into the skin (Kirjavainen et al. 1999). Current research focuses on identifying further types of skin-friendly, biodegradable surfactants that may be employed for nanoemulsion production and exhibit similarly beneficial properties for dermal drug delivery. Among others, sucrose ester mixtures have been found interesting compounds for this task (Klang et al. 2011a;

Calderilla-Fajardo et al. 2006; Cazares-Delgadillo et al. 2005).

In first studies, synthetic surfactants such as polysorbate 80 were found to be more effective permeation enhancers in comparative in vitro experiments than a hydrophilic sucrose laurate ester of comparable HLB value (Hoeller et al. 2009). This was ascribed to the enhancement mechanism of the different surfactants: while sucrose laurate interferes with the lipid chains and increases their fluidity, polysorbates may increase skin penetration both by interacting with intercellular lipid domains and keratin filaments, thus disturbing both the lipid matrix and the protein domain of the corneocyte layers (Nokhodchi et al. 2003). Later studies showed that sucrose stearate mixtures of similar HLB value as lecithin mixtures were equally suitable in terms of drug delivery from nanoemulsions and even superior in terms of physical stability (Klang et al. 2011a).

As already discussed, surfactants can be used to modify the surface charge of the droplets. Cationic surfactants such as stearylamine, oleylamine, chitosan or cetyltrimethylammonium bromide can be employed for this task. Apart from the amphiphilic phospholipids, nonionic surfactants such as poloxamers or polysorbates are frequently employed (Yang and Benita 2000). Polysorbate-type surfactants possess voluminous groups that provide for increased steric stabilisation. Likewise, surface-active polymers such as the cellulose ether hydroxypropyl methylcellulose (HPMC) can be employed to stabilise nanoemulsions as additional or even main surfactants (Schulz and Daniels 2000).

Importantly, the nature of the interfacial film surrounding the oil droplets may affect drug release. Mixtures of surfactants may lead to the formation of more compact interfacial films, which consequently form a more efficient barrier against drug release (Ibrahim et al. 2009). Interfacial films composed of lecithin alone do not represent a strong interfacial transport barrier, thus usually showing burst release properties. However, by creating mixed interfacial films by including further surfactants or nanoparticles, controlled release properties can be achieved for topical application. Recently, enhanced dermal accumulation of all-trans-retinol could be achieved by addition of oleylamine and silica nanoparticles to a conventional nanoemulsion, thus producing more complex interfacial films (Eskandar et al. 2009).

18.2.3.2 Role of Additional Compounds

Natural, semi-synthetic or synthetic lipids, fatty acids and oils can be employed for the production of oil-in-water nanoemulsions. For dermal application, skin-friendly cosmetic oils such as soybean oil, jojoba oil, castor oil, PCL liquid or squalene are popular choices. The presence of free fatty acids in the oil phase is beneficial for nanoemulsion stability, as reported for castor oil (Jumaa and Mueller 1998). In addition, the polarity of the oil phase affects the system's stability against Ostwald ripening as well as drug release properties. Squalene not only acts as a ripening inhibitor due to its hydrophobic nature but also allows for the production of smaller droplet sizes in lecithin nanoemulsions than most other oils, which in turn may affect skin interaction and drug release (Fox 2009; Chung et al. 2001).

Variation of the amount of incorporated oil may affect physical system properties such as droplet size as well as drug release rates. Higher amounts of oil may lead to decreased drug release rates due to higher retention capacity (Hung et al. 2007). The microviscosity of the system may likewise be influenced by variations in the oil content. As a result, the wetting properties of nanoemulsions may be superior to those of conventional marketed products, thus exhibiting an increased therapeutic effect particularly in mucosal drug delivery (Ibrahim et al. 2009). In addition, viscosity-enhancing additives may increase the contact time between formulation and the skin. For this reason, nanoemulsions with or without addition of gelling agents may show improved penetration of incorporated drugs when compared to aqueous drug solutions or dispersions (Mou et al. 2008).

If required, penetration enhancers such as propylene glycol or oleic acid can be incorporated into nanoemulsions to further enhance skin penetration (Fang et al. 2004). Addition of

cosurfactants such as polysorbates, sucrose esters or alkyl polyglucosides can serve the same purpose (Schwarz et al. 2012; Hoeller et al. 2009; Klang et al. 2010). Recent studies have shown that cyclodextrins, i.e. cyclic polysaccharide molecules, may likewise act as penetration enhancers for specific drugs when incorporated into nanoemulsions. This effect is most likely caused by improved solubilisation and increased availability of the drug at the skin surface and/or involvement of the cyclodextrins in oil droplet formation (Klang et al. 2010, 2011a).

Conclusion

In summary, the benefits of nanoemulsions for dermal application lie with their high skin friendliness and their excellent physical stability. The employed surfactants may serve to promote skin penetration of incorporated drugs. Enhanced in vivo efficacy of different drugs has been observed for nanoemulsion formulations due to their specific morphology and composition. Additional factors such as the droplet surface charge and viscosity of nanoemulsions can be modified with the help of different additives to further improve skin interaction and drug delivery. The possibility to incorporate various lipophilic as well as hydrophilic drugs by means of novel strategies renders nanoemulsions interesting vehicles for dermal and cosmetic applications.

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Pickering Emulsions for Controlled Drug Delivery to the Skin

19

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19.1 Introduction

Pickering emulsions are emulsions of any type, either oil-in-water (o/w), water-in-oil (w/o), or even multiple, stabilized by solid particles in place of surfactants (Aveyard et al. 2003; Binks 2002; Binks and Horozov 2006). Although such emulsions did not receive much development towards their application, their properties are quite attractive and deserve special attention. The interest lays in the fact that Pickering emulsions essentially remain emulsions, i.e., they share most properties of emulsions with their conventional surfactant-based homologues. Additionally, they have also few specific properties that are advantageous in the field of drug delivery to the skin. The solid stabilizing particles behave in quite a similar way as surfactant molecules: one part is adsorbed at the oil-water interface and the residual part is remaining in the continuous phase according to the adsorption equilibrium. Solid particles form a dense coating that can be seen by optical microscopy when the particles are large enough (Fig. 19.1).

The name "Pickering emulsion" was given after their early disclosure by S.U. Pickering (1907). Actually, the adsorption of solid particles at the air-water interface has been reported earlier (Ramsden 1903). However, the merit has been given to S.U. Pickering, because his paper specifically dealt with emulsions stabilized by solid particles; he reported improved stability for these

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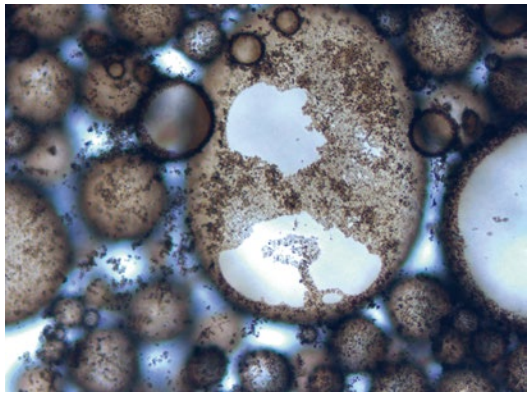

Fig. 19.1 Optical microscopy picture of an o/w Pickering emulsion of silicone oil stabilized by zinc oxide (ZnO) particles. Addition of a large concentration of sodium chloride (NaCl, 1 mol·L^{-1}) caused the partial flocculation of ZnO particles into large aggregates that could be observed at the surface of oil droplets by optical microscopy. The oil droplets are covered by a dense coating of ZnO particles over the brown areas where large aggregates are also visible. The white areas are bare. The picture also shows free ZnO particles dispersed in the aqueous phase which are seen as aggregates in between the oil droplets

emulsions with respect to surfactant-based emulsions and provided a definite proof for the adsorption of solid particles, being the origin for such stabilization.

Pickering emulsions are essentially emulsions, so that their main properties are common with classical emulsions stabilized by surfactants (emulsifiers). Pickering emulsions are prepared using the same manufacturing processes as for classical emulsions. There might be a specific process for Pickering emulsification; however, the most often used preparation methods are the same as for classical emulsions. As a consequence, the application domains of Pickering emulsions are the same as for classical emulsions, and an application based on classical emulsions can easily be switched to Pickering emulsion. Pickering emulsions show some improved properties compared to classical emulsions. Adsorbed solid particles act as a more effective barrier against coalescence than surfactants. Such important benefit opens the possible stabilization of coarse emulsions and multiple emulsions. The "surfactant-free" character makes them attractive to applications to life sciences where surfactants often cause either irritancy or hemolysis.

Applications of Pickering emulsions in pharmaceutical and cosmetic fields rely on either their specific properties manifested in vivo (low irritancy related the surfactant-free character, specific interactions with biological interfaces) or the development of new dosage forms having improved ex vivo properties (emulsion stability, thickening with no polymeric thickener) over surfactant-based emulsions. The present chapter is dealing with percutaneous penetration-enhancing properties of Pickering emulsions and covers the former aspect where drug-loaded Pickering emulsions modify the transport of drug molecules into/through skin. This includes the classical "penetration enhancer" action similar to that of "penetration enhancer" chemicals and also any modification of the drug transport induced by the formulation in a Pickering emulsion. The behavior of Pickering emulsions is discussed with respect to a reference surfactant-based emulsion.

The physicochemical properties of Pickering emulsions are shortly reviewed in the first part of the chapter. The second part reports on the skin absorption behavior of drugs followed by a discussion of the mechanism of the effects induced by Pickering emulsions compared to surfactant-based emulsions.

19.2 Physical Chemistry of Pickering Emulsions

The origin of emulsion stabilization is the adsorption of solid particles at the surface of emulsion droplets. The mechanism of adsorption is partial wetting of the particles by oil and water, which is very different of surfactants; the solid particles are not amphiphilic. Stabilization takes place by preventing destabilization events: coagulation, coalescence, and Ostwald ripening. There are strong similarities with classical surfactant-based emulsions; and there are few differences that provide specificities to Pickering emulsions. The manufacturing processes of Pickering emulsions used so far are the same as those of classical emulsions.

19.2.1 Adsorption of Solid Particles at Interfaces and Stabilization of Emulsions

Particles adsorbed at the oil-water interface are wet both by oil and water. Wetting conditions depend on interfacial tensions of the solid-water, solid-oil, and oil-water interfaces, $\gamma_{s\text{-}w}$, $\gamma_{s\text{-}o}$, and $\gamma_{o\text{-}w}$. Under partial wetting conditions, the contact angle in water, θ_w, is given by the Young's law:

$$\cos\left(\theta_w\right) = \frac{\gamma_{s\text{-}o} - \gamma_{s\text{-}w}}{\gamma_{w\text{-}o}} \qquad (19.1)$$

Partial wetting is quite common. Complete wetting by water occurs for very hydrophilic surfaces; such particles remain dispersed in the aqueous phase. This is the case of silica that cannot be used as stabilizing particle for most common oils (Frelichowska et al. 2009a). Silica is very often used however because there are commercially available grades of "hydrophobized" silica having organosilanes chemically grafted to their surface (Barthel 1995). Wetting of such silica surfaces is controlled by the grafting degree of the organosilane. Adsorption of organic molecules such as surfactants, polymers, or even small surfactant-like organic molecules, to the solid particles, allows adjusting the surface properties of the stabilizing particles so as to achieve the desired emulsion type and optimum stability (Gelot et al. 1984; Hassander et al. 1989; Midmore 1998a, b, 1999; Ghouchi Eskandar et al. 2007, 2011; Akartuna et al. 2009; Drelich et al. 2010). However, surface-active molecules that adsorb at the surface of solid particles may also adsorb at the oil-water interface and contribute to the emulsion stability in the same way as classical surfactants do. Adsorption of solid particles is very strong when partial wetting conditions are met. The strongest adsorption and the maximum stability of emulsions are reached when the contact angle is 90° (Binks and Lumsdon 2000a). Obviously, large particles having a larger area contacting oil and water show larger adsorption strength. Even small solid nanoparticles also adsorb to the oil-water interface quite strongly. Stabilizing solid particles do not show surface activity, which is a definite difference compared to surfactants (Vignati et al. 2003; Dong and Johnson 2003).

Stabilization of Pickering emulsions occurs because the layer of adsorbed solid particles forms a rigid coating that acts as a mechanical barrier against coalescence. Such a rigid protective coating has been compared to an eggshell. The origin of its mechanical strength is the two-dimensional aggregation of solid particles at the droplet surface by means of capillary forces. A supplementary three-dimensional aggregation takes place in some instances, building a thick solid layer of solid particles. Therefore, the stability of Pickering emulsions is very high compared to classical emulsions. It allows the preparation of either concentrated emulsions (high internal phase ratio) or coarse emulsions (emulsion with droplet size in the millimeter range) that conventional surfactants would not be able to stabilize with sufficient efficiency. Solid particles may also prevent coagulation if they cause thickening of the continuous phase. This effect is similar to the stabilizing action of polymeric thickeners that are often included in the formulation of conventional emulsions.

Numerous particles are able to stabilize Pickering emulsions. They are either inorganic or organic. Hydrophobized silica and clay (montmorillonite, laponite, kaolin) are the most common inorganic particles. Latex and carbohydrate nanocrystals (cellulose, chitin) are examples of suitable organic particles.

19.2.2 Emulsion Type, Emulsion Inversion, and Double Emulsions

The emulsion type is controlled by the wettability of the solid particles (Aveyard et al. 2003; Binks and Lumsdon 2000): hydrophilic particles stabilize o/w emulsions and hydrophobic particles stabilize w/o emulsions. Examples of hydrophilic particles are silica, clay, titanium dioxide, and zinc oxide. Examples of hydrophobic particles are "hydrophobic" silica (grafted with organic silanes), "hydrophobic bentonite" (montmorillonite coated with

fatty quaternary ammonium salts) polystyrene, and polytetrafluoroethylene (PTFE, Teflon®). Too much hydrophilic (or hydrophobic) particles are totally wet by water (or oil) however; partial wetting is an absolute requirement for solid particles adsorption at the oil-water interface. This is very similar to the Bancroft rule for emulsifiers stating that a hydrophilic emulsifier (high HLB) gives rise to an o/w emulsion type, while a hydrophobic emulsifier (low HLB) gives rise to a w/o emulsion type. Accordingly, a HLB-like rule has been introduced for solid particles by Kruglyakov (2000). The relevant quantity is the contact angle in water, θ_w, defined by the Young's law (Eq. 19.1): o/w emulsions form when $\theta_w < 90°$, and w/o emulsions form when $\theta_w > 90°$. In contrast with the behavior of emulsifiers, the contact angle for optimum stability is close to $\theta_w = 90°$. Therefore, "balanced particles" are efficient stabilizers, whereas "balanced surfactants" behave as poor emulsifiers. As an example, the well-known lecithin is a "balanced surfactant" that cannot stabilize emulsions; it needs being mixed with either a hydrophilic surfactant in order to stabilize o/w emulsions or with a hydrophobic surfactant for o/w emulsions. In other words, optimum emulsifiers are shifted apart from the HLB of "balanced surfactants"; conversely, "balanced particles" are at optimum. As a corollary, the same type of solid particles meets the stabilization criteria of either o/w or w/o emulsion type. The Bancroft rule for solid particles is less a clear-cut rule than for emulsifiers. Phase inversion does not take place when the contact angle is close to 90°. Catastrophic phase inversion upon progressive addition of the continuous phase does not happen as readily as for surfactants. Apart from the wetting behavior, supplementary parameters such as the relative water and oil contents, the medium where the solid particles have been initially dispersed, have a definite influence on the emulsion type (Binks and Lumsdon 2000a, b, c; Binks and Rodrigues 2003). Another consequence of both o/w and w/o emulsion types being at optimum for $\theta_w = 90°$ is a strong hysteresis in phase inversion experiments (Binks and Rodrigues 2003; Kruglyakov and Nushtayeva 2004).

Multiple emulsions of the w/o/w type are attractive dosage forms for encapsulation of hydrophilic drugs such as proteins and nucleic acids. The stabilization of multiple emulsions using emulsifiers is a difficult task because the stability is lost when the emulsifiers adsorbed at the surfaces of the w/o internal and w/o/w droplets mix together. The strong adsorption of solid particles to the various oil-water interfaces is an obvious benefit because particles keep retained at the right interfaces and do not mix.

A classical two-step process was used to prepare w/o/w multiple emulsions of medium-chain triglycerides: a primary w/o emulsion was stabilized by hydrophobic silica particles (coated with grafted dimethylsilyl groups at 49 % coverage); the obtained primary w/o emulsion was then dispersed in water using hydrophilic silica particles (coated with dimethylsilyl groups at 21 % coverage) as stabilizing particles, yielding a stable w/o/w emulsion (Barthel et al. 2003).

19.2.3 Control of Emulsion Properties by Formulation and Process Parameters

Once the emulsion type and the nature of the ingredients have been chosen, the droplet size and the rheological behavior are the main physicochemical properties of emulsions that matter in regard to their skin delivery. Such properties are controlled by the formulation (the type and concentration of the ingredients) and the emulsification process.

19.2.3.1 Droplet Size of Pickering Emulsions

The concentration of solid particles controls the droplet size. This meets expectations as it is similar to the effect of the concentration of surfactants. Indeed, a larger amount of stabilizing species allows a larger interfacial area to be formed, thus smaller droplets. Under conditions such that solid particles adsorb as a dense monolayer, the total oil-water interfacial area is in proportion to the amount of solid particles, and the mean droplet diameter is given by the following relationship (for an o/w emulsion) based on simple geometrical considerations (Wiley 1954; Arditty et al. 2003; Frelichowska et al. 2010):

$$Diam = \frac{6}{\rho_{oil}\alpha_{solid}} \frac{M(\text{oil})}{M(\text{solid})} \quad (19.2)$$

where M(oil)/M(solid) is the mass ratio of oil (dispersed phase) to solid particles, ρ_{oil} is the density of oil (kg m^{-3}), and a_{solid} is the interfacial area covered per mass of adsorbed solid particles (m^2 kg^{-1}). Such a linear relationship has often been experimentally verified (Arditty et al. 2004, 2005); but cases of departure from Eq. 19.2 have also been reported (Wang and Hobbie 2003; Binks and Whitby 2004; Frelichowska et al. 2010). Incomplete coverage by solid particles may also lead to stable Pickering emulsions; however, there are several microscopic observations of such emulsions (Fig. 19.1) (Binks and Kirkland 2002; Horozov and Binks 2006; Destribats et al. 2010).

The mechanisms being responsible for the droplet size in surfactant-based and Pickering emulsions are different. The reduction of droplet size by increasing surfactant concentration results from two phenomena: on one hand, the simple geometrical argument given above holds; on the other hand, fast surfactant adsorption during the emulsification process causes a decrease of the interfacial tension that makes the droplets fragmentation easier under shear. On the contrary, adsorption of solid particles does not change the interfacial tension (Vignati et al. 2003; Dong and Johnson 2003) (unless the particles contain surface-active impurities or they are associated with a surfactant).

In the case of a low amount of solid particles, a "limited coalescence" ripening phenomenon may occur: the poorly stabilized emulsion droplets undergo coalescence till the total interfacial area matches the area that the solid particles can stabilize (Wiley 1954; Arditty et al. 2004). Successful limited coalescence requires that the emulsion remains stable during the ripening process; otherwise emulsification fails as a part of the oil is released (Frelichowska et al. 2010; Avendaño Juárez and Whitby 2012). Under conditions of low particle content, large droplets can only be stabilized. A suitable emulsification process yields stable coarse emulsions having droplets within millimeter size range (Zhai and Efrima 1996; Arditty 2003). Formation of poorly stabilized Pickering emulsions is controlled by both formulation and emulsification process parameters.

On the basis of the geometrical relationship between droplet diameter and oil/solid mass ratio (Eq. 19.2), very fine emulsions might be prepared at a high content of solid particles. Such expectation is based on the hypothesis of droplet-size control by the amount of stabilizing particles. A high-power emulsification process is required for the fragmentation of droplets. In the case of lack of power, the smallest droplets that the process can create do form, and only a part of the stabilizing particle is used for reaching full coverage of the droplets. The remaining part of the stabilizing particles is not used for the stabilization; such excess particles are left free in the continuous phase.

As a summary, three distinct regimes are encountered depending on the mass ratio of solid particles to the dispersed phase (Fig. 19.2). In the first regime at low solid content, either emulsification fails or coarse emulsions are prepared provided a suitable process has been designed. In the second regime, the droplet size is controlled by the ratio M(particles)/M(oil) according to Eq. 19.2. The third regime is reached when the emulsification process is not able to create the interfacial area that the amount of solid particle might stabilize. The droplet size gets controlled by the emulsification process parameters such as homogenizer type, design of the instruments (propeller, rotor-stator, etc.), and stirring speed. The crossover from the second to the third regime depends on the power of the emulsification process.

19.2.3.2 Rheology of Pickering Emulsions

The viscosity of emulsions is an important property regarding their use as formulations for topical administration. Indeed it controls the materials transfer from the emulsion droplets to the skin. When all solid stabilizing particles are adsorbed to the emulsion droplets, the rheological behavior of Pickering emulsions is the same as that of surfactant-based emulsions. Emulsions are fluid as long as the concentration of droplets remains low; strong thickening appears in concentrated

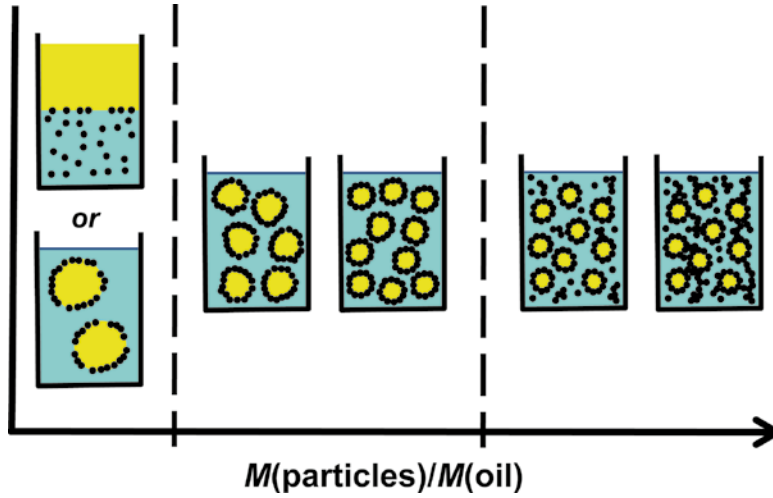

Fig. 19.2 Scheme of the different behaviors taking place according to the mass ratio *M*(particles)/*M*(oil). In the first regime at low particle/oil ratio, either emulsification process fails or a stable coarse emulsion is formed. Full adsorption of solid particles takes place in the second regime where the droplet size is controlled by the formulation (amounts of solid particles and oil). The droplet size is controlled by the emulsification process in the third regime at high particle/oil ratio; excess solid particles is dispersed in the aqueous phase and possibly particles aggregate, forming a percolating continuous network in case of strong aggregation or large amount of excess particles

emulsions when the volume fraction of droplets reaches 50–60 %. High internal phase ratio emulsions (HIPEs, gel emulsions) are viscoelastic materials found for concentrations above 60–70 %. A specific feature of Pickering emulsions is the thickening action of excess of free particles (non-adsorbed) present in the aqueous phase (of o/w emulsions). In general, the solid particles that are able to adsorb onto oil droplets also tend to self-aggregate in water because they are not hydrophilic enough to remain well dispersed. As an example, the hydrophobic fumed silica particles used for the stabilization of Pickering emulsions have been primarily made commercially available as thickening agents (Barthel 1995).

A nice example of gelation induced by solid particles in Pickering emulsions is given by Abend and Lagaly (1998, 2001). A percolating network of flocculated solid particles has been built by heterocoagulation of two types of particles. The adsorbed particles prevented coalescence; the remaining part caused thickening and prevented coagulation. Interestingly, X-ray microscopy observations (Fig. 19.3) have shown that the oil droplets coated by solid particles were stuck to the network of solid particles, so that the oil droplets were part of the gel (Neuhäusler et al. 1999; Thieme et al. 1999).

The flocculation of excess solid particles can be induced in a controlled manner by physicochemical parameters such as salinity of the aqueous phase. Thus, low salinity leave well-dispersed or weakly flocculated particles and the emulsion is fluid (either Newtonian or rheo-thinning fluid); high salinity induces the formation of a continuous network of solid particles in the continuous phase that causes gelation of the emulsion. The rheological properties of practical relevance, in particular the yield stress, can be adjusted by means of simple addition of electrolytes (Horozov et al. 2007; Whitby et al. 2011).

19.3 Skin Delivery of Drugs

Topical application of drugs loaded in Pickering emulsions is of particular relevance for pharmaceutical and cosmetic applications. It shows advantage due to the surface properties of droplets covered by solid particles and the possible controlled drug release through the barrier of solid particles present

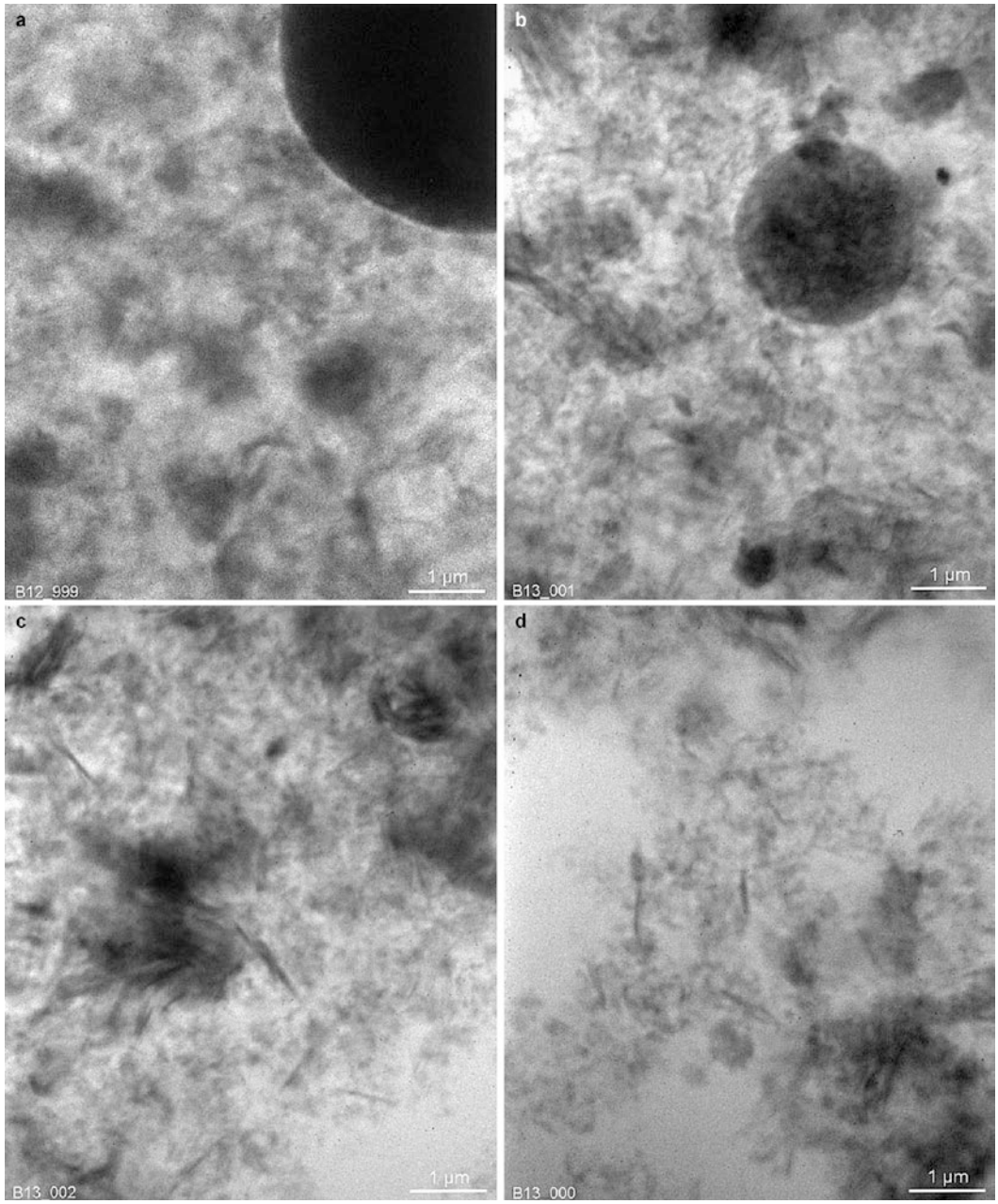

Fig. 19.3 X-ray microscopy images of o/w Pickering emulsion stabilized by mixed montmorillonite and layered double hydroxide particles that underwent heterocoagulation. A continuous network of aggregated particles is seen in the continuous phase, as well as entrapped oil droplets appearing as large black circles. Montmorillonite particles appear as platelets and layered double hydroxide appear as particles of nearly spherical shape (Thieme et al. (1999), with permission)

at the surface of droplets. An important benefit for cosmetic applications is the surfactant-free character of these emulsions which enables the avoidance of irritancy caused by surfactants. Skin delivery of drugs by Pickering emulsions has been approached in comparative investigations of Pickering and surfactant-based emulsions by several groups as reported in the following.

Pickering emulsions behave as penetration enhancers in some instances where the skin permeation of a drug loaded inside the droplets of a Pickering emulsion was faster than for a conventional emulsion or a homogeneous solution. Although the mechanism of accelerated transport is not clearly established yet, it is quite obvious that Pickering emulsions do not behave as classical penetration enhancers that increase the permeability of the stratum corneum by fluidization of the intercellular lipids. Pickering emulsions cause a faster permeation in some instances. They may also cause accumulation of drugs inside the stratum corneum. In conclusion, there are definite differences with respect to surfactant-based emulsions. Such effects are discussed in the following as being a generalized penetration enhancement behavior.

19.3.1 O/W Pickering Emulsions

Skin penetration of a hydrophobic drug, all-trans retinol, from o/w Pickering emulsions has been compared to the penetration from a classical surfactant-based emulsion (Frelichowska 2009b). For the comparison to make sense, the fundamental properties of both emulsions were set identical: the two emulsions had the same chemical composition, but the stabilizing ingredients were different (either solid particles or surfactant molecules), and they had the same droplet size and the same viscosity. The purpose was to investigate the influence of the droplet coating composed of solid particles better than comparing a Pickering emulsion to an emulsion taken from a commercial product.

Owing to its high lipophilic character ($\log P_{\text{octanol}} = 5.68$), the main part of retinol remains stored in the lipidic medium of stratum corneum. As consequence, permeation is negligible for both Pickering and classical emulsions. Accumulated amounts of retinol have been measured in the skin layers of excised pig skin after 24 h exposure to o/w Pickering and classical emulsions of medium chain triglycerides loaded with 0.1 % retinol and a solution in oil as reference. The skin penetration from emulsions was much larger than from the solution. A significant contribution to faster absorption from classical emulsion has often been ascribed to the penetration enhancer action of the emulsifiers (Brinon et al. 1998; Montenegro et al. 2008; Otto et al. 2009). Such an effect cannot hold for Pickering emulsions. That a surfactant-free emulsion causes high penetration rate casts doubts on the role of emulsifiers as the origin of improved skin penetration from conventional emulsions. The total amounts of retinol found in the skin were identical for both emulsions, but the distribution of retinol along the various skin layers showed large differences. Thus, retinol absorbed from the Pickering emulsion was strongly retained in the stratum corneum, and it consequently reached the viable epidermis and dermis to a lesser extent in comparison to the surfactant-based emulsion. Tape-stripping experiments have shown that storage of retinol in stratum corneum took place especially in its outermost layers when Pickering emulsion was used (Fig. 19.4).

A very similar investigation of skin delivery of retinol has been carried out independently at the same time from a medium chain triglycerides emulsion stabilized by mixed particle/surfactant (fumed silica nanoparticles + either anionic soybean lecithin or cationic oleylamine) (Ghouchi Eskandar et al. 2009). Interestingly, similar conclusions were reached although the stabilizing system of the Pickering emulsion was different. Retinol was accumulated in the outermost layers of the skin. The in-depth distribution of retinol in the skin was measured by cutting the skin into several horizontal 100 μm thick slices that were analyzed for their retinol content. A full comparison with the study by Frelichowska et al. (2009b) is difficult because of the low in-depth spatial resolution of the method based on parallel slicing compared to that of tape stripping (Touitou et al. 1998). Thus, the first slice containing the largest amount of retinol included the full stratum corneum and a large part of the viable epidermis. The "Pickering" emulsions were basically surfactant-based emulsions to which silica was added, so that it is difficult to conceive the relative contribution of solid particles and surfactant molecules. The skin absorption was larger than in the study by Frelichowska et al. (2009b) pertaining to true Pickering emulsions (free of surfactant); in particular, the second and third slices corresponding to 100–300 μm depth contained

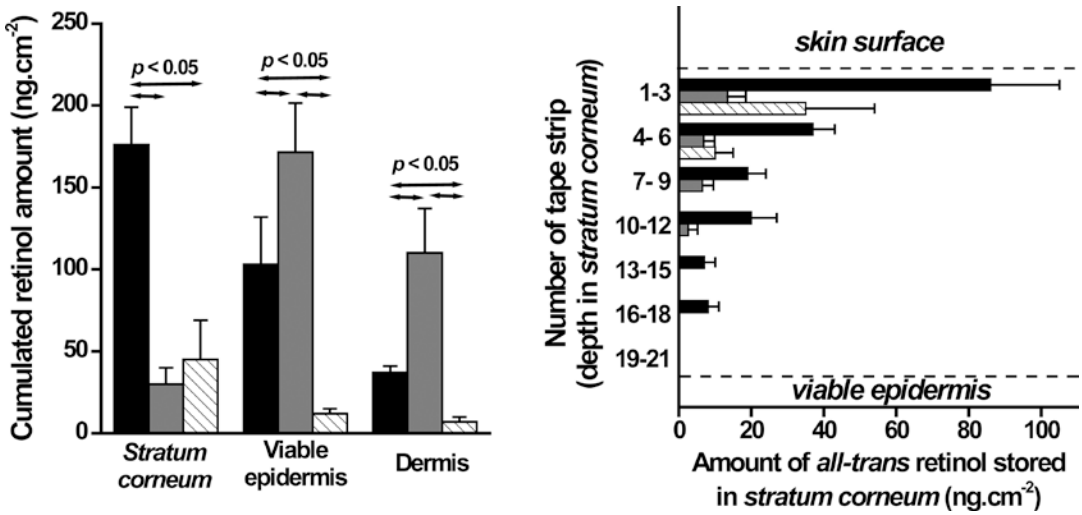

Fig. 19.4 Distribution of retinol in skin layers after 24 h exposure to Pickering emulsion (*black*), classical emulsion (*gray*), and solution in medium chain triglycerides (*dashed*). *Left*: Cumulated amount in stratum corneum, viable epidermis, and dermis (applied dose = 400,000 ng·cm^{-2}). *Right*: Distribution inside the stratum corneum obtained by the tape-stripping technique

large amounts of retinol. Such differences with respect to surfactant-free Pickering emulsions were probably caused by the penetration-enhancing action of the surfactants (especially the oleyl-chained oleylamine) present at a fairly high concentration (1 % for 10 % oil content). Indeed the infrared spectrum of stratum corneum lipids was altered by the presence of the oleylamine surfactant in the emulsion. The presence of silica particles significantly increased the absorption of retinol with respect to the silica-free emulsion (only surfactant-based). Silica could be considered as a penetration enhancer in such systems or silica acted in synergy with the surfactants, which showed definite difference with the surfactant-free Pickering emulsions.

Accelerated skin absorption of drugs has recently been shown for o/w Pickering emulsions loaded with molecules of medium polarity. The steady-state permeation flux of methyl salicylate (log$P$=2.5) loaded in a Pickering emulsion was twice higher than the aqueous solution (Marku et al. 2012). The permeation and accumulation inside skin of the fluorescent probe acridine orange 10-nonyl bromide (AONB) was enhanced by the presence of silica particles adsorbed at the surface of an o/w emulsion stabilized by the lecithin emulsifier (Ghouchi Eskandar et al. 2010). AONB loaded in the lecithin-stabilized emulsion was mainly retained in the stratum corneum, whereas its penetration was deeper in the epidermis when loaded in the emulsion stabilized by mixed silica/lecithin. However, the effects of solid particles were hardly perceptible in the same experiments performed with oleylamine as emulsifier instead of lecithin. Two effects explain this result: loading of the cationic AONB inside oil droplets was low because of the electrostatic repulsions with oleylamine emulsifier, and the strong penetration-enhancing effect of oleylamine screened the possible effect of the presence of solid particles.

As summary, accumulation inside the stratum corneum and less penetration to the deeper skin layers has been observed for the highly hydrophobic retinol. This is not a general phenomenon however. Less hydrophobic drugs loaded in o/w Pickering emulsion showed also accelerated penetration to the dermis and permeation into receiver compartment.

19.3.2 W/O Pickering Emulsions

Skin delivery of a hydrophilic drug, caffeine, from a w/o Pickering emulsion has been studied in the same way as the retinol absorption from

o/w emulsions. Permeation of caffeine was measured from a Pickering emulsion compared to a surfactant-based emulsion having the same chemical composition (but the stabilizing layer being composed of emulsifier molecules instead of silica nanoparticles), the same droplet size, and the same viscosity (Frelichowska et al. 2009c). Results of the in vitro drug diffusion through excised pig skin revealed a higher transdermal flux of caffeine through excised skin (Fig. 19.5). Such higher permeation in an in vitro experiment suggests that a larger delivery to the deep dermis and hypodermis would occur with regard to application to full skin in vivo. A higher concentration in the dermis correlates with the larger permeation.

19.3.3 Mechanisms of Enhanced Skin Absorption

The origin of the faster penetration has mainly been discussed by Frelichowska (2009c) for the skin absorption of caffeine from w/o Pickering emulsions. Several transport phenomena have been considered; experiments have been performed in order to evaluate their relative contributions and finally disclose the main origin of the faster permeation through the skin. The different pathways of the drug molecule from the emulsion droplets to the deep skin layers are considered.

The first step is drug release from inside the emulsion droplets to the surrounding medium; the release behavior is often referred to as the bioavailability. Since the dense coating of solid particles around the emulsion droplets efficiently stabilizes the emulsion with respect to emulsifier-based emulsions, it is presumed that such layer acts as a barrier to diffusion of molecules. The Pickering emulsion droplets may behave as an encapsulation system that delays the release of drugs to the continuous phase. Experimental evidence of sustained release has been given for particular o/w emulsions made of cross-linked polydimethylsiloxane coated with adsorbed silica particles (Simovic and Prestidge 2007). Sustained release of caffeine from w/o emulsion droplets to a bulk aqueous phase has been indeed measured by Frelichowska (2009c). Transfer of the drug from Pickering emulsion droplets to the aqueous phase was slowed down by a factor 1.5 with respect to the emulsifier-based emulsion.

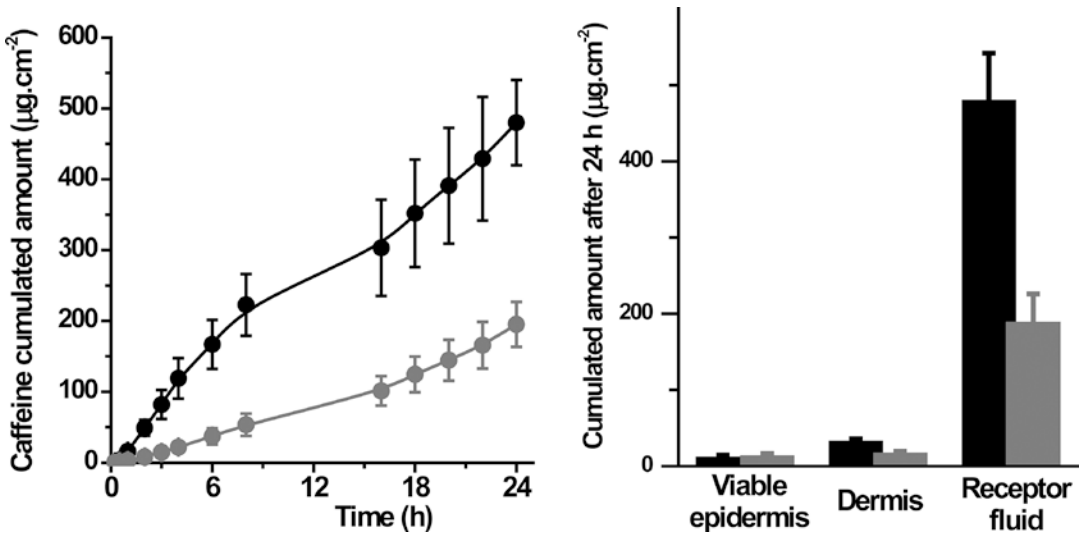

Fig. 19.5 Skin absorption of caffeine from w/o emulsions: Pickering emulsion (*black*) and classical emulsion (*gray*). *Left*: Permeation profile over 24 h exposure to different emulsions. *Right*: Distribution of caffeine inside skin layers after 24 h exposure to different emulsions (applied dose = 3,000 µg.cm^{-2})

Permeation of the drug through the skin should have been slower accordingly; on the contrary, it has been measured to be faster.

The direct transfer of drug molecules from emulsion droplets to the skin surface appeared the most relevant contribution to the accelerated permeation of caffeine. Such direct transfer occurs when droplets come in contact with the skin surface. Stronger adhesion of the w/o Pickering emulsion droplets to the skin has been disclosed from measurements of adhesion energy of water drops to the skin surface immersed in an oil medium. Thus, the adhesion energy was 3.3 $J \cdot m^{-2}$ for drops covered with silica particles ("Pickering drops") emulsions against 0.27 $J \cdot m^{-2}$ for drops from emulsifier-coated drops (and 3.3 $J \cdot m^{-2}$ for bare drops). Higher adhesion energy meant a longer contact time of the water droplets to the skin surface, and consequently a faster transfer of the caffeine molecules contained in water droplets to the skin. According to such mechanism of transfer during the contact of droplets with the skin surface, emulsion droplets are not considered as carriers, and no penetration of emulsions droplets into the skin is involved. It is believed that emulsion droplets cannot penetrate the skin as intact particles. Accelerated transfer of the drug from the emulsion droplets to the skin overcompensates the encapsulation-like sustained release of caffeine into the continuous phase, as well as the possible penetration-enhancing effect of the emulsifier contained in the classical emulsion, so that the overall effect is an acceleration of the drug delivery by Pickering emulsions.

Penetration of silica particles into the skin gives a possible supplementary transport mechanism for the drug. Indeed, the polar caffeine molecule may adsorb at the surface of silica particles that penetrate the nonpolar medium of the stratum corneum. Penetration of silica particles is limited however as discussed in the next section. The depth of penetration of silica particles upon 24 h exposure to a Pickering emulsion was 5 μm, that is, half of the thickness of the stratum corneum. Therefore, the contribution of this phenomenon is limited.

For the o/w emulsions, storage of silica particles in the stratum corneum may be the origin of a higher storage of retinol in the stratum corneum. Indeed retinol probably adsorbs at the surface of silica particles in the lipidic medium of stratum corneum in the same way as fatty alcohols do in nonpolar organic solvents. The larger storage of retinol in the stratum corneum after exposure of the skin to the o/w Pickering emulsion may result from such adsorption of the drug to silica particles that do not penetrate deeper. In case where silica particles penetrate the skin deeper, the transport of drug adsorbed to silica particles may occur. Such situation was claimed by Ghouchi Eskandar et al. (2010) on the basis of a correlation between concentration profiles of fluorescent penetrant molecule and silica nanoparticles measured from confocal fluorescence microscopy and SEM on histological transversal sections, respectively.

Easier direct transfer from emulsion droplets to the skin may also be operative as in the case of w/o Pickering emulsions. Indeed, it should compensate the penetration-enhancing activity of the surfactant in the classical emulsion taken as reference, so that the total amounts of absorbed retinol measured for Pickering and classical emulsions are the same. Although the mechanism of improved transfer coming from a higher adhesion of emulsion droplets has not been studied for o/w emulsions, this phenomenon is likely operating in the same way as for w/o emulsions. This holds for o/w Pickering emulsions loaded with drugs of medium polarity that are able to permeate through the skin (Ghouchi Eskandar et al. 2010; Marku et al. 2012).

The measurement of the adhesion energy of emulsion droplets to the skin surface can be performed quite easily taking macroscopic drops as mimicking the emulsion droplets (Frelichowska 2009c). The size of macroscopic drops is ~1,000 times larger than the size of emulsion droplets. The adhesion energy of a droplet deposited on a (skin) surface is

$$E_{\text{Adhesion}} = \gamma_{\text{w-o}} \left(1 + \cos\theta_{\text{w}}\right) \qquad (19.3)$$

where $\gamma_{\text{w-o}}$ is the interfacial tension between oil and water in the presence of either solid particles or surfactant molecules and θ_{w} is the contact

angle of the drop of dispersed phase deposited on the (flat and smooth) skin surface immersed in the continuous phase. Both $\gamma_{w\text{-}o}$ and θ_w can be easily measured with the standard laboratory equipment (drop shape analysis tensiometer).

19.3.4 Fate of the Stabilizing Solid Particles

The deep skin penetration of solid particles may be considered as an issue regarding possible health concerns of nanoparticles. Such topic has been largely addressed concerning inorganic particles used in sunscreen formulations, titanium dioxide, and zinc oxide (Mavon et al. 2007; Nohynek at al. 2008; Bolzinger et al. 2011). There is a wealth of experimental data showing that such inorganic particles do not penetrate the skin deeply and that their penetration is restricted to the stratum corneum upon reasonable exposure durations (hours to days). The same trend has been observed for silica particles used for the stabilization of Pickering emulsions. Figure 19.6 shows scanning electron microscopy (SEM) pictures of corneocytes collected by means of tape stripping after 24 h exposure of the skin to a w/o Pickering emulsion. Silica particles are readily visible at the surface of corneocytes; their abundance decreases as a function of the skin depth

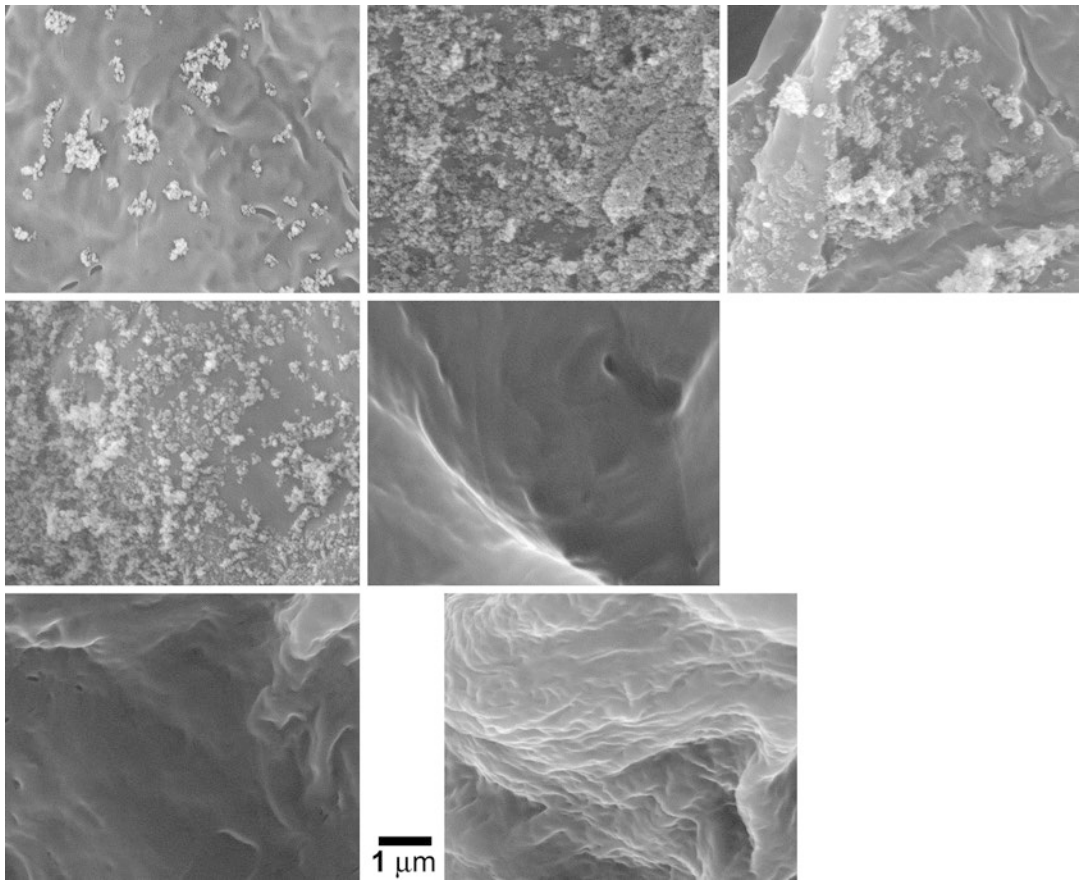

Fig. 19.6 SEM pictures of corneocytes peeled off the stratum corneum by the tape-stripping technique after 24 h exposure to Pickering emulsion. A less amount of silica particles is observed upon going deeper into the stratum corneum; no more particles are detected beyond the 10th strip corresponding to half the thickness of stratum corneum. From *top* to *bottom*: skin surface, 1st, 7th, 10th (2 pictures), 15th, and 19th strip

and no more silica particles have been detected beyond the 10th strip corresponding to 5 μm skin depth (half of the stratum corneum thickness).

Ghouchi Eskandar et al. (2010) reached the same conclusion from observations of histological sections of the skin. Silica particles appeared as white areas in SEM pictures under backscattered electron detection. The images revealed a uniformly white layer corresponding to the stratum corneum that contained a high amount of silica, and several white spots dispersed at random in the viable epidermis and dermis. The amount in the stratum corneum was obviously much higher than the cumulated amounts in the deeper layers of skin. Unfortunately, the quantitative analysis of silicon by EDX has been averaged over the whole histological section, so that a quantitative assessment of the fractions of silica in the stratum corneum and viable layers of skin was not available. Their measurements are particularly interesting because in their studies drug penetration was favored by other ingredients of the formulation that acted as penetration enhancers. The stabilizing layer was made of mixed surfactants and solid particles, so that the solid particles might have penetrated easier because of the penetration-enhancing properties of the surfactant, in particular oleylamine, which acts as a penetration enhancer.

Alternatives to inorganic nanoparticles are biodegradable organic particles. Biodegradable Pickering emulsions are attractive for skin delivery applications, as well as for other fields such as food applications, development of emulsifier-free environmentally sustainable formulations, etc. Several types of such particles can stabilize Pickering emulsions of edible or biodegradable oils: fat crystals (Rousseau 2000), cellulose (Kalashnikova et al. 2011, 2012), chitin (Tzoumakia et al. 2011), or block copolymer micelles (Laredj-Bourezg et al. 2012).

Conclusions

Solid particles can be advantageously used as emulsifiers for the stabilization of Pickering emulsions. They also influence the skin absorption of drugs loaded inside the droplets of dispersed phase. The parameters allowing the control of the emulsion properties and skin absorption pertain to both the formulation (choice of the ingredients and their concentration) and the emulsification process. The physicochemical properties of the skin surface are such that Pickering emulsion droplets show strong adhesion to the skin, thus allowing faster transfer of drugs to the skin. The penetration-enhancing activity of Pickering emulsions mainly relies on this adhesion phenomenon. Other phenomena specific to Pickering emulsions modulate skin absorption, even if they are not sensu stricto penetration enhancer actions: lower bioavailability due to sustained release and penetration of solid particles inside the stratum corneum causing immobilization of adsorbed drug molecules.

Manufacture of Pickering emulsions can be easily implemented since the replacement of classical emulsifiers by solid particles is quite a direct substitution, and fabrication process is the same as for conventional emulsions.

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Part VI

Gels as Vehicles for Skin Delivery

Hydrogel Vehicles for Hydrophilic Compounds

20

Teresa Cerchiara, Federica Bigucci, and Barbara Luppi

Contents

20.1 Introduction

Hydrogels date back to 1960 when Wichterle and Lim first proposed the use of hydrophilic networks of poly(2-hydroxyethylmethacrylate) (PHEMA) in contact lenses (Wichterle and Lim 1960). Since then, the use of hydrogels has extended to various biomedical (Hoffman 2002; Peppas et al. 2006; Kopeceka 2007) and pharmaceutical (Peppas et al. 2000) applications. In particular, due to their physical properties, similar to those of human tissues (water content, soft and pliable consistence), hydrogels have been used for different administration routes such as oral, rectal, ocular, epidermal, and subcutaneous (Peppas et al. 2000; Guy 1996; Jatav et al. 2011).

Hydrogels are composed of hydrophilic macromolecules forming three-dimensional insoluble networks able to imbibe large amounts of water or biological fluids (Peppas and Mikos 1986). Commonly, the polymers utilized to make hydrogels are insoluble due to the presence of permanent or reversible cross-links (Berger et al. 2004). Permanent cross-linked hydrogels (Wichterle and Lim 1960; Xiao and Zhou 2003; Brasch and Burchard 1996) are characterized by covalent bonds forming tie points or junctions, whereas reversible cross-linked hydrogels (Watanabe et al. 1996; Wang et al. 1999; Qu et al. 1999) present ionic, hydrophobic, or coiled-coil physical interactions. These kinds of cross-links

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in the polymer structure yield insoluble materials able to swell in aqueous environments retaining a significant fraction of water in their structure, up to thousands of times their dry weight in water.

Hydrogels can be divided into homopolymer or copolymers based on the preparative method, but they can also be natural polymers, synthetic polymers, or derivatives. In nature hydrogels can be found in plants (pectin, pullulan), various species of brown seaweed (alginic acid, agar, carrageenan), crustaceans (chitin), and animal tissue (hyaluronic acid, collagen, fibrin). Typical simple synthetic materials applied for general-purpose hydrogels are poly(ethylene oxide), poly(vinyl alcohol), poly(vinyl pyrrolidone), poly(hydroxyethyl methacrylate), and poly(N-isopropyl acrylamide). Moreover, the synthetic pathway offers more possibilities to create hydrogels with modified functional properties. In fact, several physiologically responsive hydrogels are obtained from chemical or physical modifications of natural and synthetic polymers and tested for use in the so-called intelligent biomaterials (Hoffman 1991; Miyata et al. 2002; Murdan 2003; Chen et al. 2004) because they are capable of reacting to various environmental stimuli (temperature, pH, ionic strength, solute concentration, electric radiation, light, sound, etc.).

Hydrogels can be homogeneous, when the pores between polymer chains are the only spaces available for mass transfer and the pore size is within the range of molecular dimensions (a few nanometers or less) or porous when the effective pore size is over 10 nm. In homogeneous hydrogels the transfer of water or other solutes is achieved by a pure diffusional mechanism, which restricts the rate of absorption and to some extent the size of species that are absorbed.

Porous hydrogels can be made by different polymerization methods in the presence of dispersed porosigens (ice crystals, oil, sucrose crystals) which can be removed later to leave an interconnected meshwork, where the pore size depends on the size of the porosigens (Hickey and Peppas 1995). The introduction of a porosigen reduces mechanical strength significantly making porous hydrogels weaker than homogeneous hydrogels.

In medical, engineering, and pharmaceutical technology, hydrogel degradation is considerable important. In fact, investigators have focused on controlling degradation behavior of hydrogels to design polymers able to be cleared from the body once they complete their roles (Anderson and Shive 1997; Timmer et al. 2002): for this reason labile bonds are frequently introduced in the gels. These bonds can be present either in the polymer backbone or in the cross-links used to prepare the gel. The labile bonds can be broken under physiological conditions either enzymatically or chemically, in most cases by hydrolysis (Damink et al. 1996; Eliaz and Kost 2000; Lee et al. 2004).

20.1.1 Physical and Chemical Properties of Hydrogels

An important property of hydrogels is their swelling behavior: it depends upon the polymer, extent of cross-linking, temperature, polymer-solvent interactions, and extent of ionization (Khare et al. 1992). In particular the extent of cross-linking can be changed to achieve a relatively strong and yet elastic hydrogel. Long-chain cross-linkers and low cross-linking ratios (the ratio of moles of cross-linking agent to the moles of polymer repeating units) produce extremely weak hydrogels, while short-chain cross-linkers and high cross-linking ratios produce extremely tight hydrogels. Tightly cross-linked hydrogels will swell less than the same hydrogels with low cross-linking ratios or long cross-linkers chains.

The presence of hydrophilic or hydrophobic groups in the chemical structure of the polymer affects the swelling behavior of hydrogels. When a dry hydrogel begins to absorb water, the first water molecules entering the matrix will hydrate polar hydrophilic groups. As the polar groups are hydrated, the network swells exposing hydrophobic groups which also interact with water molecules, leading to hydrophobically bound water. Finally an equilibrium swelling level is reached when the network imbibes additional water ("free water") which fills the space between the polymer chains. As hydrophobic groups minimize their exposure to the water molecule, hydrogels containing hydrophobic groups will swell much less than hydrogels containing hydrophilic groups.

As stated earlier, the dissolution of polymer chains and consequently hydrogel swelling ability is prevented by the presence of cross-linking in the three-dimensional network. Different chemical and physical cross-linking methods have been employed to prepare hydrogels (Hennink and van Nostrum 2002). In chemically and physically cross-linked gels, dissolution is prevented by covalent bonds and physical interactions between different polymer chains, respectively. Chemically cross-linked gels can be obtained by radical polymerization of low molecular weight monomers in the presence of cross-linking agents, chemical reaction of complementary groups, and high-energy irradiation. Physically cross-linked gels can be obtained by ionic interactions, hydrogen bonds, crystallization, and aggregation of the hydrophobic segments of multiblock copolymers or graft copolymers. An example of cross-linking by radical polymerization is the synthesis of hydrogels of Wichterle and Lim (1960), a copolymerization of HEMA with the cross-linker EGDMA (ethylene glycol dimethacrylate) in the presence of AIBN (2,2′-azo-bis-isobutyronitrile), the radical initiator (Fig. 20.1). Chemical cross-linking agents, such as acyl dichlorides (Fig. 20.2), can establish covalent linkages with functional groups of polymers, such as activated hydroxylic groups of poly(vinyl alcohol) (Orienti et al. 2000). Polyaldehydes are utilized to cross-link proteins such as albumin (Sahin et al. 2002) and gelatin (Draye et al. 1998) or natural polysaccharides such as hyaluronic acid (Luo et al. 2000). However, a significant disadvantage of chemical cross-linking agents is their toxicity. Among various methods applied for the production of hydrogels, the radiation technique (Safrany 1997) is a simple, efficient, clean, and environment-friendly process (Fig. 20.3). Hydrogels can be obtained by radiation technique in a few ways, including irradiation of solid polymer (Nedkov and Tsvetkova 1994), monomer (in bulk or in solution) (Rosiak 1991), or aqueous solution of polymer (Kabanov 1998). For irradiation technologies, the main irradiating sources include gamma rays from

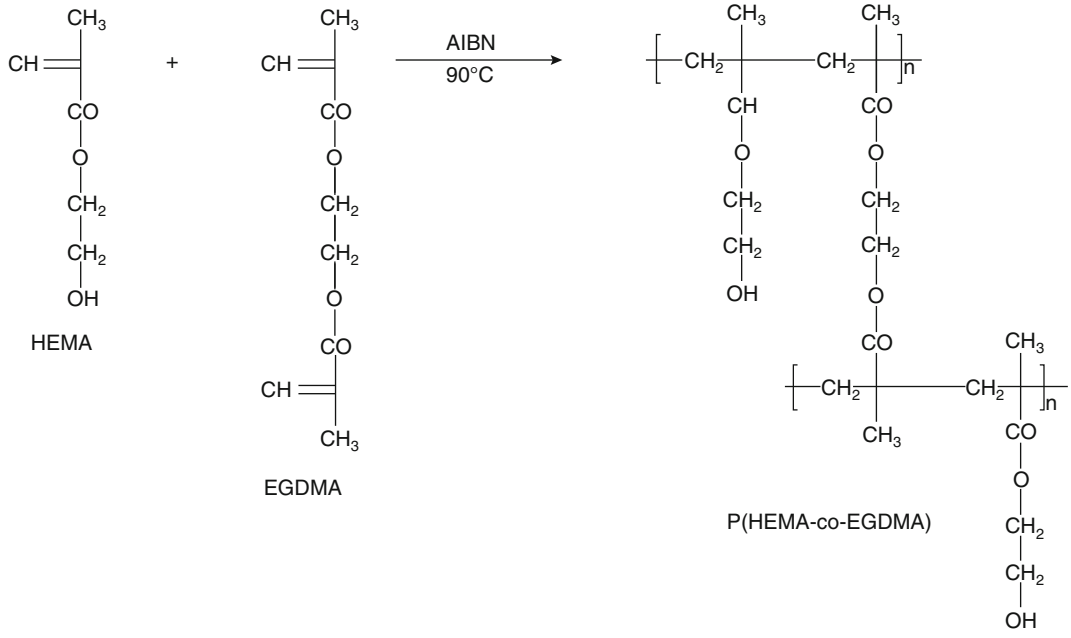

Fig. 20.1 Schematic representation of radical polymerization. Hydrogels are formed by the copolymerization of HEMA with EGDMA using AIBN as the radical initiator

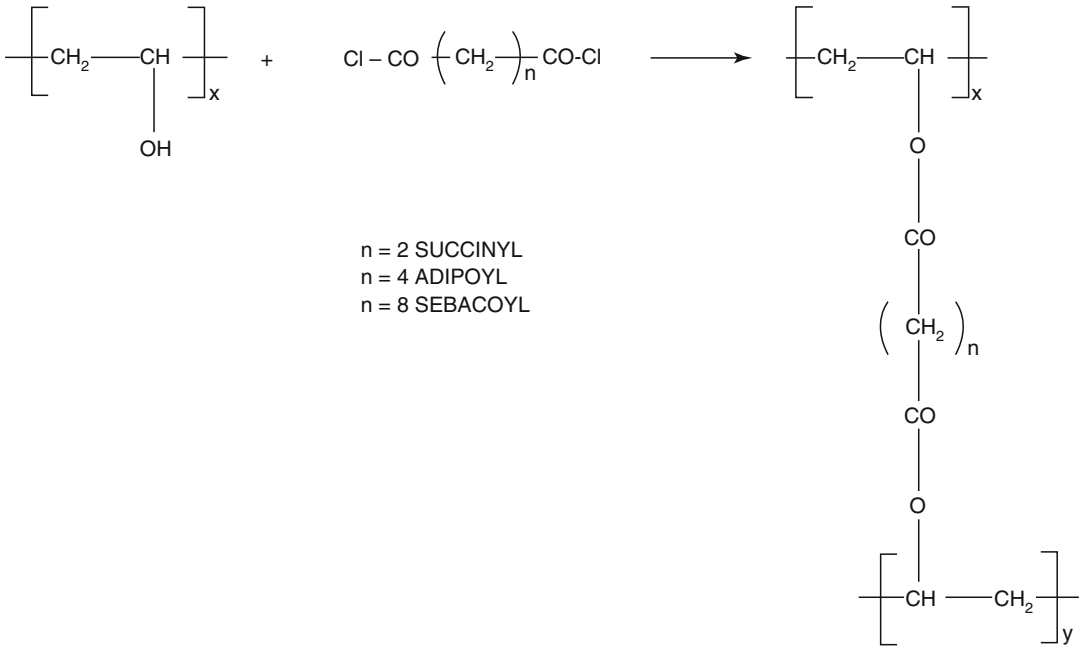

Fig. 20.2 Preparation of PVA hydrogels cross-linked by acyl dichlorides

radioactive isotopes such as cobalt 60, electron beams from electron accelerators, and X-rays converted from electron beams.

Physical cross-linking of hydrogels also avoids the use of chemical cross-linking agents. Such agents can potentially inactivate the active principle and covalently link it to the hydrogel network. Examples of ionically cross-linked alginate hydrogels have been reported (Grant et al. 1973). Alginate is a family of linear polysaccharides composed of mannuronic acid (MA) and guluronic acid (GA). The chemical composition and sequence of MA and GA residues depend on the source from which the alginate has been extracted. The gelation of alginate is mainly achieved by the exchange of sodium ions with divalent cations such as Ca^{2+}, Cu^{2+}, Zn^{2+}, or Mn^{2+}, which can form cation bridges between adjacent molecules. The "egg-box" model of Grant et al. (1973) is generally taken into consideration to explain the formation of a rodlike cross-linked complex due to the bounding of the divalent cations in the interchain cavities. Some polymeric complexes can be held together by hydrogen bonds: poly(acrylic acid) and poly(methacrylic acid) provide physically cross-linked hydrogels with poly(ethylene glycol) due to the formation of hydrogen bonds between the oxygen of poly(ethylene glycol) and the carboxylic groups of the acrylic polymers (Eagland et al. 1994). Another physical method for producing physically cross-linked hydrogels is the formation of crystalline regions in the polymer network, obtained by casting dilute, aqueous solutions of poly(vinyl alcohol), then cooling to −20 °C, and thawing back to room temperature several times (Stauffer and Peppas 1992). These freeze/thawed gels have demonstrated enhanced physical properties, such as high mechanical strength and high elasticity, that make them suitable for biomedical applications.

Finally, physically cross-linked hydrogels can be obtained by hydrophobic modification of polymers and in particular of polysaccharides such as chitosan, dextran, pullulan, and carboxymethyl curdlan (Noble et al. 1999; Sludden et al. 2000; Cerchiara et al. 2002). Glycol chitosan substituted with palmitoyl chains is an example of a hydrophobized polysaccharide. The attachment of hydrophobic groups to glycol chitosan yields an amphiphilic polymer capable of self-assembly into vesicles (Uchegbu et al. 1998). Non-covalent cross-linking is achieved by the

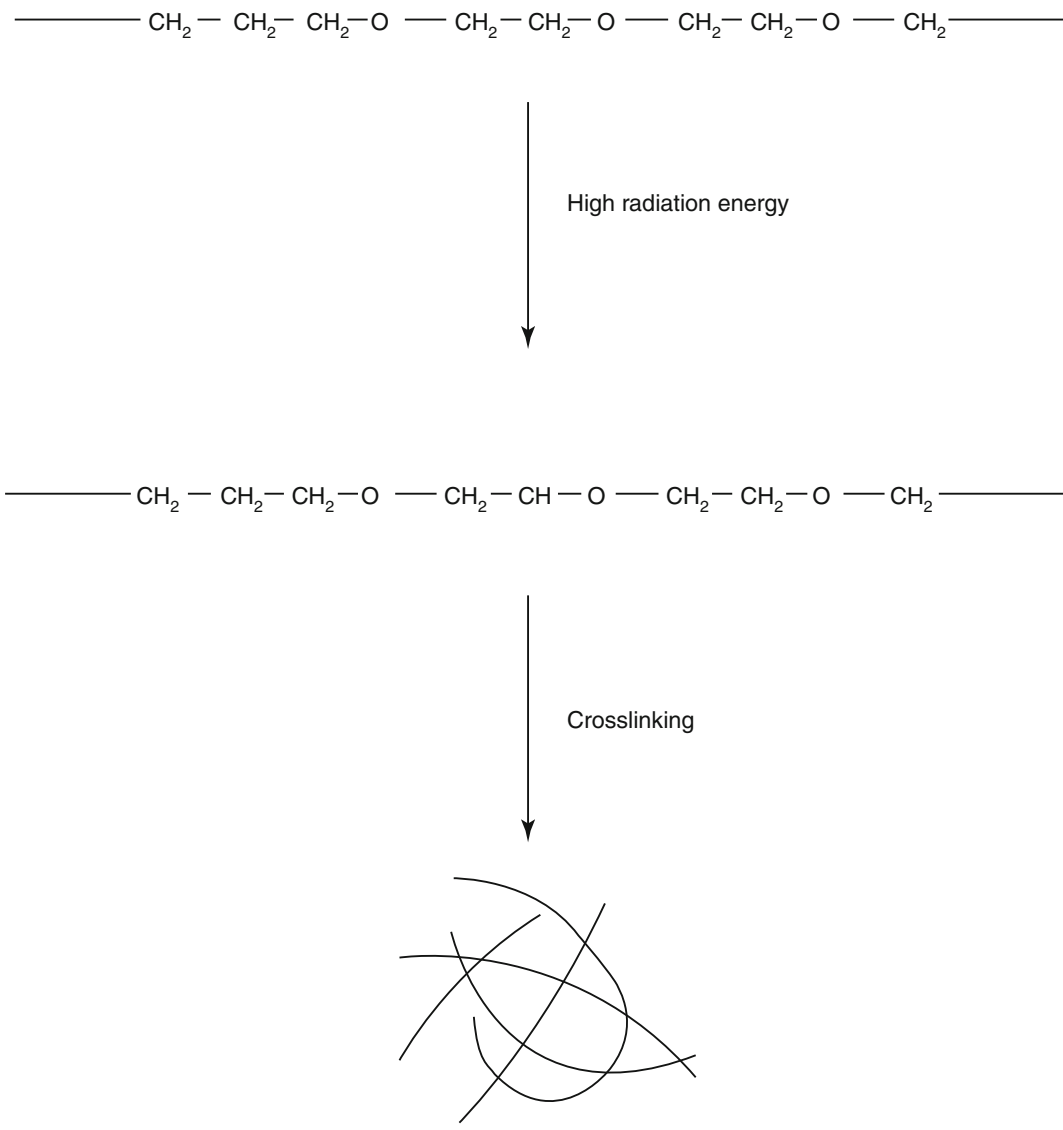

Fig. 20.3 Schematic representation of the radiation method to design hydrogels

hydrophobic interactions of the palmitoyl groups and a gel matrix is formed. Finally, our research group (Cerchiara et al. 2002) reported physically cross-linked chitosan hydrogels with lauric, myristic, palmitic, or stearic acid prepared by freeze drying and studied for transdermal use (Fig. 20.4). These polymers produce hydrogels with different functional properties related to the different acyl chains introduced in the polymer structure. In particular, the permeation of hydrophilic substances through the skin can be modulated by increased or decreased drug solubility due to the interaction of the different acyl chains with the stratum corneum.

20.2 Applications of Hydrogels in Transdermal Drug Delivery

Transdermal delivery is an attractive and promising alternative compared to conventional administration routes (e.g., oral and injectable) for transport through the skin into the blood circulation of drugs

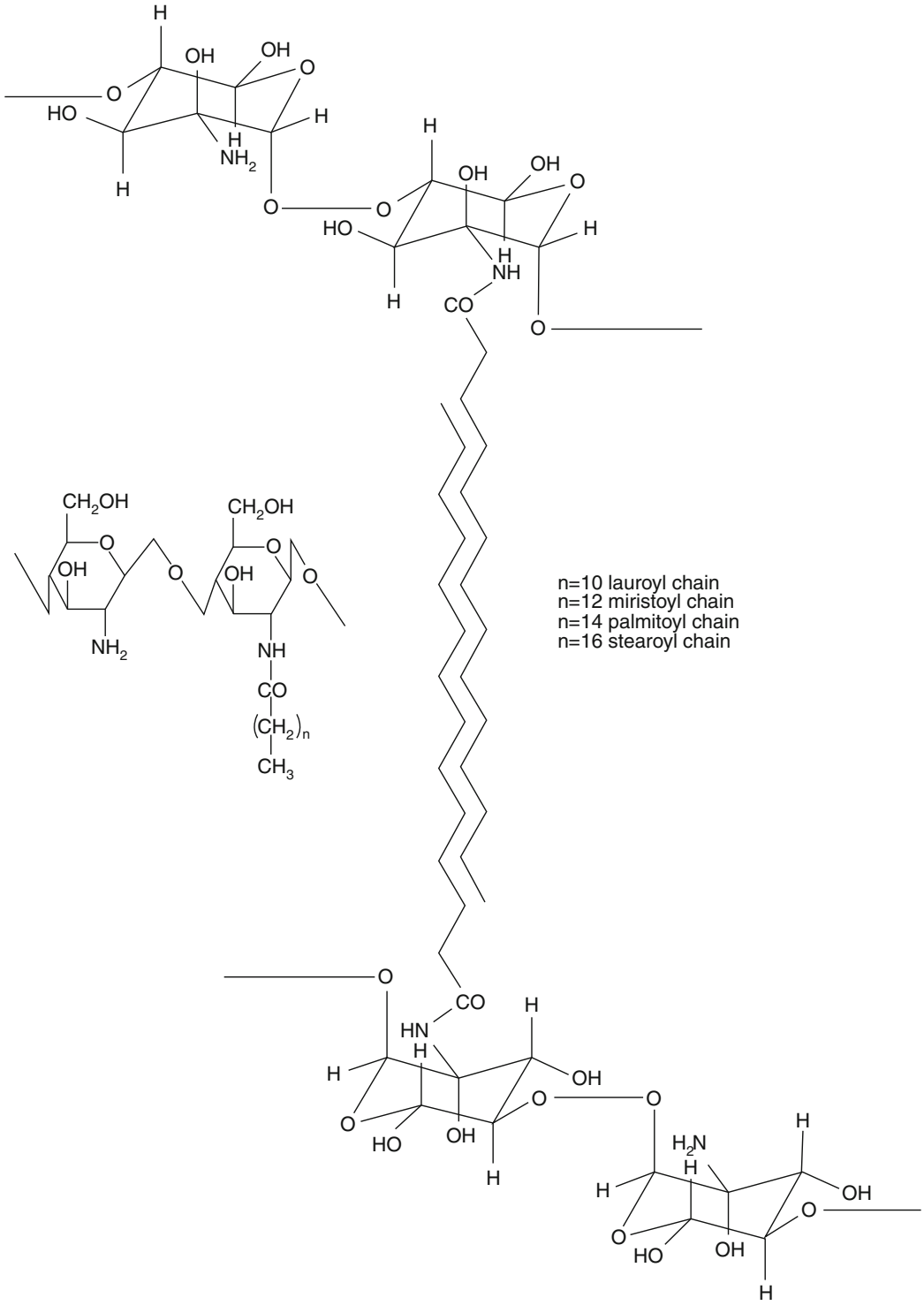

Fig. 20.4 Structural representation of physically cross-linked chitosan hydrogels

such as hydrophobic small molecules, hydrophilic molecules, and macromolecules. In fact, this route offers several advantages over conventional routes (Peppas et al. 2000; Brown et al. 2006; Prausnitz and Langer 2008):

- Is noninvasive and pain-free, with no trauma or risk of infection (Denet et al. 2004), thus resulting in an enhanced patient compliance
- Maintains constant drug levels in the blood, eliminating plasma peaks and valleys associated to oral and injectable administration
- Avoids gastrointestinal tract and circumvents hepatic first-pass metabolism, thus resulting in a lower drug amount administered and in a reduction of systemic side effects
- Is inexpensive

In spite of the advantages, major disadvantage of transdermal drug delivery is that the drug itself or the materials used to fabricate the vehicles may sometimes induce an irritation or sensitization reaction of the skin (Kurihara-Bergstrom et al. 1991; Murphy and Carmichael 2000; Ale et al. 2009; Wohlrab et al. 2011). Moreover, few molecules have been successfully delivered transdermally, mainly due to the stratum corneum that forms a barrier to the permeation of hydrophilic drugs, especially macromolecules such as proteins, peptides, and vaccines.

Consequently, research trends are focusing on approaches to overcome the barrier presented by the skin, including physical penetration enhancers such as microneedles (Henry et al. 1998), iontophoresis and electroosmosis (Pikal 2001), electroporation (Hu et al. 2000), radiofrequency energy (Sintov et al. 2003), and chemical penetration enhancers such as sulfoxides, alkanones, alcohols, polyols, amides, fatty acids, fatty acid esters, surfactants, terpenes, organic acids, and cyclodextrins (Thong et al. 2007). Recent studies have introduced a new category of transdermal penetration enhancers such as positively charged polymers. In fact, Taveira et al. (2009) described that chitosan interacts with negative charges in the skin improving drug penetration in the deeper layers, while He et al. (2008, 2009) demonstrated that chitosan and its derivatives such as N-trimethyl chitosan and mono-N-carboxymethyl chitosan are able to change the secondary structure of keratin in stratum corneum leading to a less organized structure of this protein and enhancing transdermal permeation of drugs.

As regards the transdermal drug delivery systems, the traditional formulations as plasters, cream, and ointments have been replaced in order to minimize skin irritation, promote adhesion properties, guarantee dosage flexibility, enhance patient acceptability, and improve ease of use. In this context, particular attention has been paid to the formulations based on hydrogels such as semisolid systems and film-based systems (matrix-type systems, membrane-coated systems, film-forming solution).

20.2.1 Semisolid Vehicles

Hydrogels are three-dimensional networks based on linear hydrophilic polymers that are able to absorb large amounts of water, remaining insoluble due to the presence of chemical or physical cross-links (Peppas et al. 2000). The relatively high water content makes them a good alternative to other dosage forms such as creams, ointments, and patches, enhancing skin moisturization and elasticity and providing a better feel when applied to the skin. The most important and well-known polymers used for preparing these hydrogels are biopolymers such as polysaccharides (starch, cellulose, chitin, alginate, hyaluronate) or proteins (collagens, gelatins, caseins, albumins) and synthetic polymers such as polyvinyl alcohol, polyvinylpyrrolidone, polyethylene glycol, and polyacrylates.

Chitosan, a polysaccharide comprising copolymer of glucosamine and N-acetylglucosamine, derived by the partial deacetylation of chitin, is a nontoxic and bioabsorbable polymer (Muzzarelli et al. 1988; Luppi et al. 2010a) extensively studied for the release of many drugs. Our research group (Cerchiara et al. 2002) described physically cross-linked chitosan hydrogels with lauric, myristic, palmitic, or stearic acid able to enhance the skin permeation of propranolol hydrochloride selected as a hydrophilic model drug. The aim of the work was to improve the permeation of drugs through biological membranes, using hydrogels

made of amphiphilic polymer (Noble et al. 1999). The concomitant presence of hydrophobic and hydrophilic groups in the polymer influenced the swelling properties. So, at pH 7.4 all hydrogels swelled slowly and their behavior influenced the drug release. Among the different chitosan gels, chitosan laurate and chitosan myristate enhanced drug permeation through the skin with respect to chitosan palmitate and chitosan stearate hydrogels (flux values (mg/h cm^2) of propranolol hydrochloride from physically cross-linked chitosans oversaturated hydrogels through porcine skin were 1.00 ± 0.02 for chitosan laurate, 0.87 ± 0.05 for chitosan myristate, 0.47 ± 0.03 for chitosan palmitate, and 0.37 ± 0.01 for chitosan stearate). This could be explained by the interaction of the hydrogels with the stratum corneum, increasing the solubility of the drug in the skin.

Another example of hydrophilic and biocompatible polymer used to design hydrogels able to release hydrophilic drugs through the skin is polyvinyl alcohol. Polyvinyl alcohol cross-linked with succinyl, adipoyl, or sebacoyl chloride was employed as a supporting material to release propranolol hydrochloride. In particular, these hydrogels increased the transdermal permeation of drug, and as described in the previous work (Cerchiara et al. 2002), this effect seems to be linked to an increased drug solubility in the skin, probably produced by the interaction of the polymer with the stratum corneum. Moreover, the maximum enhancement of the drug permeation has been observed in the presence of the higher degree of cross-linking and the shorter length of the cross-linker acyl chain (Orienti et al. 2000).

Luppi and coworkers (2003) used also the cross-linked poly(methyl vinyl ether-co-maleic anhydride) (GZ) as a topical vehicles for pyridoxine hydrochloride, selected as a hydrophilic model drug. In particular, poly(methyl vinyl ether-co-maleic anhydride) was cross-linked with ethylene glycol (GZ-ET), butanediol (GZ-BUT), 1,6-exandiol (GZ-EX), 1,8-octanediol (GZ-OCT), 1,10-decanediol (GZ-DEC), or 1,12-dodecanediol (GZ-DOD). In vitro permeation studies were influenced by the nature of the cross-linker: the decrease in cross-linker acyl chain length provides vehicles accelerating drug permeation through the skin. In fact, flux values (mg/h cm^2) of pyridoxine hydrochloride from hydrogels through porcine skin were 1.29 ± 0.12 for GZ, 5.83 ± 0.22 for GZ-ET, 4.91 ± 0.14 for GZ-BUT, 4.32 ± 0.10 GZ-EX, 3.82 ± 0.09 GZ-OCT, 3.25 ± 0.11 GZ-DEC, and 2.42 ± 0.12 GZ-DOD.

20.2.2 Film-Based Vehicles

Film-based vehicles are the widest utilized and studied transdermal delivery systems based on hydrogels. They are generally distinguished in matrix-type systems, membrane-coated systems, and film-forming solution.

The matrix-type systems are essentially a polymeric layer containing the drug, eventually added with an adhesive layer to enhance the bioadhesion and an impermeable layer to force the release to the skin. In absence of the adhesive layer and in order to assure a controlled delivery of the drug, it is necessary to choose polymers able to guarantee an intimate and prolonged contact with the skin after the application and flexibility and elasticity sufficient to follow the movements of the skin. An adhesive hydrogel patch based on a hydrophilic matrix of poly(N-vinylpyrrolidone) and oligomeric short-chain poly(ethylene glycol) was reported by Feldstein and coworkers (1996). They observed that the delivery rates of drugs with various chemical structures (propranolol, glyceryl trinitrate, isosorbide dinitrate) from the hydrophilic transdermal systems were higher than from the hydrophobic ones (stirene-butadiene rubber/mineral oil, polydimethylsiloxane/ silicone oil, polyisobutylene/mineral oil), and the drug delivery from the hydrophilic matrix across human cadaver skin epidermis or skin-imitating Carbosil membrane in vitro was characterized by zero-order drug delivery kinetics up to the point of 75–85 % drug release from initial contents in matrix. Drug delivery rates from the hydrophilic matrix were controlled by the skin or skin-imitating membrane permeability and may be described by Fick's law (Iordanskii et al. 2000).

Ethylene-vinyl acetate (EVA) matrix was tested as a system for transdermal delivery of atenolol in the presence of plasticizers able to increase the rate of drug release. The effects of drug concentration, temperature, and plasticizers on drug release were investigated. The release rate from EVA matrix was enhanced increasing temperature and drug concentration. In particular, the release rate of drug increased about 1.72-fold when the temperature of release system was raised from 32 to 42 °C and about 1.24-fold when the drug loading dose was increased from 0.5 to 1.5 %. Moreover, drug release from the polymeric matrix followed a diffusion-controlled model, where the quantity released per unit area was proportional to the square root of time. Among the plasticizers used such as alkyl citrates and phthalates, tributyl citrate showed the best enhancing effects: flux values (μg/cm^2/h$^{1/2}$) were 8.056 and 5.327 for EVA matrix containing tributyl citrate and EVA matrix without plasticizer, respectively. The results obtained confirmed that ethylene-vinyl acetate matrix could be used for transdermal delivery of hydrophilic drug (Kim and Shin 2004).

Padula and coworkers (2003) proposed a polyvinyl alcohol film not adhesive in the dry state, but bioadhesive when applied on wet skin. The film was applied to the skin in the presence of a certain amount of water. Water swelled the film on the surface in contact with the skin, transforming a dry polymeric matrix into a jellified polymer layer. This particular film is flexible, is mechanically resistant, and can avoid skin occlusion because of its permeability to water vapor. Compared to a typical patch, the bioadhesive film has a monolayer structure which includes backing, adhesive, and drug reservoir functions. This simple delivery system composed of a smaller number of layers simplifies the preparation procedure of transdermal patches and represents a great innovation in the field of transdermal patch.

Another polymer that represents a good candidate as a starting material for film-based vehicles because of its good film-forming properties is chitosan (Mengatto et al. 2012). Frequently, chitosan is modified by cross-linking reactions to achieve films with improved mechanical properties and obtain an efficient control of drug delivery. In this context, films based on polyelectrolyte complexes were developed for topical and transdermal administration of drugs by Silva et al. (2008). The complexes were prepared with chitosan and different polyacrylic acid polymers, cross-linked with allyl pentaerythritol (Carbopol 71G NF®) or divinylglycol (Noveon AA-1®) at different cross-linking densities. The interaction between the polymers was maximized controlling the preparative conditions of complexes, and the film properties were improved by means of different plasticizers (glycerol or polyethylene glycol 200), a moisturizing agent (Hydrovance®), and an hydrophilic pressure sensitive adhesive (polyvinylpyrrolidone/polyethylene glycol 400). Between the different vehicles obtained, the film prepared by cross-linking with Noveon, plasticized with glycerol and covered with adhesive, has shown very good flexibility, resistance, and bioadhesion, making it a good candidate for further incorporation of drugs for topical and transdermal administration. Recently, chitosan-polyvinyl alcohol blend reticulated with glutaraldehyde has been utilized to prepare transdermal film suitable for insulin release in diabetes chemotherapy (Zu et al. 2012). The hydrogel obtained had a honeycomb-like structure and showed good mechanical and thermal properties. Moreover, the in vitro release studies showed that insulin release is comply with Fick's first law of diffusion showing a high permeation rate (4.421 μg/(cm^{2}h)).

Transdermal delivery represents also an important opportunity for vaccine administration (Prausnitz and Langer 2008). In fact, although vaccines are generally large entities such as macromolecules or viral particles, their small dose facilitates transdermal administration. Ishii et al. (2008) describes simple, easy-to-use, noninvasive transcutaneous vaccination system, formed by an adhesive matrix. This patch is composed of cross-linked acrylic medical adhesive, octyldodecyl lactate, glycerin, and sodium hyaluronate and delivers antigenic proteins to Langerhans cells resident in the epidermal layer without destroying or removing the stratum corneum and induces Th2 (Type 2 helper T cells)-dominant immune response (production of neutralizing immunoglobulin G1 antibodies), effectively preventing viral and bacterial infection.

Finally, Luppi et al. (2010b) formulated transdermal hydroxypropyl methylcellulose-based films containing chlorpromazine hydrochloride for the treatment of psychotic disorders. Film composition was modified by incorporating a chemical permeation enhancer or binary enhancer combinations (oleic acid or polysorbate 80, or both) and a plasticizer (propylene glycol). Both oleic acid and polysorbate 80 had significant effect on drug permeation with respect to the control formulation and films containing a mixture of oleic acid and polysorbate 80 provided the best enhancement activity for chlorpromazine. In fact, the amount of chlorpromazine hydrochloride permeated through pig ear skin from hydroxypropyl methylcellulose films after 100 h were 15.2 ± 0.7 mg/cm^2 for film containing oleic acid (2.15, % w/w on dry basis) and polysorbate 80 (2.15, % w/w on dry basis) and 6.5 ± 0.3 mg/cm^2 for control formulation (film with the same composition, but without permeation enhancers). Moreover, also the hydroxypropyl methylcellulose type and the different concentration of drug and plasticizer contributed to modulate drug permeation. A decrease of hydroxypropyl methylcellulose viscosity, as a function of its molecular weight, and an increase in propylene glycol and chlorpromazine content provided higher cumulative amounts of drug permeated. These results confirm that chlorpromazine permeation can be easily modulated by varying the composition of hydroxypropyl methylcellulose-based films.

Another type of film-based vehicles is membrane-coated systems that consist of a drug depot and an hydrogel-based membrane able to control drug release. Tacharodi and Panduranga Rao (1995) proposed a patch consisting of a chitosan membrane cross-linked with different concentrations of glutaraldehyde and a chitosan gel as propranolol hydrochloride reservoir. Drug release can be easily tailored by changing cross-link density within the membrane and strongly depends on the area of the device. In fact, devices characterized by a diameter of 2.5 cm and chitosan membrane having low cross-link density released about 12 mg of drug within 24 h, while devices with diameter of 2.5 cm and membrane having high cross-link density released 7.5 mg within 24 h. Moreover, devices having a diameter of 2.5 cm and characterized by uncross-linked chitosan membrane released about 15 mg after 24 h, while the same device having a diameter of 1.5 cm released about 4 mg after 24 h. Finally, all the devices delivered propranolol hydrochloride in a near zero order fashion suggesting that these chitosan membranes might be used successfully for the fabrication of membrane-controlled transdermal delivery systems.

A more innovative approach in transdermal drug delivery is represented by film-forming solutions. These systems are essentially polymer solutions, containing dispersed or dissolved drug. After application onto the skin, solvent evaporation guarantees the formation of a bioadhesive and thin film. Compared with matrix-type vehicles and membrane-coated vehicles, these systems are very easy to prepare and they possess higher dosage flexibility, less irritation of the skin, better cosmetic appearance associated with high ease of use. Schroeder et al. (2007) tested a broad range of polymers (acrylates, polyurethane-acrylates, cellulose derivatives, polyvinylpyrrolidones, silicones) as film-forming materials. Polymers at different concentration were solubilized in a volatile solvent (ethanol or volatile silicone), added with different amounts of plasticizer (triethyl citrate, triacetin, dibutyl phthalate) and possibly with a cross-linking agent (succinic acid). Formulations with adequate properties for the application on the skin (low viscosity, short drying time, low outward stickiness, high cosmetical attractiveness, and integrity on the skin for a prolonged time) were evaluated in terms of mechanical properties (tensile strength and elongation at break), water vapor permeability, and transepidermal water loss. The positively evaluated preparations resulting from these experiments provide the basis for the development of film-forming polymeric solutions as a transdermal dosage form.

A novel organic-inorganic hybrid film-forming agent for transdermal drug delivery was prepared by a modified poly(vinyl alcohol) (PVA) gel using γ-(glycidyloxypropyl)trimethoxysilane) (GPTMS) as a cross-linking agent, poly(N-

vinyl pyrrolidone) as a tackifier, and glycerol as a plasticizer. The obtained gels can be applied to the skin by a coating method and in situ form very thin and transparent films with good flexibility and adhesive properties. Furthermore, the skin irritation tests showed that the formulations produced no skin irritation after topical application, while the in vitro release studies revealed that these films are able to release both hydrophilic drugs such as 5-fluorouracil both lipophilic molecules such as ibuprofen. The cumulative release of hydrophilic 5-fluorouracil was much higher than hydrophobic ibuprofen and introduction of adequate GPTMS amount (GPTMS/(PVA + GPTMS) ratio = 20–30 %) into PVA matrix can decrease the crystalline regions of PVA and enhance drug diffusion (Guo et al. 2011).

Conclusions

Transdermal delivery is a major administration route for drugs that are destroyed by the liver when taken orally (Langer 2004). Recently, much attention has been paid to the hydrogels as vehicles for transdermal drug delivery, and their success can be mainly attributed to the possibility to modulate drug release kinetics (Kim et al. 1992). Currently, few drugs have been successfully delivered through the skin utilizing hydrogels, and new opportunities may be envisaged.

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Lecithin Organogels in Enhancing Skin Delivery of Drugs

21

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21.1 Introduction

In the past three decades, LOs have emerged as one of the most effective drug delivery systems for the transdermal delivery of hydrophobic, hydrophilic, as well as amphoteric drugs, including enzymes. LOs are gels formed by the addition of trace amounts of water to organic/nonaqueous lecithin solution. LOs are thermodynamically stable, clear, viscoelastic, biocompatible, isotropic gels composed of lecithin, an organic solvent and a polar liquid. It was Scartazzini and Luisi who first provided information about LOs

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in an article published in 1988. They observed that adding a small quantity of water into a non-aqueous solution of naturally occurring lecithin resulted in drastic rise in viscosity (Scartazzini and Luisi 1988). Two years later, Schurtenberger et al. (1990) published their findings on structural and dynamic properties of LOs. Following these reports, LOs have gained attention from the researchers across the globe. Willimann and Luisi (1991) were the first to unleash the potential of LOs as a matrix for transdermal delivery of drugs. They investigated the ability of LOs as a means to deliver scopolamine across the skin. A tenfold increase in the percutaneous absorption of scopolamine was observed when delivered using LOs as compared to the aqueous solution of scopolamine. Since then, many studies have been conducted by various research groups with LOs as transdermal drug delivery systems. Although LOs have demonstrated their efficacy and safety as delivery systems at the lab scale, their utility as a drug delivery system needs to be further demonstrated in clinical settings. For this purpose, the full biocompatibility profile (efficacy in humans and long-term toxicity) of LOs needs to be explored. Once clinically proven, LOs will emerge as a promising pharmaceutical carrier system for the transdermal delivery of drugs and will replace most of the conventional delivery systems used for the transdermal delivery of drugs.

21.2 Lecithin Organogels (LOs)

LOs are, as aforementioned, thermodynamically stable gels which are composed of lecithin (Fig. 21.1), an organic solvent and a polar liquid. LOs exhibit viscoelasticity, biocompatibility, and isotropicity. They are generally clear in appearance (Willimann and Luisi 1991; Schurtenberger et al. 1990). LOs are formed by a three-dimensional network of entangled reverse cylindrical micelles (Motulsky et al. 2005). First, the lecithin spherical reverse micelles are formed when lecithin is solubilized in nonpolar organic liquids such as isopropyl myristate, isopropyl palmitate, and others (Walde et al. 1990). Later, hydration of these spherical reverse micellar leads to the formation of elongated tubular micelles. These then entangle to form a three-dimensional network in the solution (Shchipunov 2001). The latter serves to immobilize the external organic phase, thus producing a gel form of the initial nonviscous solution, keeping the transparency and optical isotropy of the organogel same as the original (Capitani et al. 1996). The supramolecularly associated micellar aggregates in the entangled state are similar to uncrossed polymers in semi-dilute or concentrated solutions, due to which these systems are often called polymer-like micelles. They are also termed as living or equilibrium polymers, worm-like or threadlike micelles (Shchipunov 1995; Shchipunov and Shumilina 1995).

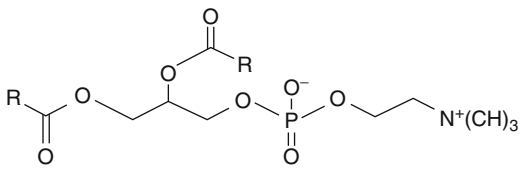

Fig. 21.1 Structure of lecithin

21.3 Physicochemical Properties of Lecithin Organogels

21.3.1 Viscoelasticity

The viscous and elastic nature of LOs follows the Maxwell model of viscoelasticity (Toshiyuki et al. 2003; Shikata et al. 2003). LOs are the three-dimensional structures formed as a result of physical interactions of lecithin molecules with each other. LOs show an elastic property at low shear rate. As the shear rate increases, the physical interactions among lecithin molecules start getting weak, and thereby the three-dimensional fibrous structure of LOs is distorted. Finally, the shear stress reaches its threshold and destroys the LOs' structure. This behavior is termed as plastic flow behavior (Abdallah et al. 2000). The viscoelastic behavior of LOs depends on the concentration of lecithin and the type of organic solvent (precisely fatty acid ester) used. The higher the lecithin concentration, the higher is the viscosity (Schurtenberger et al. 1989). The long-chain fatty acid esters, such

as isopropyl palmitate or cetearyl octanoate, produce LOs with high viscosity, whereas short-chain esters, such as ethyl and propyl acetate, produce LOs of relatively lesser viscosity. It is worthwhile to note that LOs can also be prepared by utilizing mixtures of solvents rather than using a single solvent. Using a blend of organic solvents, we can tailor the viscoelastic properties of LOs based on our needs. Nastruzzi et al. (1994) has developed LOs using a combination of organic solvents and investigated its effect on the viscoelastic properties of LOs. The viscosity of LOs has an impact on the release profile of the drug encapsulated into LOs. In general, the higher the viscosity, the slower is the release of the drug (Shchipunov and Shumilina 1995; Kumar and Katare 2005). Also, medicated LOs are reported to have slightly lesser viscosity than placebo LOs of similar composition (Shaikh et al. 2009; Nastruzzi and Gambari 1994).

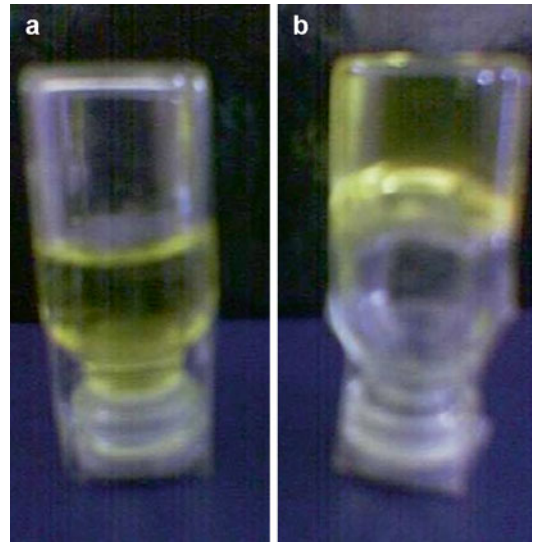

Fig. 21.2 Thermal behavior of the organogels. (**a**) Sol state of the LO at 60 °C and (**b**) semisolid state of the LO at room temperature

21.3.2 Non-birefringence and Optical Transparency

The LOs when viewed under polarized light appear as a dark matrix. This is because the isotropic nature of organogels does not cause passage of polarized light through the matrix. This property of organogels is termed as non-birefringence (Kantaria et al. 1999; Nasseri et al. 2003). Optically, LOs are transparent and provide the benefit of visual inspection so that the presence of any particulate matter can be easily recognized (Kumar and Katare 2005).

21.3.3 Thermoreversibility/ Thermostability

Heating above critical temperature causes LOs to convert from gel state to sol state (Fig. 21.2). The temperature at which sol-gel transition takes place is called as gelation temperature. The gelation temperature varies depending on the solvent system used when preparing LOs. The gel-sol transition in LOs occurs when increased thermal energy within LOs disrupts physical interactions between lecithin molecules, and subsequent cooling of hot LOs causes regain of physical interaction between lecithin molecules which results in sol-gel transition (Avramiotis et al. 2007). The gelation temperature can be determined visibly. It is essential to determine the gelation temperature of the LOs containing drugs as it serves as a guide for recommending storage conditions for drug-loaded medicated LOs (Díaz et al. 2008; Dasgupta et al. 2009; Guenet 2006).

21.3.4 Biological, Physical, and Chemical Safety

Lecithin is an important component of all living cells, and it is recognized by the Food and Drug Administration (FDA) as Generally Regarded as Safe (GRAS) (21 CFR 184, 1400). Compatibility studies with human skin revealed that topical use of LOs is safe (Dreher et al. 1997; Shchipunov et al. 2001; Schurtenberger et al. 1990). Vehicles (organic solvents, such as fatty acid esters, e.g., isopropyl myristate, ethyl oleate) used for preparing LOs are also GRAS excipients. LOs are moisture insensitive, and due to their organic nature, they resist microbial contamination. Thermodynamic stability, ease of preparation and

scale-up, easier quality monitoring, and enhanced topical performance along with biocompatibility and safety upon applications for a prolonged period make the organogels a vehicle of choice for dermal and transdermal drug delivery.

21.4 Salient Features of Lecithin Organogels (LOs)

- LOs can encapsulate various substances with diverse physicochemical characters, i.e., having a different solubility, molecular weight, and size.
- Self-assembled supramolecular arrangement of surfactant molecules imparts spontaneity to organogel formation and hence the process becomes simpler.
- LOs remain structurally integrated for a longer period of time due to their thermodynamic stability.
- LOs are not moisture sensitive; they also resist microbial contamination due to their organic nature.
- Being well balanced in hydrophilic and lipophilic character, they efficiently partition into the skin and enhance the skin penetration and transport of molecules. LOs also provide the desired hydration of the skin in a lipid-enriched environment.
- The use of biocompatible, biodegradable, and non-immunogenic materials for their formulation makes them safe for long-term applications (Kumar and Katare 2005).[1]

21.5 Preparation of Lecithin Organogels

The LOs are readily obtained by adding a minimal amount of polar solvent, such as water, to a solution of lecithin in organic solvents. They are formed by three different components, including organogellator (lecithin), a nonpolar organic solvent as external or continuous phase, and a polar agent, usually water. The transfer into jelly-like state has been demonstrated only for nonaqueous solutions of naturally occurring unsaturated lecithin (Walde et al. 1990; Shchipunov 2001).

21.5.1 Organogellator: Lecithin

Lecithin is a trivial name for 1, 2-diacyl-sn-3-phosphocholine. It belongs to a biologically essential class of substances termed phosphoglycerides or phospholipids. Lecithin is a complex mixture of acetone-insoluble phosphatides, which mainly consist of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol combined with different amounts of other substances such as triglycerides and fatty acids (Reynolds 1996). The main sources of lecithin are soya beans and egg yolk. Lecithin varies greatly in its physical form, from viscous semiliquid to powder depending on the content of free fatty acids. It may also vary in color from brown to light yellow depending on whether it is bleached or unbleached (Wade et al. 1994). Lecithin is commercially available on the market under trade name of Epikuron (Lucas Meyer, Hamburg, Germany), Lipoid S100 (Lipoid GmbH, Ludwigshafen, Germany), and Capcithin (Lucas Meyer, Hamburg, Germany), which are derived and purified from either soya bean or eggs. The desired gelation in organic solvent occurs only when the lecithin contains more than 95 % phosphatidylcholine and is free from fat as well as moisture. Lecithin is a multifunctional surface-active agent. The lecithin molecule consists of two portions: the nonpolar tail (fatty acid portion) and the polar head (phosphoric acid portion). Because of these two aspects, lecithin molecules arrange themselves at the boundary between immiscible liquids such as oil and water. This arrangement reduces the interfacial tension between oil and water and makes relatively stable emulsions (Scartazzini et al. 1988). Its unique lipid molecular structure performs versatile functions. It has a wide variety of roles in pharmaceuticals, cosmetics, and food industries as an emulsifier, viscosity modifier, stabilizer, and solubilizer and penetration enhancer (Szuhaj 1989). They form the lipid matrix of biological membrane and play a key

[1] The above content is adapted and modified from (Kumar and Katare 2005), with permission.

role in the cellular metabolism (Hanahan 1997). Due to its biocompatibility, it is widely used in human and animal food, medicine, cosmetics, and other various industrial applications (Wendel 1995). No systematic research has been done till date in order to investigate the effect of unsaturation in phospholipids on organogelling ability. However, it has been reported that unsaturation in phospholipid molecules affect the nature of self-assembly in which the phospholipid molecules associate and form the microstructures. The property of unsaturation can be interpreted in terms of the degree of hydration of phospholipid molecules that it provides. Unlike saturated hydrogenated phospholipids, unsaturation in phospholipid molecules would result in better hydration of the polar head group, thereby increasing the area per lipid polar head group. Hence, a larger area-to-volume ratio would favorably alter the spontaneous curvature of lipid monomers for the formation of micelles and subsequently their self-assembly to form the micellar network (Shchipunov 2001).

21.5.2 Organic Solvents

Organic solvent plays a vital role in organogels by providing the desired solvent action for the drug (hydrophobic) as well as for lecithin (Kumar and Katare 2005; Moore 1982; Sato et al. 1988). More than 50 organic solvents have been reported to form organogels with water as an aqueous phase. Among them, there are linear, branched, and cyclic alkanes; ethers and esters; fatty acids; and amines. Specific examples include ethyl laureate, ethyl myristate, isopropyl palmitate, cyclopentane, cyclooctane, trans-decalin, trans- pinane, n-pentane, n-hexane, n-hexadecane, and tripropylamine. Among these, the fatty acid esters have been widely used for LO formation (due to better skin feel), but structural investigations have only been performed on hydrocarbons (such as isooctane, cyclohexane). Natural oils including soya bean oil, sunflower oil, rapeseed oil, and mustard oil are proposed as potentially useful organic solvents for preparing LOs (Shchipunov 2001).

21.5.3 Polar Solvent

The third component, a polar agent, acts as a structure forming and stabilizing agent. A series of polar solvents have been studied in order to check their suitability for producing the thickening effect on hydrocarbon and fatty acid ester solutions of lecithin. Water has been used extensively as a polar solvent for organogel formation. However, it has been established that glycerol, formamide, and ethylene glycol have the ability to induce gelation. The gel-forming ability of the polar solvent is governed by its physicochemical properties (Shchipunov and Shumilina 1995, 1996; Shchipunov and Hoffmann 1998). The ability to promote thickening of lecithin solutions has been correlated with the polarity of the solvent used for preparing LOs. This correlation is particularly pronounced in the series of structurally related solvents such as glycerol and ethylene glycol. In a proposed model of organogels, the solvent molecules bridge phosphate groups of neighboring lipid molecules, allowing their association into tubular aggregates through an extensive ribbonlike hydrogen bonding network (Shchipunov 2001). Figure 21.3 depicts schematic diagram of the preparation of LOs.

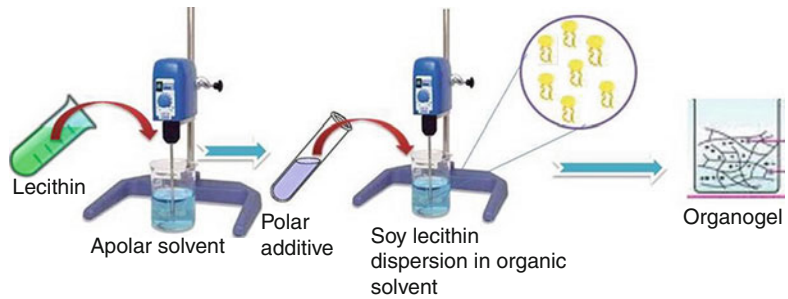

Fig. 21.3 Schematic diagram of the preparation of LOs

It is important to understand that LOs cannot be formed by random mixing of lecithin, an organic solvent and polar solvent. It depends on the mixing of a fixed quantity of these three components. Selection of suitable amounts of these three components can be done by making use of the pseudo ternary phase diagram. A detailed description on how to estimate the determination of the required amount of lecithin/water/organic solvent is described in the next section.

21.6 Identification of Appropriate Quantities of Lecithin/ Organic Solvent/Water Using Phase Diagram

The phase diagram provides information for the boundaries of the different phases as a function of composition variables. The phase behavior of a ternary system of lecithin/organic solvent/polar solvent is mainly governed by the concentration of polar solvent and lecithin (Shchipunov 2001; Shchipunov and Schmiedel 1996). It is defined in terms of a parameter, molar ratio of polar solvent to lecithin (*nw* = [polar solvent]/[lecithin]). When the polar solvent is water, *nw* is also termed w_o (w_o = [water]/[lecithin]). *Nw* is the critical molar ratio of polar solvent to lecithin molecules where gel formation and entrapment of external organic phase occur.

For LOs prepared using different organic solvents but fixed lecithin concentration, the *nw* value varies from solvent to solvent. For isopropyl myristate-based LOs with a lecithin concentration of 200 mM, *nw* value is reported to be 3, whereas for ethyl myristate-based LOs with a lecithin concentration of 200 mM, *nw* value is reported as 5 (Nastruzzi and Gambari 1994). LOs exist in a narrow characteristic range of water-to-lecithin molar ratio, which is reported as *ncr*. It is the critical molar ratio of polar solvent to lecithin molecules at which complete gel formation takes place and entrapment of maximum amount of external organic phase is observed. When *nw* is equal to *ncr*, LOs with maximum viscosity are produced, and when *nw* exceeds *ncr*, phase separation of the LOs occurs (Shchipunov 2001; Shchipunov and Schmiedel 1996a, b). The rheological measurements or visual and optical observations of a ternary system between cross polarizers are widely used to determine n_{cr} values. For a better understanding on the construction of a phase diagram, we wish to describe an example from our reported paper on LO-containing aceclofenac (Shaikh et al. 2009).

A pseudo ternary phase diagram was constructed to determine the concentration range of lecithin, organic solvent (ethyl oleate), and water required for aceclofenac containing LO. This study was carried out for the lecithin concentration ranging from 10 to 60 % w/v because lecithin in proportion above 60 % w/v could not be solubilized. Initially, water in oil microemulsion was stabilized by lecithin micelles formed with a low concentration of water, which is characterized by optical transparency and low viscosity (Fig. 21.4a). As the amount of water was increased, the microemulsion turned to a viscous gel (Fig. 21.4b). Further increase in water resulted in turbidity appearance (Fig. 21.4c) and finally phase separation (Fig. 21.4d). Organogel

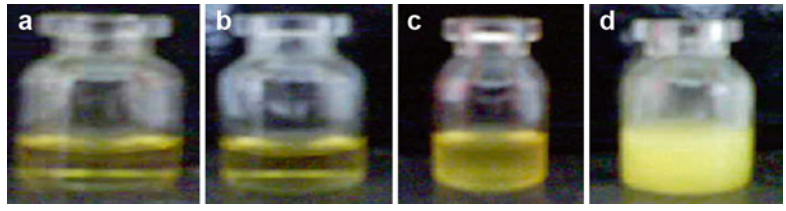

Fig. 21.4 Changes in lecithin-/ethyl oleate-based system with addition of water. (**a**) Water in oil microemulsion with low viscosity and optical transparency, (**b**) microemulsion turned into viscous transparent gel at wo = 4, (**c**) turbidity appearance wo > 4, and (**d**) phase separation at wo > 5

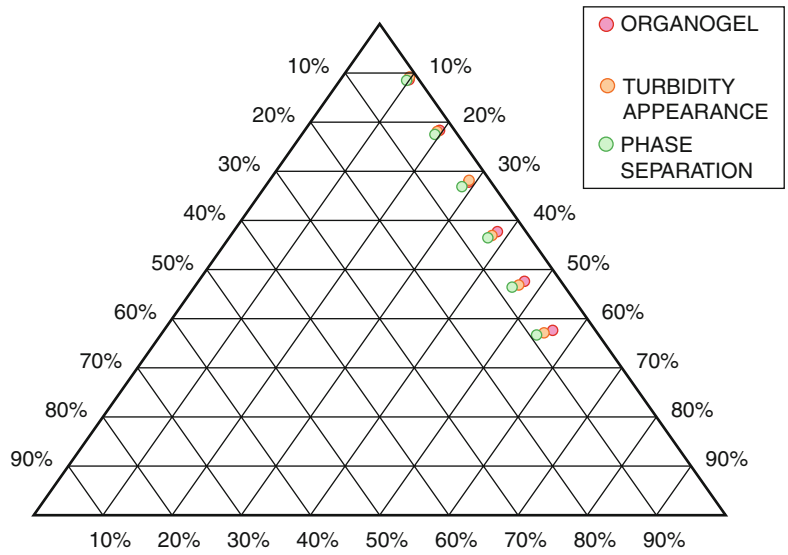

Fig. 21.5 Ternary phase diagram for ethyl oleate/lecithin/water system

existence area was obtained from the above pseudo ternary phase diagram (Fig. 21.5).

Based on the outcome of the phase diagram experiment, it was inferred that the occurrence of the gel phase was lecithin concentration dependent. The water-holding capacity of lecithin organogel increases with the increase in lecithin concentration. Lecithin concentrations ranging from 10 % to 60 % w/v were found to be optimum for preparing LOs.

21.7 Mechanism of Formation Lecithin Organogels (LOs)

The first prerequisite for gel formation is the balance of intermolecular interaction among the gelator molecules (e.g., H bonding, van der Waals interactions, etc.) and between gelator and solvent molecules. A comparative increase in the intermolecular attraction among the gelator molecules and a comparative decrease in the interaction between the gelator molecules and solvent lead to the formation of a molecular dispersion, which further results in the formation of a three-dimensional network in which the solvent molecules are trapped (Fig. 21.6).

Lecithin forms reverse spherical micelles spontaneously when dissolved in apolar solvent alone at a concentration of ~0.01 mM (Shchipunov et al. 1998). The enormous uniaxial growth of these spherical reverse micelles and subsequent transformation into tubular or cylindrical micellar aggregates (sphere-to-cylinder transformation) is triggered by the addition of small and critical amounts of polar additive as shown in Fig. 21.7.

The molecules of polar solvent, on addition, bind in stoichiometric ratios to the hydrophilic head portion of the lecithin molecules in such a way that two adjacent lecithin molecules are bridged together by one polar molecule (Walde et al. 1990; Shchipunov and Shumilina 1995). This leads to the formation of linear networks resulting due to hydrogen bonds formed by the polar molecules and phosphate groups of lecithin molecules and, in turn, to the 1-dimensional uniaxial growth of lecithin reverse micelles. If polar additive concentration is increased further, flexible and long tubular micelles from 2.0 to 2.5 nm in radius and nanometer length are formed (Shchipunov and Schmiedel 1996a, b). After reaching a critical length, these extended micelles begin to overlap, entangle themselves, and build up a transient 3-dimensional network (Shchipunov and Hoffmann 2000; Voit and Shchipunov 2000; Shchipunov et al. 2001).

This marks a crossover to a system characterized by increased viscosity and viscoelastic properties. The organogel formed contains a considerable

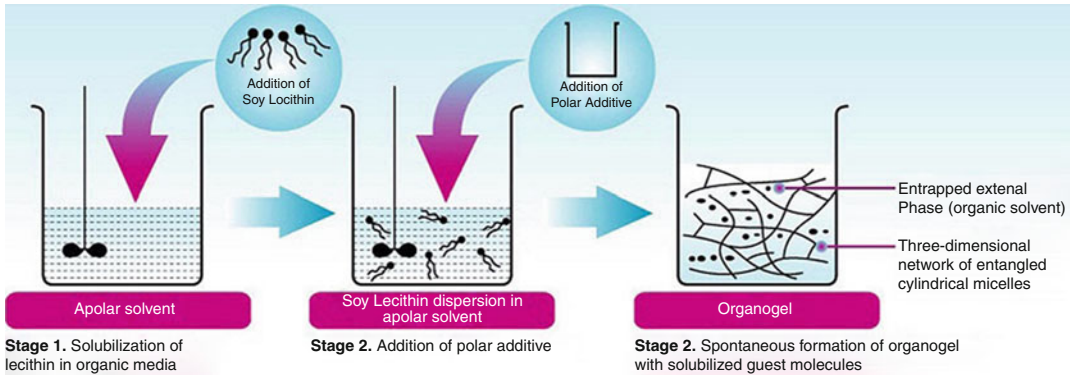

Fig. 21.6 Entanglements of cylindrical reverse micelles to form gel

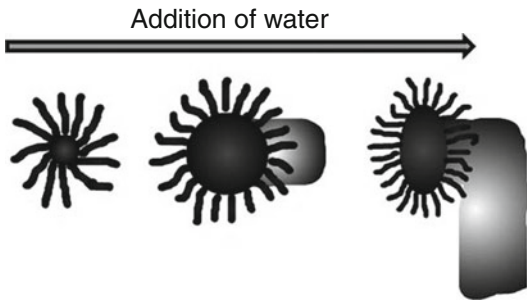

Fig. 21.7 Transformation of spherical micelles into cylindrical micelles

amount of external organic phase entrapped in the spaces between the entangled reverse micelles. The hydrogen bonding network built up by molecules of polar additive and phosphate groups is also accompanied by stiffness of the phospholipid molecule in the region of phosphate group and glycerol residue, which stabilizes the micellar aggregates.

21.8 Characterization of Lecithin Organogels (LOs) (Kumar and Katare 2005)[2]

Interior structural design due to self-associated supramolecules makes the characterization of LOs complicated. These microstructures are formed due to the number of polar-nonpolar interactions. Hence, they become highly sensitive and impart difficulties in investigative studies. It was found that a number of physicochemical properties of LOs such as rheological behavior, physical and mechanical stability, and drug release behavior depend upon the arrangement of molecules within LOs to provide specific structural network (Willimann and Luisi 1991; Schurtenberger et al. 1990; Capitani et al. 1996).

21.8.1 Structural Features

Structural elucidation is important for any organogel system. The isotropic nature and the optical clarity of LOs make their study feasible by various spectroscopic techniques, namely, nuclear magnetic resonance (NMR) spectroscopy (i.e., ^{2}H NMR, ^{31}P NMR), and Fourier transformed infrared (FTIR) spectroscopy. Various techniques have been employed to find the nature of binding forces responsible for the association of monomers to form self-assembled structures, and FTIR spectroscopy has been found to be successful in establishing the hydrogen bonding as one of the major driving forces for the self-assembly of organogelator molecules in the organic solvents (Aboofazeli et al. 2002; Bonina et al. 1995).

The molecular packing of organogelator molecules inside the organogel network was studied by scanning and transmission electron microscopy (SEM and TEM, respectively) (Willimann and Luisi 1991), dynamic and static light scattering

[2]The content of Sect. 21.8 is partly adapted from the reference and suitably modified with due permission.

(elastic or quasielastic light scattering (QLS) techniques) (Schurtenberger et al. 1990, 1993; Capitani et al. 1996; Sato et al. 1988; Aboofazeli et al. 2000), small-angle neutron scattering (SANS), small-angle X-ray scattering (SAXS), and atomic force microscopy (AFM) (Zemb et al. 1990; Terech and Weiss 1998; Simmons et al. 2001; Gronwald et al. 2002). These techniques allow many features of organogels to be studied at 1–1,000 nm scale. Recently, SAXS and AFM are used to study the molecular arrangement of LOs (Gronwald et al. 2002; Abdallah et al. 2000).

The scattering information using SAXS and SANS measurements on organogels combined with mathematical analysis provides details about the static correlation length ξ, mesh size of the network (or the number density of entanglements "ν"), and diffusion coefficients. They also give information on the flexibility of the fibrous network, along with the structural features of the cross sections of LOs (Terech and Weiss 1998; Simmons et al. 2001; VanEsch and Feringa 2000; McAllister et al. 2002). The direct visualization of the gel in its naïve state is possible using AFM, which allows observing the microstructures of the fibrous network throughout the gel mass. It also provides structural details where micellar fibers or chains aggregate into large-sized bundles. Thus, multiple instrumental techniques based on microscopy along with spectroscopic and scattering analysis can help reveal the structural details of the LO systems.

21.8.2 Rheological Behavior

LOs have been studied extensively for their rheological attributes and have been determined to be viscoelastic in nature (Shchipunov 2001; Shchipunov and Hoffmann 2000). The critical parameters such as spreadability, adhesiveness, cohesiveness, and gel consistency need to be modified in a favorable manner. It has been reported that the Maxwell rheology model holds good for systems with supramolecular organization, consisting of a temporal three-dimensional network of entangled micelles. Also, the desired viscoelastic property can be managed by modifying the various formulation components (i.e., selecting the type of organic solvent, concentration of gelator or cosurfactant, or the type or amount of polar agent), which significantly influence the structural stability and rheological behavior of organogels (Voit and Shchipunov 2000).

21.8.3 Phase Transition Temperatures

The phase transition temperature (PTT) gives an idea about the nature of microstructures which are found in the network of organogels. The phase behavior of organogels varies on changing temperature conditions. PTTs not only help in optimizing the organogels' composition (Jibry et al. 2004) but also reveal the microstructural homogeneity of the prepared organogel system. For example, a narrow PTT range (i.e., 3–5 °C) indicates homogenous microstructures within the gel (Terech 1985). Accurate and sensitive methods like hot stage microscopy (HSM) and high sensitivity differential scanning calorimetry (HSDSC) are used to measure PTTs. However, the inverse flow method, a simple technique based on visual observations, has also been employed (Couffin-Hoarau et al. 2004).

21.8.4 Water Content

The water content of an organogel system is important to be maintained, as evaporation of water can cause a consequent decrease in viscosity, thus affecting the gel stability. Near-infrared (NIR) spectroscopy has been proposed as a simple, rapid, and nondestructive technique for determining the water content in organogels (Nastruzzi and Gambari 1994). This technique can also identify syneresis in organogels.

21.9 Mechanism of Penetration Enhancement of Lecithin Organogels (LOs)

Drugs administered by conventional means often have undesirable side effects and are many times ineffective. Transdermal delivery of drugs

provides advantages like avoidance of first-pass metabolism, increased drug efficacy, etc. But as the skin is an exceptionally effective barrier to most chemicals, very few drugs can penetrate it in a manner sufficient to deliver therapeutic dose. Therefore, drug delivery systems that have the ability to traverse the skin by transient opening of pores and thereby delivering the drugs to their site of action are of great interest. Traditionally; to formulate such drug delivery systems; chemical penetration enhancers are incorporated in the formulations. However; long term use of chemical penetration enhancers are associated with skin irritation and sensitization. Thus; long term use of these chemical penetration enhancers are avoided. Lecithin is a biocompatible and biodegradable surfactant as well as a cellular component, which acts as a penetration enhancer (Schneider 1997). A wide variety of molecules, such as vitamins A and C, hormones, peptides, amino acids, local anesthetics, and antifungal agents were reported to have increased permeation when delivered using LOs as a matrix for transdermal/topical drug delivery. The permeation enhancement induced by LOs is attributed to (a) lecithin and (b) organic solvents used for LO preparation.

Lecithin is reported to disorganize the structure of the skin; it opens up the pores of the skin transiently and thus increases the penetration of various drugs (Natsuki and Takabatake 1987). Although the exact mechanism of how lecithin causes skin alteration is not yet clearly understood, it is anticipated that it could be due to the interaction between the skin lipids and the lecithin's phospholipids. Mahjour et al. (1990) studied the effects of commercial lecithin, Epikuron 135f (Lucas Meyer, Hamburg, Germany), in tetraglycol and egg yolk lecithin in propylene glycol on the in vitro permeation of procaterol, dextromethorphan, oxymorphone, and diphenhydramine using hairless mouse skin as a model membrane. Vehicles (tetraglycol and propylene glycol) without lecithin were considered as control. The average flux for procaterol in the control preparation (tetraglycol alone) was 0.05 μg/cm^2/h and that for commercial soya bean lecithin-containing formulation (Epikuron plus tetraglycol) was 5.06 μg/cm^2/h. Similarly, the average flux for procaterol in the control preparation (propylene glycol alone) was 0.14 μg/cm^2/h and that for egg yolk lecithin containing formulation (egg yolk lecithin plus propylene glycol) was 3.47 μg/cm^2/h. As exemplified, their research suggested that both commercial soya bean lecithins and egg yolk lecithins are effective skin penetration enhancers and that the control preparations resulted in significantly decreased permeation of the drug molecules. They reported that the enhancement effect could be mainly due to the reduction of skin resistance to drug permeation induced by surfactant action of phospholipids present in lecithin. Bentley et al. (1997) investigated the permeation of hydrocortisone acetate from poloxamer gels containing penetration enhancers, such as lecithin (8 % w/v) or urea (12 % w/v). Lecithin was found to significantly increase the permeation of hydrocortisone acetate as compared to urea. They investigated the mechanism of penetration enhancement by differential scanning calorimetry (DSC) and FTIR spectroscopy using the SC from hairless mouse treated with solutions of lecithin and urea. The DSC and FTIR results suggested that lecithin has a more pronounced effect with the intercellular lipids than urea and thereby aids in increased skin penetration of hydrocortisone acetate.

As to *organic solvents*, there are a wide range of organic solvents, such as ethyl laureate, ethyl myristate, isopropyl palmitate, isopropyl myristate, cyclopentane, cyclooctane, and n-hexadecane, that can be used for LO preparation. Organic solvents containing fatty acid esters give better feel to the skin as compared to the organic solvent containing hydrocarbon oils and, thus, are preferred for preparing LOs. Moreover, fatty acid esters have shown to increase the permeation of drugs, such as aceclofenac, scopolamine, and broxaterol (Shaikh et al. 2009; Willimann and Luisi 1991). The exact mechanism of skin penetration enhancement effect of fatty acid esters is not known, but it is proposed that these solvents enhance skin permeation by solubilizing the SC (Fujii et al. 1996; Dreher et al. 1996, 1997).

21.10 Improved Skin Penetration Enhancement and Enhanced Efficacy of Drugs with Lecithin Organogels (LOs) as Drug Carriers

LOs have shown great promise as a drug matrix for enhanced skin penetration of drugs. There is another drug delivery system related to LO, i.e., Pluronic Lecithin Organogel, or PLO, that has shown the ability to enhance the dermal/transdermal delivery of various drugs. However, in this section, we will be dealing only with the application of LOs as skin penetration-enhancing drug matrices. The readers are encouraged to refer to reviews on PLO from Kumar and Katare (2005), Murdan (2005), and Almeida (2012) for details on preparation and applications of PLO. As to LOs and their application as complex penetration enhancers, examples are given below:

- Willimann et al. (1992) have reported LOs containing scopolamine. LOs were prepared with lecithin/IPP/water. They investigated the comparative ability of LOs with aqueous solution of scopolamine as a means to deliver scopolamine across the skin. They observed a tenfold increase in the percutaneous absorption of scopolamine when delivered using LOs as compared to the aqueous solution of scopolamine.
- Nastruzzi and Gambari (1994) have reported the transdermal delivery of the aromatic polyamidine TAPP-Br (compounds with antitumor activity) formulated in LOs. LOs were developed using lecithin/IPP/water. They demonstrated the reduction of the tumor mass developed in nude mice injected with a Ha-ras-1 transformed cell line when treated with LOs containing TAPP-Br.
- Bhatnagar and Vyas (1994) have reported LOs containing propranolol. LOs were developed with lecithin/isooctane/water. They conducted a comparative estimation of propranolol permeation with LOs and petroleum jelly. They inferred that the permeation rate of propranolol was increased by tenfold with LOs when compared with petroleum jelly.
- Bonina et al. (1995) have reported an enhanced in vitro and in vivo skin penetration of methyl nicotinate for LOs and lecithin-based liposomes as compared to phospholipid free vehicles (carbomer- and carboxymethyl-based hydrophilic gels). LOs were prepared by using lecithin/IPP/water. Their results emphasized the significance of having lecithin in transdermal preparations so as to get better effects. The results showed rapid induction and intense persistence of methyl nicotinate-induced erythema when LOs were tested in human subjects. The author proposed the supramolecular aggregation structure of lecithin (which leads to a strong interaction of lecithin with the SC) as a probable cause of enhanced methyl nicotinate skin penetration from LOs.
- Dreher et al. (1996) have reported their study on diclofenac incorporated in LOs. LOs consisted of lecithin/IPP/water. Improved skin penetration of diclofenac by 3.5-fold was observed for LOs as compared to the vehicle (IPP alone) alone. In addition, Grace et al. (1999) have assessed the therapeutic efficacy of a 2 % diclofenac LO by conducting a randomized clinical trial in patients suffering from osteoarthritis. A significant reduction of knee pain in osteoarthritic patients was reported with the 2 % diclofenac LOs.
- Fujii et al. (1996) proved greater permeation of indomethacin from LOs through rat skin. The gels were prepared by lecithin/isocetyl isostearate/water. Their study suggested that the skin permeation of indomethacin was enhanced by fourfold when LOs was used as drug matrix as compared to isocetyl isostearate-based suspension. They attributed the higher skin penetration of indomethacin in LOs due to higher solubility and better skin permeation of the drug in isocetyl isostearate and lecithin.
- Aboofazeli et al. (2002) have reported a significant improvement in the transdermal delivery of nicardipine with LOs as compared to pure solvent-based formulations. LOs were prepared by lecithin/isopropyl myristate/

propylene glycol/oleic acid/dimethyl isosorbide/water. The cumulative amount of nicardipine released from gel matrix was approximately threefold higher than that from a tertiary solvent formulation (propylene glycol/oleic acid/dimethyl isosorbide) containing nicardipine. This suggested that the gel matrix possesses a higher skin penetration enhancement effect compared to the pure solvent formulation. The author attributed the skin enhancement effect of LOs to lecithin as well as the solvent mixture (propylene glycol/oleic acid/dimethyl isosorbide)

- Nasseri et al. (2003) developed LOs containing varied concentrations of lecithin/isopropyl myristate/water for topical application of ketorolac tromethamine (6.5 % w/w). They tested the in vitro release profile of various LO preparations containing ketorolac on guinea pig skin. Their study suggested that LOs prepared with a lecithin/IPM weight ratio of 40:60 and 0.6 % w/w of water provided maximum skin penetration of ketorolac tromethamine.
- Agrawal et al. (2004) have developed lecithin-/IPM-/water-based LOs containing piroxicam. They reported increased solubility of piroxicam in LOs and its improved in vitro skin permeation. In addition, they investigated the anti-inflammatory action of LOs by conducting the carrageenan-induced rat paw edema test and compared it with that of marketed piroxicam gel. The outcome of the rat paw edema test indicated that after 3 h of study, the LOs are twofold more effective than the marketed gel in reducing the edema. They attributed this enhanced performance of LOs to the lecithin present in the formulation.
- Shaikh et al. (2009) have reported enhanced skin penetration of aceclofenac from LOs as compared to aceclofenac from hydrogels. LOs were prepared using lecithin/ethyl oleate/water. They reported higher drug flux from LO as compared to hydrogels. It was proposed that skin penetration enhancement of aceclofenac could be due to lecithin and ethyl oleate in LOs. LOs not only provided increased penetration but also improved the solubility of aceclofenac by 13-fold as compared to the pure ethyl oleate. Moreover, the paw edema test conducted in mice demonstrated LOs as a better formulation than hydrogels in treating the inflammation.

21.11 Safety Profile of Lecithin Organogels (LOs) as Drug Carrier

Lecithin organogels (LOs) contain a fairly high level of surfactants and organic solvents. Therefore, it is important to consider the safety and irritancy of the formulation on prolonged use. Skin (human and mice) compatibility studies using LOs have been evaluated and some examples are reported below:

1. Willimann et al. (1992) have conducted a comparative safety study of LOs containing isopropyl myristate and control samples (physiological NaCl solution) on human skin using microscopic (light) investigation. During the study, human skin was treated with LOs and control sample and observed for 3 days. The outcome of the study indicated that there were no significant alterations in the skin before and after treatment of LOs and the LOs applied skin sample was comparable with that of control samples.
2. Dreher et al. (1996) has assessed the skin irritation potential of LOs (containing isopropyl palmitate and lecithin) by conducting cumulative irritation tests. A cumulative irritation study was conducted by applying LOs in 20 volunteers. The volunteers were observed for skin irritancy for a period of 21 days. The parameter "IT50" (irritation time of 50 % of test population) was used as an index for the safety level. Results indicated a very low cumulative skin irritation potential of LOs (IT50 of 13 days) and suggested the suitability of LOs as a safe dermal and transdermal drug delivery system for long-term applications.
3. The safety of ethyl oleate-based LOs in hairless mouse skin was investigated by conducting histopathological studies. The histopathological investigation of mouse skin after application of the LOs demonstrated that the formulation has no toxic effects on the

skin and all the surface epithelium lining and the granular structure remained intact. This ensures the safety of LOs as a formulation for the delivery of drugs via the skin (Shaikh et al. 2009; Jadhav et al. 2009).

Conclusion

Ease of preparation, biocompatible components, the ability to accommodate hydrophobic or hydrophilic micro/macromolecules, thermoreversible nature, skin permeation-enhancing ability, and long shelf life have resulted in wide acceptance of LOs as dermal and transdermal drug delivery systems. The skin permeation-enhancing ability of LOs is attributed to both lecithin and the organic solvent used for preparing LOs. The effect of addition of small amounts of conventional penetration enhancers into LOs on skin penetration needs to be investigated. Further, the effect of using two organic solvents for preparing of LOs instead of one solvent on formulation attributes can also be explored, and its effect on skin permeation can be studied. In addition, LOs have demonstrated their efficacy and safety as a drug delivery system at the lab scale. However, their utility as a drug delivery system needs to be further demonstrated in clinical studies. For this purpose, the full biocompatibility profile (efficacy in humans and long-term toxicity) of LOs needs to be explored. Once clinically proven, LOs will emerge as a promising pharmaceutical carrier system for enhancing the skin delivery of a wide range of drug candidates.

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Thermosensitive Hydrogels in Dermatology: A Multidisciplinary Overview

22

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22.1 Introduction

The hospital triad formed by the dermatologist, the pharmacist, and the nurse is involved in the prescription, the preparation, and the administration of adapted, efficient, and safe medicines. Each professional defines specific requirements concerning dermatological treatments which might be schematically conceptualized in Fig. 22.1. However, many medical and nursing requirements concerning topical treatments imply, for hospital pharmacist stuck "between the

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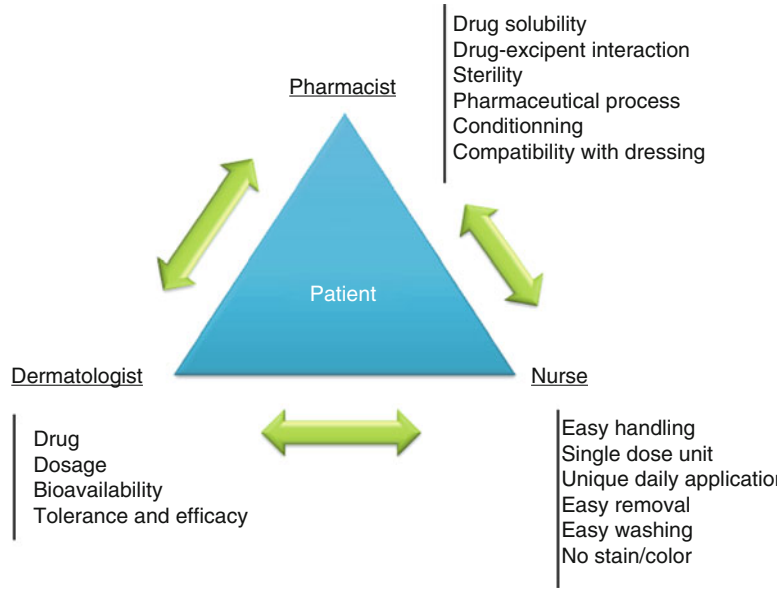


Fig. 22.1 The hospital triad involved in dermatological medicine management and major outcome that should be considered by each protagonist

mortar and the pestle", numerous practical considerations.

Firstly, active pharmaceutical ingredients (APIs), currently prescribed by dermatologist, exhibit low aqueous solubility (e.g., class II, diclofenac; class IV, sulfadiazine) and low permeability (e.g., class III, acyclovir, and class V, acetazolamide) which complicate the selection of excipients for compounding. Therefore, pre-formulation studies are usually necessary for screening appropriate excipients when compounded preparations prescribed are not detailed or indexed in the Pharmacopeia and the Formulary (i.e., national compendia for chemical and biological drug substances, dosage forms, and compounded preparations, excipients, medical devices, and dietary supplements). Both national compendia display substantial heterogeneity in their contents over the world.

Secondly, although medical and scientific literature detailed many original excipients, alone or in combination, enabling the formulation of APIs for topical treatment, few topical preparations are reported in the Pharmacopeia and the Formulary.

Thirdly, the suppliers of pharmaceutical grade excipients necessary for dosage forms and compounded preparations are (1) scarce, (2) sometimes located in foreign country limiting importation and/or exportation of pharmaceutical products, (3) not permanently approved by local health and safety regulatory authorities, and (4) not scaled for small production, packaging, and shipment of excipients and APIs to health-care hospital or clinical establishments.

Fourthly, the safety of excipients is recurrently questioned by authorities from the analysis of notable adverse effects imputable to excipients shifting their status from inactive to mystery ingredients, reducing again the width of the field of choice (Noiles and Vender 2010).

Fifthly, the conservation, the packaging, and storage of topical formulations is a major concern since the use and reuse of the preparation is an obvious source of human and exogenous contamination, a factor of physicochemical degradation (e.g., hydrolysis, oxidation) of APIs and excipients, and an issue for formulation instability (e.g., syneresis, creaming, sedimentation).

Again, the degree of purity of excipients, from different origins (i.e., from biological or mineral to chemical-based synthesis), is often weakened by concomitant components or processing aids, and the final use of excipients is not always known by the supplier. Therefore, the choice of appropriate excipients for topical compounding is also a com-

promise between pharmaceutical state of the art and the regulatory and availability status of excipients. Facing (i) the pharmaceutical compounding challenge, (ii) the inherent restrictions of available, authorized, and harmless excipients, (iii) the package features, surely, the simplest drug-excipient combination for ready-to-use and easy-handling product is highly needed for the formulation development of topicals.

However, the pharmacist experiences that, at some points, the development of topical preparation leads to consider top-ten recommendations:

1. Avoiding the use of many excipients, to prefer straightforward process of preparation where APIs are quickly dissolved, miscible, or suspended in aqueous solvent supplemented by not more than three excipients
2. To choose excipients insuring both physical and chemical stability of APIs, excipients, and formulation
3. To reduce pH variation of formulation over time during skin exposure (skin surface – pH ~5.5)
4. To check the probability to reuse and to avoid contamination of formulation
5. To guarantee easy spreading and removal, sustainability, and aesthetical acceptability (i.e., feel, color, fragrance, absorbability) of formulation
6. To permit optimal API penetration into skin structures (dermal delivery)
7. To permit optimal API permeation through skin structures (transdermal delivery)
8. To favor or to limit the buildup of APIs and excipients into the skin
9. To improve the cutaneous tolerance to APIs and excipients
10. To improve the efficacy of APIs into the skin or after percutaneous delivery

Therefore, few excipients might fulfill prerequisites detailed above. Among likely candidates, excipients forming thermosensitive (also called thermoresponsive or thermoreversible) hydrogels offer many advantages which have been extensively detailed in reviews published in the last decade (Jeong et al. 2012; Klouda and Mikos 2008; Ruel-Gariépy and Leroux 2004). The main intrinsic advantages of thermosensitive hydrogel are as follows: (1) high water content, (2) solubilizing properties for hydrophobic APIs, (3) control of swelling properties and gelling temperature, (4) adaptation for tailor-made formulations in specific dermatologic diseases, and (5) versatile skin drug delivery from either surface application, intradermal or subcutaneous injection.

In the followings sections, physicochemical properties of current and innovative thermosensitive polymers are presented, and then the actual and prospective dermatological applications of thermosensitive polymer-based formulations are emphasized.

22.2 Thermosensitive Polymers

22.2.1 General Considerations About Hydrogels

The gelation in the aqueous solvent is a complex phenomenon where a polymer initially soluble in water becomes more hydrophobic by (1) interaction with mineral ions (e.g., gellan gum, natural anionic heteropolysaccharide, sodium alginate, natural polysaccharide), (2) variation of pH (e.g., polymers carrying carboxylic acid, phosphoric acid, and amine groups) leading to a change of conformation and swelling behavior (Schmaljohann 2006), or (3) modification of temperature. As a result, a transparent or translucent semisolid polymeric matrix is obtained where the fluid flow is limited by entrapment and immobilization of the solvent molecules and possesses remarkable mechanical properties (deformation, viscoelastic properties) which facilitate further cutaneous spreading.

The regional ionic strength upon the outermost layer of the skin, the *stratum corneum*, is likely insufficient to elicit gelation with ionic-responsive polymers (i.e., making necessary pregelation of formulation containing appropriate ionic strength) (Aust et al. 2012), while acidic pH (~5.5) at the skin surface do not allow a gelation of common acidic polymer (e.g., carbomer).

Besides, the regulation of body temperature, one of the major skin functions in homeostasis, might be exploited for the successful development of thermosensitive hydrogels.

Moreover, interactions between skin and thermosensitive polymers have been of growing interest in the past decades as (1) intimate properties and mechanics of such polymers were gradually documented and (2) skin is regarded as a promising alternative to traditional oral or parenteral routes for the administration of active pharmaceutical ingredients. Furthermore, interesting parallels between skin or subcutaneous tissues and hydrogels in terms of chemical and physical characteristics draw exciting perspectives for future developments in experimental and clinical fields (Lee et al. 2009).

22.2.2 General Considerations About Thermosensitive Hydrogels

The ability for a solution of polymer to modify its bulk viscosity in response to temperature variation is called thermosensitivity. Generally natural polymer solutions form gels at low temperature and liquefy when temperature rises, but chemically modified polymers or synthetic polymers may exhibit opposite behavior defined as reverse thermosensitivity. As the physical state (i.e., free flowing or non-flowing during usage time) can be controlled by thermal modulation, formulas containing those polymers may have innovating pharmaceutical applications due to control of solute transport abilities and biocompatibility. Various polymeric molecules exhibit thermosensitive properties such as natural polymers (e.g., gelatin, agarose, carrageenans), modified natural polymers (e.g., cellulose derivatives, chitosan, dextran, xyloglucan), synthetic polymers (e.g., N-isopropylacrylamide and its copolymers), or poloxamers (i.e., poly(ethylene oxide)/poly(propylene oxide), polyethylene glycol/polyester copolymers) (Table 22.1) (Jeong et al. 2012; Klouda and Mikos 2008; Ruel-Gariépy and Leroux 2004).

Figure 22.2 shows the literature content and patent applications over the last decade underlying the importance of natural and modified natural polymers in the dermatological research and subsequent clinical outcomes. Although well known and considerably used, cellulose derivatives and chitosan still motivate some intellectual property issues.

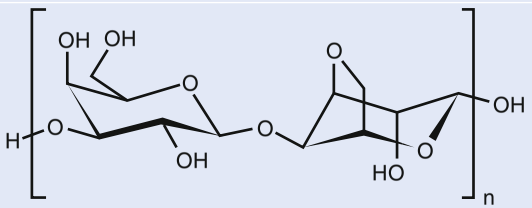
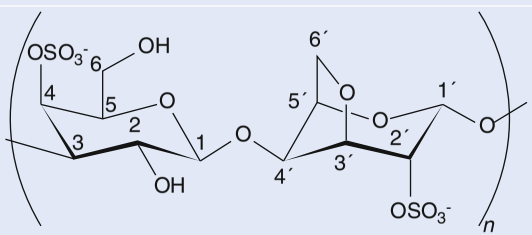
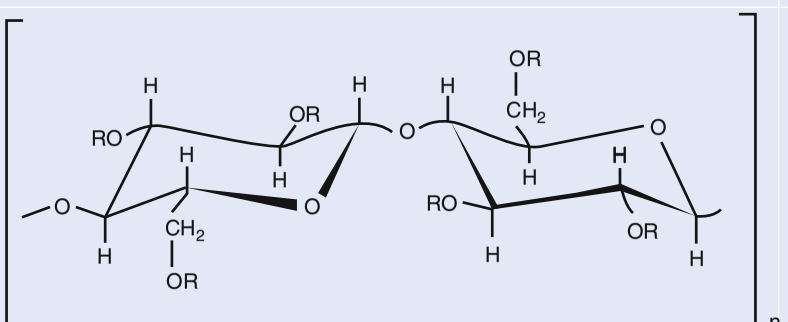
22.2.3 Physicochemical Characteristics of Thermosensitive Hydrogels

Many thermosensitive polymers are amphiphilic block copolymers with A-B or A-B-A type structures (A: hydrophilic block; B: hydrophobic block) that will form micelles in aqueous solvents. Polyethylene glycol (PEG) is commonly used as hydrophilic block due to biocompatibility and high water solubility. Hydrophobic block usually forms the drug-binding core of the system in hydrogels showing a large variety of structures such as polypropylene glycol and polyesters such as poly(lactide-co-glycolide) and poly(lactide-co-caprolactone) (Rijcken et al. 2007). Usually, polymer concentration in solvent will determine gelation temperature (Lenaerts et al. 1987). Nature and ionic content of thermosensitive polymer gel solvents is of great importance as it can significantly modify drug release properties (Pandit and Wang 1998; Ur-Rehman et al. 2010) and gelation ability (Pandit and Kisaka 1996). Also, ionic content has an influence on pH and drug ionization state which is an important issue to consider regarding skin-formulation interactions.

22.2.4 Rheological Properties of Thermosensitive Hydrogels

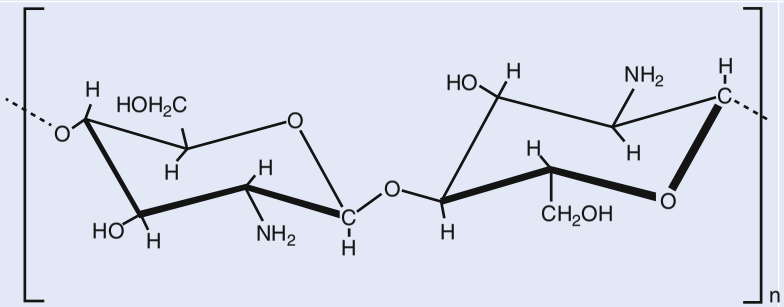
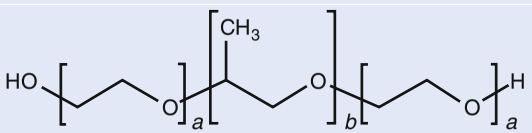
The mechanism of this viscosity change, called sol→gel transition when viscosity increases or gel→sol transition when it decreases, is dependent on molecular interactions within the polymer solution. Natural polymer solutions mostly form a gel phase when temperature is low and liquefy on heating. In these hydrogels, polymer molecules arrange in a partial helicoidal architecture that destructures in a random coil conformation when temperature rises (Ruel-Gariépy and Leroux 2004).

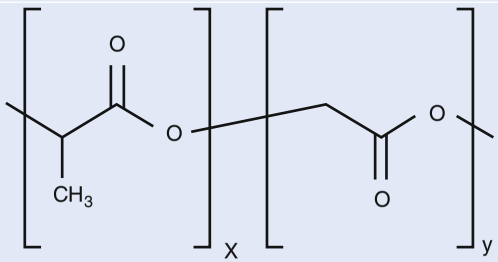
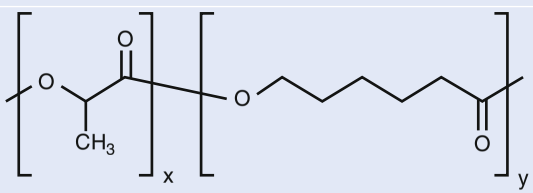
Table 22.1 Structures and formulas of polymers currently used for thermosensitive hydrogel formulations in commercial products or pharmaceutical compounding

Polymers	Formula	Molecular weight ($g.mol^{-1}$)	USP/Eur. Ph	CAS number
Gelatin[2]	e.g., -Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro-	15,000–250,000	Referenced/ referenced	9000-70-8
Agarose[2] (Agar)		306 • n	Referenced/ referenced	9063-31-4
Carrageenans[2]		451 • n	Referenced/*not referenced*	9000-07-1
Cellulose derivatives	 R = -H, $-CH_3$, $-CH_2CH_2OH$,$-CH_2CH(OH)CH_3$ $-CH_2OCH_2COONa$			

(continued)

Table 22.1 (continued)

Polymers	Formula	Molecular weight ($g.mol^{-1}$)	USP/Eur. Ph	CAS number
Methylcellulose[④]		10,000–220,000	Referenced/ referenced	9004-67-5
Hydroxyethylcellulose[②]		–	Referenced/ referenced	9004-62-0
Hydroxypropyl-cellulose[①]		50,000–1,250,000	Referenced/ referenced	9004-64-2
Hydroxyethyl methylcellulose[①]		–	*Not referenced/not referenced*	9032-42-2
Hydroxypropyl-methylcellulose[①]		10,000–1.500,000	*Not referenced/* referenced	9004-65-3
Carboxymethyl cellulose sodium[②]		90,000–700,000	Referenced/ referenced	9004-32-4
Chitosan[②]		320 • n	*Not referenced/* referenced	9012-76-4
Poloxamers				
124[⑤]	*a:* 10–15; *b*: 18–23	2,090–2,360	Referenced/ referenced	9003-11-6
188[⑤]	*a*: 75–85; *b*: 25–30	7,680–9.510	Referenced/ referenced	9003-11-6
237[⑤]	*a*: 60–68; *b*: 35–40	6,840–8,830	Referenced/ referenced	9003-11-6

338®	*a*: 137–146; *b*: 42–47	12,700–17,400	Referenced/ referenced	9003-11-6
407®	*a*: 95–105; *b*: 54–60	9,840–14,600	Referenced/ referenced	9003-11-6
Polyesters copolymers[a]		2,000–100,000 or higher	*Not referenced/not referenced*	
Poly(lactide-co-glycolide)				30846-39-0 26780-50-7
Poly(lactide-co-caprolactone)				70524-20-8 65408-67-5

Reference of polymers in the United States (29) and European Pharmacopoeia (7.6) as monographs is reported as well as chemical abstract service (CAS) numbers. Dynamic viscosity behavior as a function of heating is reported as follows: ① sol→gel transition, ② gel→sol transition, ③ sol→gel→sol transitions, and ④ gel→sol→gel transitions

[a]Hydrophobic moieties of polyester copolymers are presented as poly(lactide-co-glycolide) and poly(lactide-co-caprolactone). Hydrophilic moieties linked to hydrophobic polyesters determining thermosensitive properties are, e.g., polyethylene glycol blocs

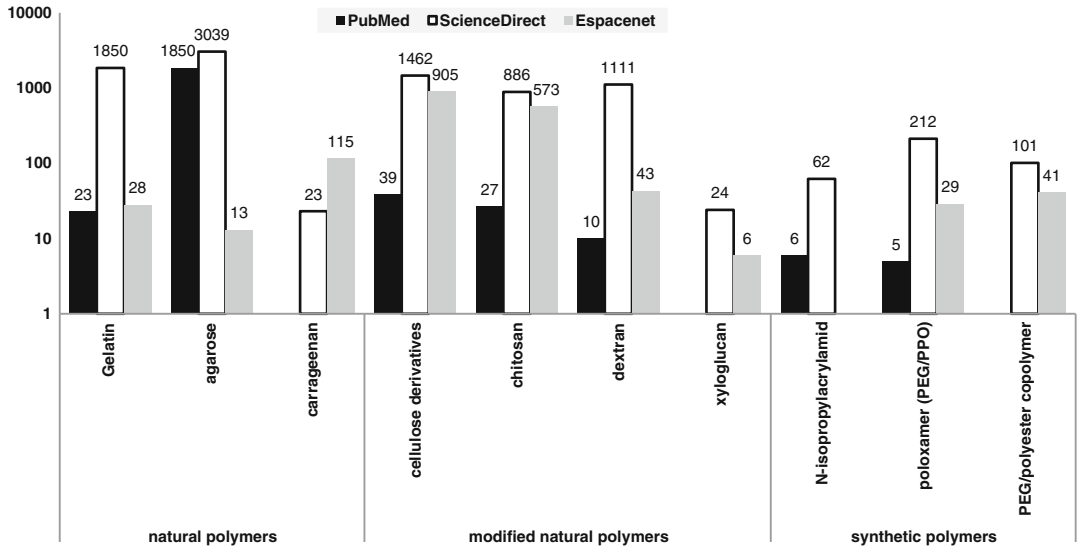


Fig. 22.2 Review of the literature was built by using bibliographic (*PubMed* and *ScienceDirect*) and patent research (*Espacenet*) databases running "*polymer* (e.g., gelatin) and skin" keywords for the last decade. In spite of the growing interest for synthetic polymers, research studies, medical applications, and intellectual property still focus on natural and modified natural thermosensitive polymers

Oppositely, reverse thermosensitive polymer solutions are liquid when temperature is low and gelify on heating. Temperature variations modify the affinity (i.e., hydrophilicity, hydrophobicity) between polymer solution components (i.e., polymer molecules and solvent molecules) resulting in changes in the intimate architecture of the solution. Globally, an increase in polymer's solvent solubility results in low viscosity (sol), whereas a decrease in polymer's solvent solubility results in polymer molecule network formation and therefore gelation (Klouda and Mikos 2008).

Experimental determination of the boundary between sol and gel states is compulsory to thermosensitive formulation characterization. The simplest method is the tube inversion method where a test tube containing polymer solution is tilted to evidence formulation flow under different temperature conditions with standardized test parameters (i.e., time, tilting rate, tube diameter, and amount of solution). Another simple method is the falling ball method where a small dense ball is deposited on the top of the gelified polymer solution. The temperature point at which the ball penetrates the solution under standardized conditions is the gel→sol transition temperature. Differential scanning calorimetry (DSC) may be used to determine transition temperature by detection of gelation endothermic peak and also gives information about enthalpy of gelation. Eventually, dynamic mechanical analysis by a rheometer in a thermally controlled environment enables to obtain precise data about sol→gel and gel→sol transition (Jeong et al. 2012).

22.3 Formulation of Thermosensitive Polymers

22.3.1 Gel Preparations

Semisolid formulations, commonly called gels, are convenient for dermatological applications as they may incorporate various APIs within their internal network structure and provide physical stability towards (1) sedimentation, (2) creaming, and (3) flocculation due to high viscosity. Nature is rich in semisolid elements (e.g., connective tissues, extracellular matrix), and many natural polymers can be used in pharmaceutical formulations. Relatively recent concerns about the environment have led to the discard of petroleum-based synthetic polymers despite their low-cost and biochemical inertness. Modern

analytical methods enable to understand the microstructure/function relation in polymers and open the way to optimized biocompatible modified natural polymers (Yu et al. 2006).

Chen et al. described a PEG/poly(lactide-co-glycolide) hydrogel matrix meant for prolonged subcutaneous delivery of porcine growth hormone (pGH). The preparation was very simple, consisting in a single homogenization of polymer and pGH in aqueous solution. Subcutaneous injection in rabbits of low dose (0.12 %) and high dose (0.42 %) pGH-loaded hydrogel matrix enabled constant prolonged delivery of pGH for about 4 weeks, i.e., 5–15-fold that of aqueous solution for subcutaneous injection. Cell viability study in growth media containing polymer extract was the same as control (Chen and Singh 2008).

Furthermore, poloxamers can be used to enhance aqueous solubility of poorly soluble drugs such as anti-inflammatory drugs. In an earlier study, poloxamer 407 aqueous formulation containing various cosolvents and surfactants allowed a 2,000-fold increase in tolfenamic acid solubility. The release profile of API was independent of bulk viscosity suggesting dependence on gel microstructure. Solvent composition is known to influence gel microstructure and therefore can be adapted to obtain desired drug release profile from poloxamer 407 hydrogel formulations (Cafaggi et al. 2008; Ivanova et al. 2001).

22.3.2 Carrier Systems

Polymer incorporation into structures of vesicular systems enables to produce nano- or microscaled delivery systems that respond to environmental signal (e.g., light, temperature, chemicals, and biomolecules). Thermosensitive polymers are especially interesting in this perspective, and their properties can be combined to other responsive polymers (e.g., photosensitive, pH sensitive) to produce complex vesicular systems responding to multiple external signals. Desired properties of such polymers are (1) high selectivity in stimuli recognition, (2) amplification of the signal, and (3) transduction of the signal into changes in the system's properties (Motornov et al. 2010).

Recently, Choi et al. described a thermosensitive polymer-based nanocarrier for transcutaneous protein delivery. A chitosan-poloxamer 407-conjugated nanocarrier was able to efficiently encapsulate insulin due to thermosensitive swelling and surfactant properties of poloxamer 407. Enhanced cutaneous permeation was allowed by chitosan's properties (i.e., loosening of keratin structure in the *stratum corneum* and transient opening of tight junctions) (Choi et al. 2012).

Niosome systems formed by poloxamers allowed to improve skin permeation of class IV APIs (sulfadiazine) (Muzzalupo et al. 2001), while chlorhexidine-loaded poly-ε-caprolactone nanocapsule suspension in thermosensitive hydrogel (i.e., carboxymethylcellulose or poloxamers) was proven efficient in sustaining antimicrobial activity after an unique application onto the skin surface (Nhung et al. 2007; Pirot and Falson 2009). Interestingly, poly-ε-caprolactone nanocapsules might be freeze-dried and rehydrated *in situ* in the presence of moisture on the skin surface or wounds and reform locally thermosensitive hydrogel (unpublished data, Fig. 22.3).

22.4 Dermatological Applications

Hydrogels are easy to formulate and handle, enabling interesting drug therapy applications in dermatology (Fig. 22.4). Consequently, thermosensitive polymers are currently used in (1) cutaneous, (2) intradermal, or (3) subcutaneous commercial formulation (Fig. 22.5). At the opposite, in developing countries, compounding dermatologic preparations for treating the most common dermatologic disorders revealed a minimal use of thermosensitive polymers as compared to, e.g., white petrolatum (Lamarre et al. 2009).

22.4.1 Topical Delivery

Heilmann et al. investigated thermosensitive hydrogels (i.e., poloxamer and hydroxyethylcellulose) for opioid local delivery in patients with severe skin wounds. Such formulation

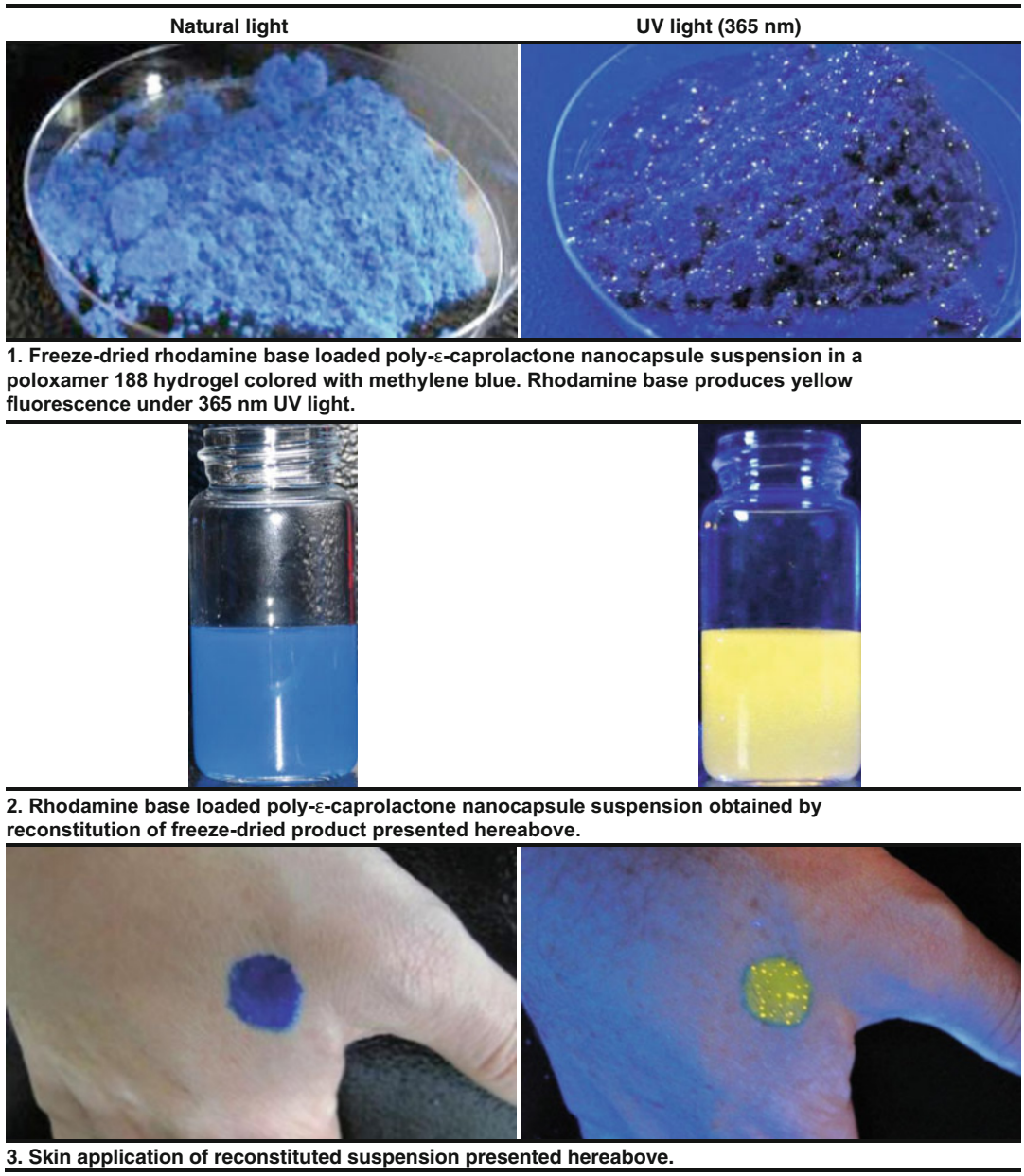


Fig. 22.3 Photographic pictures under (1) natural light and (2) UV light (365 nm) of rhodamine-base-loaded poly-ε-caprolactone nanocapsule suspension in poloxamer 188 hydrogel after (step 1) freeze-drying, (step 2) resuspension in water, and (step 3) application onto the skin

should (1) be able to deliver pain relieving amounts of morphine for a long period of time, avoiding multiple dressing changes and should (2) not impair wound healing. Hydrogels are known to provide a moist environment that favors skin reparation, and poloxamer gels have previously been described as potent "artificial skin" in the treatment of severe burns. A 25 % poloxamer 407 hydrogel was found to be an efficient topical formulation vehicle enabeling 24 hours sustained release of morphine in addition to be conveniently applicable and no impairing natural skin healing (Heilmann et al. 2013).

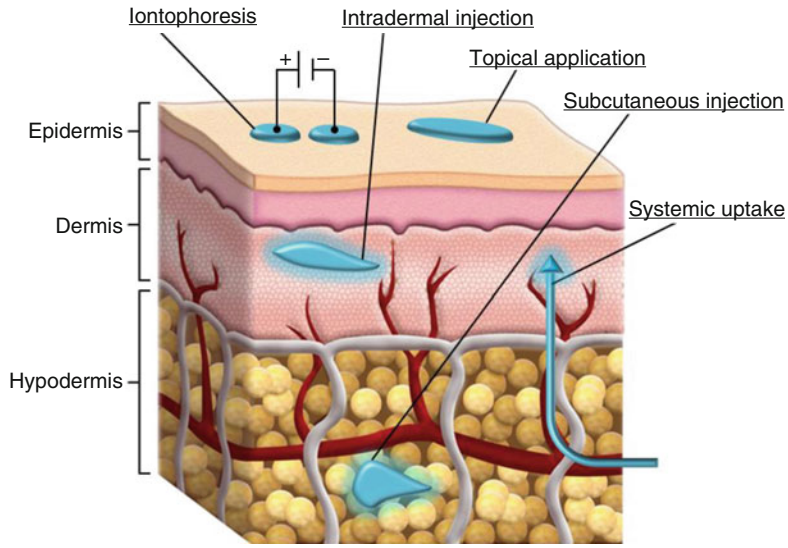


Fig. 22.4 Schematic view of possible skin-related administrations of API-loaded thermosensitive gel (*blue* spot) showing dermatological interest (Presented view is adapted from http://whgormanmd.com/plastic-surgery-educational-images/)

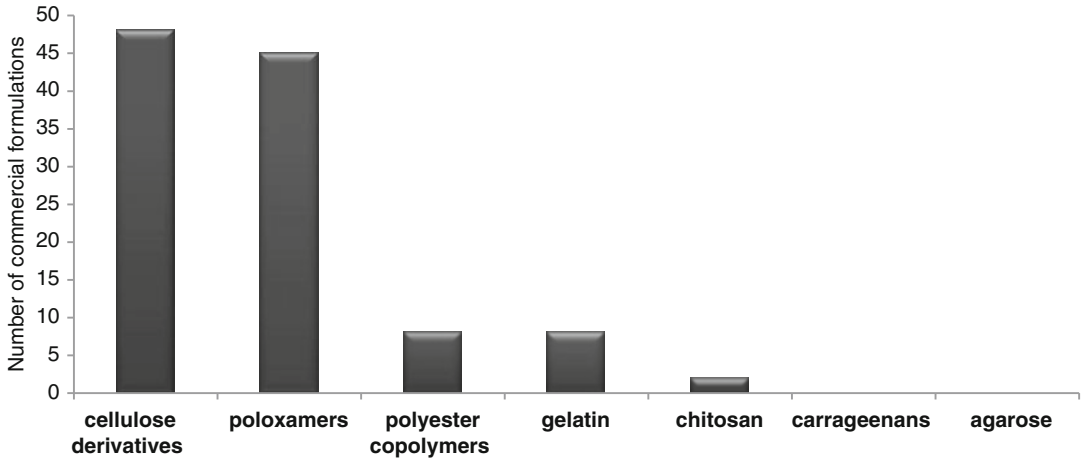


Fig. 22.5 Histogram showing the number of approved (1) cutaneous, (2) intradermal, or (3) subcutaneous commercial formulations containing thermosensitive polymers (Source www.theriaque.org)

22.4.2 *In Situ* Gelling Systems

In situ gelling systems are liquid aqueous formulations that gelify under physiological conditions. They have a wide range of pharmaceutical and medical applications, notably for (1) drug delivery, (2) cell encapsulation, and (3) tissue repair. Thermosensitive *in situ* gelling systems are polymeric solutions undergoing rapid change in viscosity upon administration as a consequence of temperature dependent rise of polymer hydrophobicity (Ruel-Gariépy and Leroux 2004). Local or systemic controlled long-lasting delivery of APIs is therefore made possible with minimal administration invasivity and frequency. Applications in local anticancer therapy (Desai et al. 2008) or hormone systemic delivery for contraception or substitution (Yoshida et al. 1991) are described in literature.

22.4.3 Iontophoresis and Electroporation

Iontophoretic delivery is a promising alternative to parenteral administration of active compounds with poor skin permeation potential, especially of high-molecular-weight molecules like proteins. However, technical issues need to be resolved to make this administration method clinically usable. Poloxamer 407 hydrogel containing insulin and permeation enhancers has been developed by Pillai et al. answering many of these technical issues. The reverse thermosensitive nature of this formulation enabled facilitated preparation and storage between +2 °C and +8 °C as it would remain liquid at such temperature. Application onto the skin and compatibility with iontophoresis device as the cool solution flowed easily onto the skin, forming a non-occlusive gel when reaching body temperature with excellent contact with the skin and device. Furthermore, the adaptability of electrical conductivity and pH of hydrogels and their ability to absorb sweat gland secretions, avoiding irritation under long-term occlusion, enabled ideal iontophoretic conditions (Pillai and Panchagnula 2003).

Electroporation consists in applying an electric field to the skin in order to temporarily create pores in the stratum corneum enabling skin permeation of unabsorbable molecules. Following the administration process, resealing of the pores to restore skin barrier function is desired to prevent further unwanted absorption. Poloxamer 188 solution applied after electroporation procedure *in vitro* enabled (1) significantly more rapid and efficient epidermal electric resistance recovery and (2) rapid skin permeability impairment of witness hydrophilic compound (i.e., glucose). The amphiphilic nature of poloxamer is believed to be responsible for this effect because of its ability to interact with skin lipids and therefore facilitates their reassembly from electroporation-induced vesicular structures to physiological lamellar structures (Burgess et al. 2007).

22.4.4 Subcutaneous Surrogates

Hydrogels exhibit physical similarities with human soft tissues. The interstitial space of all tissues, including skin, is composed of collagen fibers supporting fully charged glycosaminoglycan hydrogel at physiological pH (Porter et al. 2001). Physicochemical characteristics (e.g., osmolarity, pH, density, rheology, water content) of such hydrogel is possible to approach *in vitro* using previously described polymers, such as agarose, and adjusting their characteristics with adjuvants. Recently, Ostergaard's group described piroxicam diffusion from an oil solution into a poloxamer 407 and an agarose gel meant as subcutaneous matrix surrogates. Piroxicam diffusion inside the hydrogels was monitored in real time by UV imaging (Ye et al. 2012a, b). Yet unpublished data from our group have reported the effect of poloxamer 407 and hyaluronic acid on porcine ear *stratum corneum* and dermis hydration and water evaporation. Significant dehydration of skin samples was evidenced with 30 % poloxamer 407 gel as compared to 0.5 % hyaluronic acid or 0.9 % sodium chloride solution. The implication on wound healing after gastric endoscopic mucosal resection using poloxamer gels (Fernandez-Esparrach et al. 2009) is discussed. Such experimental approaches could lead to better understanding and *in vitro* characterization (e.g., molecular transport, tissular effect) of pharmaceutical products meant for subcutaneous or submucosal injection.

Conclusion

Thermosensitive polymeric systems forming hydrogels offer numerous applications in the field of skin-related therapy. Their versatility enables to consider various delivery systems, from a simple gel to complex nanostructured carriers. Moreover, they are relatively low-cost ingredients and might conveniently be selected and adapted to match suitable properties.

Conventional natural (e.g., gelatin) or modified natural polymers (e.g., cellulose derivatives)

are the most widely used polymers in hospital pharmacy daily practice and still provide the majority of literature content related to hydrogels. However, the growing interest in biocompatible synthetic polymers such as poloxamers and the ability to control intimately their microstructure and microreactivity make them an exciting object of study and application. The amphiphilic nature of these block copolymers supports subsequent capacity to interact with a large range of API classes (including class IV compounds and proteins of therapeutical interest) and many human body tissues and components. In the future, hydrogels, oleogels, and composite systems with responsive capacities to physiological stimuli will multiply pharmaceutical applications and open a window to long time announced and plebicited nanomedicine.

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Liposomal Gels in Enhancing Skin Delivery of Drugs

23

Lorena Tavano

Contents

Abbreviations

Chol	Cholesterol
DSPC-Chol	Distearoyl-glycero-PC and cholesterol
EPC	Egg phosphatidylcholine
EPC-Na	Egg phosphatidylglycerol sodium
HEC	Hydroxyethyl cellulose
HPC	Hydrogenated PC
HPMC	Hydroxypropyl methylcellulose
PC	Phosphatidylcholine

23.1 Introduction

Transdermal drug delivery (TDD) is an administration route used for potent, low-molecular-weight therapeutic agents which cannot withstand the hostile environment of the gastrointestinal tract and/or are subject to considerable first-pass metabolism by the liver (Prausnitz and Langer 2008). TDD provides controlled and constant administration of the drug, allows continuous input of therapeutics with short biological half-lives and eliminates pulsed entry into systemic circulation, which often causes undesirable side effects. Skin has been considered as a promising route for the administration of drugs because of its accessibility and large surface area. It is a very heterogeneous membrane that provides a very effective barrier towards the penetration of drugs both to and through it, thanks to

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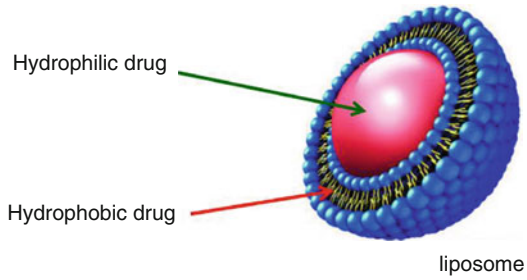

Fig. 23.1 Schematic representation of a liposome

its outermost layer, the stratum corneum (SC), a multilayered wall-like structure in which corneocytes are embed in a matrix of lipids (Bronaugh and Maibach 1985). Since this barrier represents a significant obstacle for transdermal permeation of most drugs, enhancement strategies are necessary to overcome it, thus improving drug release: among these, penetration enhancers, supersaturated solutions, physical methods and specific delivery systems such as vesicular systems (liposomes and niosomes) or vesicular hydrogels represent powerful approaches receiving considerable attention (Barry 1983).

23.1.1 Liposomes

Liposomes are microscopic vesicles that contain amphipathic phospholipids arranged in one or more concentric bilayers enclosing an equal in number of aqueous compartments (Fig. 23.1): In this form, as a spherical shell, they resemble biological membranes (Gregoriadis and Florence 1993). The ability of phospholipids to form bilayers is due to their amphipathic nature: the presence of a hydrophilic or polar region in the head (attracts water) and a nonpolar region or lipophilic tail (repels water). In this light, drug molecules can be encapsulated in the aqueous space (hydrophilic compounds) or intercalated into the lipid bilayer (lipophilic compounds) depending upon their physico-chemical characteristics. Due to their entrapping ability, biodegradable and non-toxic nature, liposomes have been reported to have several potential applications (Allen and Curtis 2013).

Particularly, liposomes have been shown to be a promising skin drug delivery system: their use may produce severalfold higher drug concentrations in the epidermis and dermis and lower systemic concentrations when compared to conventional dosage forms (El Maghraby 2008). Clearly, their topical use depends on their characteristics as size, surface, charge and chemical composition. Mezei and Gulasekharam in 1980 were the first to employ liposomes as skin drug delivery systems: they demonstrated that vesicles of dipalmitoylphosphatidylcholine (DPPC) and cholesterol (CH) (1.1:0.5, molar ratio) increased the concentration of triamcinolone acetonide in the epidermis and dermis by four- to fivefold and reduced percutaneous absorption compared with a standard ointment (Mezei and Gulasekharam 1980). Several mechanisms have been suggested for liposomes acting as skin drug delivery systems. According to the free drug mechanism, the drug may permeate the skin independently after exiting from the vesicles, since the liposomes themselves enhance the transdermal drug delivery by lowering the permeability barrier of the skin, changing the ultrastructures of the intercellular lipids and the enthalpy of the lipid-related transitions of the stratum corneum (Bernadete et al. 2011). In some cases, the vesicles may adsorb to the stratum corneum surface with subsequent transfer of drug directly from vesicles to the skin (El Maghraby et al. 2008). Moreover, vesicles have been reported to fuse and mix with the stratum corneum lipid matrix, increasing drug partitioning into the skin (El Maghraby et al. 2008). The possibility that intact vesicles penetrate human skin acting as carriers and go deep enough to be absorbed by the systemic circulation has been also suggested (El Maghraby et al. 2008).

Different novel vesicular systems derived from liposomes have been proposed as a valid alternative and among these, niosomes, nonionic surfactant-based vesicles, represent one of the most suitable options: niosomes appear to be similar in terms of their physical properties to liposomes, but they are preferred in topical delivery because of chemical stability and low cost of production (Schreier and Bouwstra 1994).

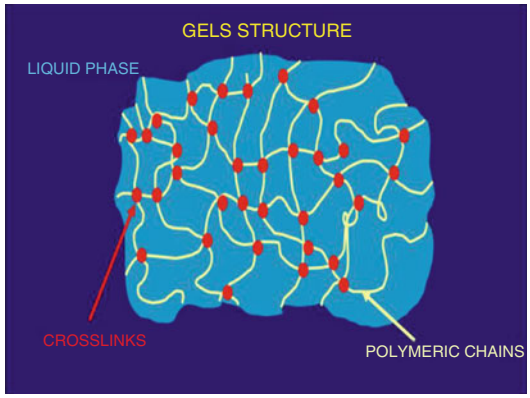

Fig. 23.2 Schematic representation of a gel system

23.1.2 Gel Systems

The term gel was introduced in the late 1800s to name semisolid systems in which a liquid phase is constrained within a three-dimensional polymeric matrix of natural or synthetic gums with a high degree of physical or chemical cross links, as shown in Fig. 23.2 (Narin 1997). Gel forming polymers include natural polymer (proteins and polysaccharides), semisynthetic polymers (cellulose derivatives), synthetic polymers (carbomers and poloxamers) and surfactants (cetostearyl alcohol and polyoxyethylene glycol alkyl ethers (Brij™, Croda International PLC)). Gel also possess a degree of flexibility very similar to natural tissue, and due to their high water content, they resemble natural living tissue more than any other type of synthetic biomaterial. Moreover, they have significant roles in pharmaceutical and cosmetical fields because of their biocompatibility, non-toxicity and good skin adhesion (Peppas 1986). Additionally, their insoluble cross-linked structure allows medium for dissolution of hydrophilic drugs, and since only the dissolved drug presented to the skin is able to enter the stratum corneum, gels could represent a strategy to enhance the percutaneous drug absorption and release across the skin in well-defined specific manner (Wichterle and Lim 1960).

Clearly, the type of vehicle used to formulate a topical dermatological product greatly influences its effectiveness. Gels prepared with organic polymers, such as carbomers, impart an aesthetically pleasing, clear, sparkling appearance to the products and are easily washed off from the skin with water; vehicles containing large amounts of oleaginous substances provide an emollient effect to dry irritated skin; bases made up of non-volatile oleaginous substances can form an occlusive barrier on the skin that prevents the escape of moisture from the site of application, causing hydration of the stratum corneum and increase of opening up of intra- and intercellular channels for easier passage of drug molecules (Hoare and Kohane 2008).

23.2 Liposomal Gel Systems

23.2.1 Introduction

Taking into account all the previously mentioned reasons, the incorporation of vesicular systems into a gel dosage form has been designed as a new strategy to improve drug percutaneous permeation: the resulting multicomponent systems, named *liposomal gel* (Fig. 23.3), may possess the advantages of the individual formulations (vesicular suspensions and gel systems) and some other important benefits (Foldvari 1996).

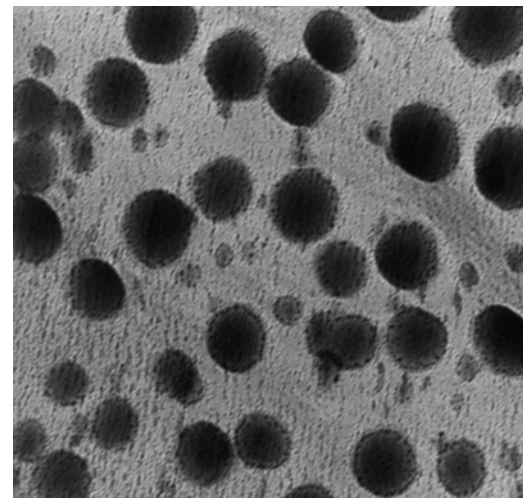

Fig. 23.3 Photomicrographs of a vesicles-gel system as seen by transmission electron microscopy (TEM) (Adapted from reference Antunes et al. 2011)

Since topically applied liposomal suspensions may leak from the application site, they could be mixed with gels in order to obtain semi-solid formulations. In addition, liposomal gels were found to enhance the skin retention of drugs and to provide higher and sustained skin concentrations of therapeutics compared to conventional gels and creams, without enhancing their systemic absorption (Pavelic et al. 2005). In addition, the stability of the liposomes (membrane integrity and mechanical stability) has been reported to increase when incorporated into a gel matrix (Mourtas et al. 2007). Additionally, liposomal gels have been demonstrated to have better rheological characteristics with respect to the liposomal dispersion (easiness of application and removal from the skin), to ensure an appropriate release of the active principle (compatibility with most active substances) and increased skin tolerance and compliance for patients. Clearly the type and concentration of the polymer forming the gel matrix has been reported to influence the stability and release rate of the active substance, whereby an assessment of the physico-chemical properties of the drugs, liposomes and polymeric gel must be made to avoid adverse effect and chemical, physical and biological incompatibility (Mourtas et al. 2008).

Moreover, liposomes in a gel can be more stable to environmental stimuli compared with bare liposomes in a dispersion, and when a drug is placed inside the liposomal core and the liposomes are included in a gel network, the drug will experience a combination of transport resistances due to the liposomal bilayer and the network itself: this results in a release of the drug over a longer period of time. Additionally, this can also avoid the problem of "burst release" seen with some polymer gels where a large bolus of drug is released initially from the gel, which can cause toxicity (Lee et al. 2012).

The first study on the incorporation of liposomes in a gel dosage form was reported by Mezei and Gulasekharam in 1982 (Mezei and Gulasekharam 1982). They compared the permeation of triamcinolone from plain and liposomal gel and they found that the application of the liposomal gel resulted in a concentration of triamcinolone acetonide approximately five times higher in the epidermis and three times higher in the dermis, than application of the conventional drug gel. The results of this study and those reported earlier by the same researchers (Mezei and Gulasekharam 1980) suggested the inherent potential of liposomes (when applied in a gel) as a drug delivery system for cutaneous application and the role of liposomes in the formation of a large drug reservoir in the skin, which is useful in local treatments. Since then, a lot of researchers explored the potential of liposomal gel systems in transdermal drug delivery (Gabrijelcic and Sentjurc 1995).

The most used polymers to obtain liposomal gels are carbomers, cellulose derivatives and poloxamers. Carbomers are polymers of acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol. They swell in water up to 1,000 times their original volume to form a gel when exposed to a pH environment above 4.0–6.0. Because the pKa of these polymers is 6.0–0.5, the carboxylate groups on the polymer backbone ionize, resulting in repulsion between the negative charges, which adds to the swelling of the polymer (Florence and Pu 1994). Carbomer polymers are very well suited for aqueous formulations of the topical dosage forms: many commercial products available today have been formulated with these polymers, as they provide numerous benefits to topical formulations. Carbomer polymer possesses low toxicity and low irritancy potential and they are non-sensitizing even upon repeated usage. In addition, due to their extremely high molecular weight, they cannot penetrate the skin or affect the activity of the drug. Because of their excellent thickening, suspending, emulsification and suitable rheological properties, good tissue compatibility and convenience in handing and ease of application, carbomer gels represent a good alternative to oil-based formulations (Jian Hwa 2003).

Cellulose polymers (hydroxyethyl cellulose, hydroxypropyl cellulose, sodium carboxymethyl cellulose) are examples of polymers that have been reported to possess adhesive properties (Jones et al. 1997). Chemically, these linear polymers are cellulose derivatives possessing various degrees of substitution and may be ionic or non-ionic; following addition to an aqueous phase,

these cellulose derivatives undergo swelling prior to dissolution. Pharmaceutically, water-soluble cellulose polymers have found widespread applications, e.g. in the formulation of solid dosage forms, aqueous disperse systems as viscosity enhancing agents and in products for topical application (Peppas et al. 2000).

Poloxamers are polymers consisting of a relatively long hydrophobic poly(propylene oxide) (PPO) middle block and two hydrophilic poly(ethylene oxide) (PEO) end blocks and are commercially available as Pluronics® (BASF, Hanover, Germany). In the presence of a either solvent selective for the hydrophilic PEO blocks, such as water, PEO–PPO–PEO block copolymers self-organize into a variety mesophases with lamellar, hexagonal or cubic structure (Batrakova and Kabanov 2008). Pluronics® have attracted particular interest in the design of dermal and transdermal delivery systems, with a view to promoting, improving or retarding drug permeation through the skin. Moreover, they possess specific pharmacological actions, in particular the dynamic PEO chains prevent particle opsonization and render them 'unrecognizable' to reticuloendothelial system (RES) and macrophages (Tavano et al. 2010). Several studies reported the advantageous interactions between Pluronics® and liposomes: in particular, these polymers have been used to sterically stabilize the vesicles and, hence, to prolong their half-life after parenteral administration (Kostarelos et al. 1999).

23.2.2 Overview on Liposomal Gel Systems Used for Dermal Drug Delivery

A summary of recent works in the area of novel liposomal gel formulations for dermal drug delivery is provided below.

Pavelic et al. in 2001 developed a liposomal gel system able to provide sustained and controlled release of calcein, as model drug, for local vaginal therapy (Pavelic et al. 2001). Traditional liposomes were prepared from egg phosphatidylcholine (EPC) and egg phosphatidylglycerol sodium (EPG-Na); they were incorporated in gels of polyacrylate, i.e. carbomer gels (Carbopol 974P NF or Carbopol 980 NF, BF Goodrich, Belgium). In vitro release of encapsulated calcein from both liposomal gels was tested and compared with that of liposomes dispersed in buffer (control). A slower release of calcein from liposomal gel was achieved: in fact, after 24 h, more than 80 % of the originally encapsulated calcein was retained in liposomes embedded in gel with respect to the control (60 %, respectively). The authors ascribed these results to the increased viscosity of the gel system which reduced migration of drug molecules, acting as drug reservoir system, while preserving the structure and integrity of liposomes.

Similar results were obtained by Glavas-Dodov and collaborators in 2002 (Glavas-Dodov et al. 2002). The authors compared the in vitro drug release properties of free and liposomally entrapped lidocaine hydrochloride hydrogels. As expected, hydrogel formulations showed higher release rate of lidocaine hydrochloride compared to liposomal gel. Moreover, the release kinetic in the case of liposomal gels can be described as diffusion controlled, while a steady-state release, achieved after the third hour, suggested that liposomes act as a reservoir system for continuous delivery of the drug and for these reasons they could have a potential as dermal delivery systems with prolonged and sustained drug release.

Another delivery system based on liposomal gels containing vitamin E acetate was designed in 2006 by Padamwar and collaborators to improve topical drug delivery (Padamwar and Pokharkar 2006). The prepared liposomal dispersion showed sevenfold increase in drug deposition in rat skin compared to the control (plain drug dispersion), and the liposomal gel formulation demonstrated sixfold and fourfold increase in drug deposition in rat skin compared to the control gel and marketed cream, respectively. Moreover, the liposomal gel formulation was found to be more stable than the corresponding liposomal in terms of drug entrapment efficiency and uniformity up to 3 months.

Mura et al. in 2007 designed and evaluated the potential of a liposomal gel formulation for the topical delivery of benzocaine as a model drug (Mura et al. 2007). Drug permeation from

liposomal dispersions (based on mixtures of phosphatidylcholine, cholesterol, ethanol and water) as such or formulated in a carbomer gel was evaluated both through artificial lipophilic membranes and excised abdominal rat skin, whereas in vivo anaesthetic effect was tested in rabbits. Liposomes were prepared with drug encapsulated in the hydrophilic core or incorporated in the hydrophobic bilayer. The results of the benzocaine release study across artificial membranes showed that the presence of the polymeric network in the case of liposomal gels gave rise to a general reduction of the drug permeation rate and allowed obtainment of a more regular release profile as a function of time, with respect to simple liposomal dispersions. Interestingly, drug-loaded gels showed a faster drug release in respect to the gel-containing liposomes with the drug in the lipophilic phase, but slower than that obtained from liposomal gels with the drug encapsulated in the aqueous phase. In permeation studies using rat skin, a higher reduction of drug permeation rate was noted, due to the more complex permeation process through rat skin than across artificial membranes. In percutaneous permeation studies across rat skin, the difference between the use of the liposomal dispersion as such or formulated in the carbomer gel was less evident, probably due to the major controlling effect exerted by the skin on the drug permeation rate. Moreover, an initial lag phase was present in respect to the permeation studies thought artificial membranes, which was attributed to the longer time necessary to saturate the skin membrane and to reach a pseudo steady-state flux condition between donor and receiver compartments. Finally, the lowest drug permeation was observed from the conventional gel in comparison to all liposomal gel formulations (containing drug concentrations ranged from 0.05 to 0.5 % w/w), confirming the hypothesized permeation enhancing effect of liposomal vesicles on drug delivery, which cannot be efficiently estimated by using the artificial membrane.

Mourtas et al. in 2007 evaluated the effect of liposomes, drugs and gel properties on drug release kinetics (Mourtas et al. 2007). They studied the release of two model compounds, one hydrophilic (calcein) and one lipophilic (griseofulvin, GRF), when dissolved directly in hydrogels (control gels) or dispersed in hydrogels in the form of liposomes (liposomal gels). Drug-loaded liposomes based on PC or DSPC/Chol were dispersed in carbomer, i.e. Carbopol® 974 (Chemix S.A, Athens, Greece), HEC (Natrosol 250 HX, Hercules Inc, Athens, Greece) or a mixture of these two hydrogels. Results demonstrated that depending on the intended use of a liposomal gel formulation, the first parameter that should be considered is whether the drug is hydrophilic or lipophilic. In fact, the release of calcein from liposomal gels was slower compared to that obtained from conventional gels and strongly dependent on the liposome-membrane rigidity. If the drug was encapsulated in DSPC/Chol liposomes and dispersed in gels, it was released significantly slower compared to the corresponding formulation based on PC liposomes. In the case of GRF, the release from liposomal gels was determined by drug loading. At high drug loading levels, GRF is released steadily from liposomal gels irrespective of liposome type (PC or DSPC/Chol). Moreover, in the case of the lipophilic drug, liposomes provided means for substantially increased drug loading in gels acting as reservoirs which released the drug in a sustained manner. Finally, the authors demonstrated that in the case of liposomal carbomer (Carbopol®) gels that behave predominantly as elastic solids and have substantially different flow properties (compared with HEC-based gels), increased release rates for free calcein and GRF, indicating easier diffusion of the compounds through this system, were obtained.

In the following year, Mourtas et al. investigated the effect of added liposomes on the rheological properties of a hydrogel containing 0.40 % w/v of carbomer, i.e. Carbopol® 974 NF (Chemix S.A., Athens, Greece) and 1.5 % w/v of HEC (Mourtas et al. 2008). PC or HPC liposomes, plain or mixed with Chol, were used to prepare liposomes. As reported by other authors, the lipid composition of liposomal bilayers was found to strongly influence the rheological properties of liposomal gels. Zero-rate shear viscosity and power law index values revealed that addi-

tion of PC liposomes to the hydrogel had the smallest effect on its rheological properties, even when the highest lipid concentration was used (20 mg/ml). Oppositely, incorporation of HPC (or HPC/chol) liposomes into gels resulted in a significant increase of the elastic character of the gel, which increased with increasing lipid concentration. Since drug rate permeation was found to be dependent on gel viscosity, authors demonstrated that liposomal gel based on HPC could be used to act as drug reservoir and to ensure a slow drug release over a prolonged time period, while liposomal gel based on PC could be used to obtain faster drug permeation.

Manosroi et al. in 2008 developed a new formulation in which elastic (containing ethanol) and conventional niosomal vesicles containing diclofenac diethylammonium (DCFD) were incorporated into a gel base containing 0.2 % w/w carbomer (Carbopol® 980, Noveon, India) (Manosroi et al. 2008). Authors reported that the cumulative amounts of drug in stratum corneum, viable epidermis and dermis and the receiving solution, obtained upon the application of niosomal gel, were higher compared to the commercial emulgel (containing 1.16 % (w/w) of DCFD) and conventional gel without vesicles. They suggested a synergistic mechanism between ethanol, vesicles and skin lipids to improve drug permeation. Moreover the developed niosomal gels showed an 18.92 % of inhibition of rat ear oedema (after 1 h of application) higher than the commercial emulgel (2.70 %) and conventional gel (5.41 %), indicating an in vivo anti-inflammatory activity enhancement of DCFD when entrapped in vesicles. This study demonstrated that the gel containing the novel vesicular system entrapped with DCFD may be a promising formulation to be used in the topical non-invasive treatment of inflammation.

The effect of incorporation of surfactants into hydrogels to increase drug loading concentration and attenuate the topical release rates was also investigated by Kapoor et al. in 2008 (Kapoor and Chauhan 2008). They demonstrated that the presence of 0.25, 0.6 and 1.5 % w/w polyoxyethylene (20) oleyl ether (Brij™ 98, Sigma–Aldrich Chemicals, St. Louis, MO) as surfactant into a polyhydroxy ethyl methacrylate (p-HEMA) hydrogel affected the loading of cyclosporine A in the matrix and controlled the drug release rate, depending on Brij™ concentration. At concentrations of Brij™ above the critical micellar concentration (CMC), the surfactant forms micellar than from the liposomal gel, but the lower value obtained by the multicomponent system was suggestive for prolonged and sustained drug release, because of the presence of several lipid bilayers that released the drug more aggregates, into which the hydrophobic drugs can partition preferentially.

The objective of the work performed by Patel in 2009 was to formulate a 1 % carbomer gel-containing ketoconazole-loaded liposomes and to study the in vitro drug release, skin retention and in vitro antifungal activity (Patel et al. 2009). The in vitro permeation of ketoconazole from liposomal gel through wistar albino rat skin was compared with that of the corresponding conventional cream and gel. From the results it can be concluded that the cumulative drug permeation was significantly higher from the conventional gel slowly. The higher skin retention of the drug achieved in the case of the liposomal gel was found to depend on the creation of the reservoir effect due to the presence of liposomes. In addition, the gel-containing liposomal ketoconazole showed the highest antifungal activity after 30 h compared to the conventional ketoconazole gel and cream formulations, by using the cup plate (or cylinder plate) method.

The main aim of the study published by Nina Dragicevic-Curic et al. in 2009 was to develop a temoporfin-loaded liposomal hydrogel which would be able to deliver the photosensitizer in an efficient dose into the stratum corneum and deeper skin layers (Dragicevic-Curic et al. 2009). Results showed that the presence of the polymeric network in the liposomal gels systems led to a slower drug release and lower permeation rate compared to liposomal dispersions and that the increase in polymer concentration from 0.5 to 1 % w/w, resulting in an increase of the viscosity of the systems, led to lower skin penetration of temoporfin. Despite this, authors demonstrated that liposomal gel formulations were able to deliver a sufficiently high amount

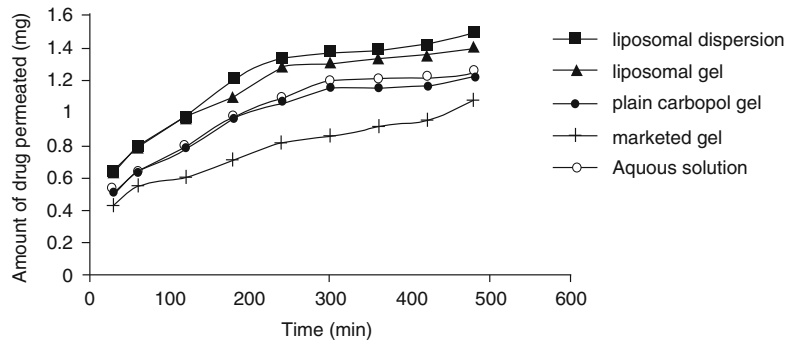

Fig. 23.4 Permeation profile of different fluconazole containing systems (Adapted from reference Mitkari et al. 2010)

of temoporfin into and also into the deeper skin layers, because they ensure long contact time of the formulation with the skin. For these reasons, these new formulations have been suggested as advantageous against cutaneous malignant (basal cell carcinoma) or non-malignant diseases (psoriasis, acne, etc.).

Several groups developed liposomal gels to be used for the dermal delivery of fluconazole. In 2007, Zhao et al. designed a new liposomal gel based on lecithin/cholesterol liposomes and carbomer (Carbopol® 941, Beijing Haidian Huiyou Fine Chemical Plant, China). They evaluated fluconazole skin permeation from the liposomal gel across rat skin and compared it to the corresponding conventional gel (Zhao et al. 2007). Results indicated that the cumulative amount of drug permeated through rat skin in the case of the liposomal gel was lower than that found upon the application of the conventional gel. Moreover, as reported in analogue studies, the addition of the drug into multicomponent systems significantly increased the deposited amounts of drug in the rat skin and resulted beneficial for topical use.

Differently, Mitkari et al. in 2010 designed a liposomal gel based on hydrogenated PC (Phospholipon 90H, Phospholipids GmbH, Germany) and carbomer (Carbopol® 934 NF, Noveon, India) and demonstrated that fluconazol skin permeation increased in the case of the liposomal gel compared to the drug solution, plain carbomer gel and marketed gel (Flucos® gel, Cosme Health Care, Goa, India) as represented in Fig. 23.4 (Mitkari et al. 2010).

In 2010, Jithan et al. formulated a diclofenac sodium-loaded liposomal gel to improve its anti-inflammatory activity and compared it to the traditional gel formulation (Jithan and Swathi 2010). In vitro drug release and ex vivo permeation studies showed that the liposomal gel provided a more sustained drug release and prolonged anti-inflammatory effect compared to the control gel formulation.

In a recent study, Lingan et al. formulated topical gel-containing clobetasol propionate vesicles to prolong the duration of the drug effect and to prevent its side effects (Lingan et al. 2011). The vesicles were prepared by varying the ratios between nonionic surfactants such as sorbitan monopalmitate, sorbitan monostearate, sorbitan monooleate (Span® 40, 60, 80, S.D. Fine chemicals, Mumbai, India) and cholesterol. Afterwards, the vesicles were incorporated into a 2 % w/w carbomer gel. The conventional carbomer gel and marketed gel (Clobevate, Stiefel Laboratories Pakistan (PVT) Pakistan) showed cumulative percentage of drug release (in respect to the initial applied dose) of about 98 % in 300 min, respectively, while the multicomponent formulation showed 51 % of drug release within 24 h. In addition, results demonstrated that the percentage of reduction in paw oedema was gradually increased in the case of niosomal gels up to 8 h, whereas in marketed gel it gradually increased up to 4 h and later it declined at 6 h and 8 h, revealing that the multicomponent systems had a sustained as well as prolonged action.

Recently, Megha et al. examined the ability of a new developed liposomal carbomer (Carbopol® 934, Noveon, India) gel loading selegiline, to deliver the active substance across the skin, and demonstrated that this novel sys-

tem provided a prolonged and sustained selegiline release rate. This gel could represent an attractive device for transdermal applications (Megha et al. 2012).

The objective of the study performed by Mansoori et al. in 2012 was to develop a ketoprofen liposomal gel to enhance the drug's anti-inflammatory activity and reduce its adverse effects (Mansoori et al. 2012). Carbomer (Carbopol® 934) gel was used as a vehicle (1 % w/w) and liposomes at different lipid concentration were incorporated. Drug release profiles of the different formulations were determined by using Franz diffusion cell up to 24 h and compared with that of a marketed gel (control) The results showed that the marketed gel released approximately 92 % of the drug within 24 h, while liposomal gel formulations showed percentage of drug release ranging from 81 to 87 %, indicating that the multicomponent formulations provided a more retarded and controlled drug release compared to the commercial gel. Finally, the ketoprofen liposomal gel showed a significantly enhanced retention of drug molecules in the skin.

The aim of a recent study performed in 2012 by Patel et al. was to develop a niosomal gel as a new transdermal device to improve the systemic availability of lopinavir (Patel et al. 2012). Skin permeation profiles of lopinavir was studied from a conventional gel, optimized niosomal and ethosomal dispersions, niosomal gel and ethosomal gel, after their non-occlusive application onto rat abdominal skin ex vivo. Interesting results were obtained: in terms of overall skin permeation of lopinavir (including percentage deposited within the skin and percentage permeated into the acceptor medium), the ethosomal gel appeared to be more efficient than the niosomal gel. However, the major fraction of lopinavir delivered via the ethosomal gel remained deposited within the skin, while the niosomal gel efficiently delivered the drug deeper into the skin and released 21.24 ± 0.23 % of the drug in 24 h, representing an amount significantly greater than that released via the ethosomal gel (11.15 ± 0.15 %). Moreover the percentage of drug permeated and deposited in the skin when using the niosomal gel was higher than that obtained from the niosomal dispersion (18.32 ± 0.18 %).

In 2011, Vyas et al. developed a new topical niosomal gel loaded with benzoyl peroxide (commonly used for the treatment of acne) to avoid drug side effects like skin redness, irritation, itching and oedema (Vyas et al. 2011). Several vesicular formulations were prepared to achieve the best performance in terms of size and entrapment efficiency and the optimized niosomal formulation was incorporated into 2 % w/w HPMC K15 (Colorcon Asia, Mumbai). Drug leakage studies revealed that vesicles incorporated in the multicomponent system were significantly more stable, because of the prevention of niosomes' fusion. In vitro drug release profiles revealed higher amount of released drug from plain gel and marketed gel in respect to niosomal gel, thus demonstrating a prolongation of drug release in the case of the multicomponent system. Drug retention in the skin has been shown to be higher (41.53 %) with the niosomal gel in respect to the plain gel (21.45 %) and marketed gel (24.88 %) after 24 h. Further, prolonged drug permeation was observed from the niosomal gel in respect to the plain gel and marketed gel, which may be due to slower diffusion of the drug across the skin and to the creation of a drug reservoir.

Recently, El-Nabarawi and collaborators developed a liposomal gel formulation to be used for transdermal paroxetine (PAX) delivery (El-Nabarawi et al. 2013). Selected drug-loaded liposomes were incorporated into HPMC E-4M gel (Tama, Tokyo, Japan) and then fabricated into transdermal patches. In vitro release profiles were evaluated and the transdermal patches were applied to rabbits for in vivo bioavailability study. Authors demonstrated that the diffusion of paroxetine from different liposomal gels in vitro through the artificial membrane was apparently dependent on the concentration of HPMC E-4M, where the increase in polymer concentration was associated with a decrease of the permeation rate due to the increase of gel viscosity. The percentages of paroxetine released from liposomal gels through artificial membrane were compared to that permeated from liposomal dispersions, and results indicated that the incorporation of liposomes into the gel resulted in a more delayed

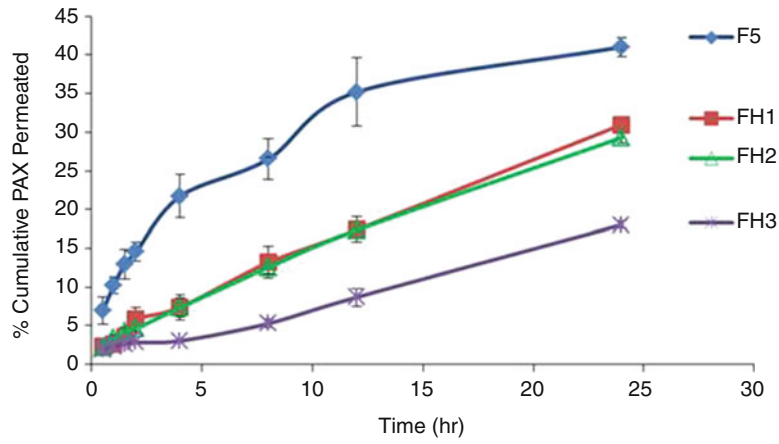

Fig. 23.5 Cumulative percentage of paroxetine (PAX) permeated from different formulations: F5 liposomal dispersion, FH1 liposomal gel (2 % of polymer), FH2 liposomal gel (4 % of polymer), FH3 liposomal gel (6 % of polymer) (Adapted from reference El-Nabarawi et al. 2013)

release due to the presence of the polymer network that act as an additional diffusion barrier to the drug release (Fig. 23.5).

In 2003, liposome gels bearing 5-fluorouracil (5-FU) as an antineoplastic agent, intended for transdermal application, have been prepared and in vitro drug release properties have been evaluated by Glavas-Dodov et al. (2003). Different liposomal formulations, prepared by varying the lipid phase composition, hydration conditions of the dry lipid film and drug concentration, were incorporated into a chitosan gel base (1 %, w/w, Katakura Chikkarin, Japan). Afterwards, 5-FU release profiles from conventional liposomes, conventional hydrogel as well as drug aqueous solutions were estimated. Results demonstrated that 5-FU was fully released from aqueous solutions within a period of 4 h, while the entrapment of 5-FU into conventional chitosan gel resulted in a prolonged release rate, due to the polymeric network of the hydrogel. Moreover, the release of 5-FU from the liposomal gel was significantly slower (40 % released within 8 h) than from all other formulations, confirming that its encapsulation into liposomes resulted in a prolonged and sustained drug release. In addition, drug release profiles of 5-FU obeyed the Higuchi diffusion model, thus liposomes have been demonstrated to act as reservoir systems for continuous delivery of the antineoplastic drug.

Nie and co-workers in 2011 designed an in situ gel system based on liposome-containing paclitaxel, by using 18 % w/w poloxamer (Pluronic® F127, BASF, Hanover, Germany) as thermoreversible gel, with the aim to control drug release and improve its antitumor efficiency (Nie et al. 2011). In vitro release experiment showed that liposomal gel exhibited the longest drug release period compared to liposomes, conventional gel and corresponding commercial gel formulation (Taxol, Bristol-Myers Squibb, China). This effect was presumably due to the increased viscosity of the liposomal gel, which has the effect of creating a drug reservoir. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and drug uptake studies showed that the treatment with the paclitaxel-loaded liposomal Pluronic® F127 gel yielded cytotoxicity, intercellular fluorescence intensity and drug concentration in keratinocyte cancer cells lines (KB cells) much higher than that of conventional liposomes.

In 2011, the influence of nonionic niosomes on the rheological behaviour of poloxamer (Pluronic® F127, BASF, Hanover, Germany) gel was investigated by Antunes and co-workers, with the aim to develop new devices for the controlled topical delivery of diclofenac sodium salt (Antunes et al. 2011). Results showed that the presence of surfactant aggregates in the polymer network clearly enhances the network strength, because of polymer-vesicle hydrophobic associ-

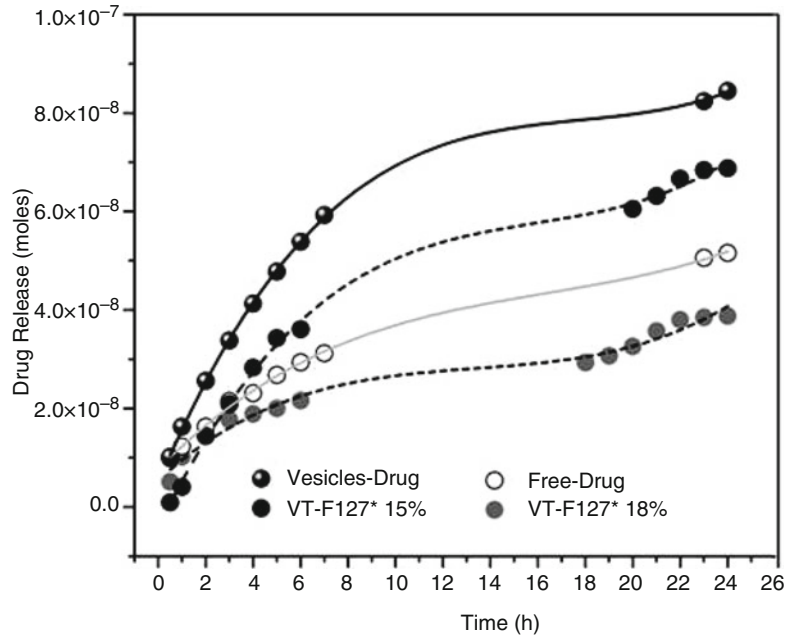

Fig. 23.6 Cumulative drug amount versus time of permeated diclofenac sodium salt at 37 °C from different formulations (*niosomal gels with 15 and 18 % w/w Pluronic® F127) (Adapted from Antunes et al. (2011))

ation and the increase of density of active links present in the polymer network. This resulted in a thickening effect, being optimal to enhance the suitability of vesicular suspensions as transdermal delivery devices, since traditional niosomal formulations are in liquid state. Interestingly, the niosomal size increased upon polymer addition, probably due to the formation of a polymer layer along the vesicle surface. As reported in (Fig. 23.6) higher percutaneous permeation of diclofenac sodium salt from niosomal formulation in comparison to drug solution has been found. Interestingly, in the study of Antunes, the drug cumulative amounts permeated from the niosomal gel decreased with increasing the polymer concentration. In fact, vesicle gel system at 15 % w/w of Pluronic® F127 provided higher amount of permeated drug compared to the mixture of 18 % w/w Pluronic® F127 gel and drug solution. The higher values of cumulative diclofenac sodium salt permeated were obtained by the niosomal dispersion. Unexpected, the cumulative permeated drug amount from niosomal gel at 18 % w/w Pluronic® F127 was found to be lower than that achieved by using drug solution due to the higher viscosity of the network gel.

Conclusions

Over the last two decades, several researchers have attempted to combine the properties of liposomes and polymer gels within the same material, embedding the vesicular systems in the polymer matrix.

These novel materials have demonstrated some attractive features, including the fact that liposomes in a gel resulted more stable to environmental stimuli compared with liposomes in solution. Moreover, some workers demonstrated that when a drug was placed inside the liposomal core and the liposomes have been included in a gel network, the drug experienced a combination of transport resistances due to the liposomal bilayer and the network itself, ensuring a release over a longer period of time and in a sustained manner.

Finally, liposomal gel formulations have been reported to provide therapeutically better effects than the conventional formulations, as topical dosage forms with prolonged and controlled drug release, which may lead to improved therapeutical effectiveness and better patient compliance.

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