Vinod P. Shah · Howard I. Maibach
John Jenner *Editors*

Topical Drug Bioavailability, Bioequivalence, and Penetration

Second Edition

Springer

Topical Drug Bioavailability, Bioequivalence, and Penetration

Vinod P. Shah • Howard I. Maibach • John Jenner
Editors

Topical Drug Bioavailability, Bioequivalence, and Penetration

Second Edition

Springer

Editors
Vinod P. Shah
Pharmaceutical Consultant
North Potomac
Maryland
USA

Howard I. Maibach
Department of Dermatology
School of Medicine
University of California
San Francisco
California
USA

John Jenner
The Defence Science and Technology
Laboratory, Biomedical Sciences
Department
Salisbury
United Kingdom

ISBN 978-1-4939-1288-9 ISBN 978-1-4939-1289-6 (eBook)
DOI 10.1007/978-1-4939-1289-6

Library of Congress Control Number: 2014957726

Springer New York Heidelberg Dordrecht London

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Contents

Part III Bioequivalence of Topical Drug Products

Part IV Bioequivalence: Targeted Drug Delivery

Part V Transdermal Drugs and Modelling

Part VI Clinical Studies and Bioequivalence

Part VII Guidelines and Workshop Report

About the Editors

Vinod P. Shah is a pharmaceutical consultant. He was Scientific Secretary of International Pharmaceutical Federation (FIP) and is now Chair of Regulatory Sciences Special Interest Group of FIP. Dr. Shah has served at the U.S. Food and Drug Administration and has developed several regulatory guidances for the pharmaceutical industry in biopharmaceutics and topical drug products. Dr. Shah is a fellow of FIP as well as the American Association of Pharmaceutical Scientists (AAPS), where he served as president for a year. He is a recipient of an honorary doctorate from Semmelweis University, Budapest, Hungary.

Howard I. Maibach is professor of dermatology at the University of California, San Francisco. He received his M.D. at Tulane University Medical School in New Orleans, Louisiana, and completed his residency and research fellowships at the University of Pennsylvania in Philadelphia, Pennsylvania. Professor Maibach is a leading authority in the fields of dermatotoxicology and dermatopharmacology, in which he has conducted research and written extensively. Prof. Maibach has served on the editorial boards of more than 30 scientific journals and is a member of many professional societies, including the American Academy of Dermatology and the International Commission on Occupational Health.

John Jenner is a principal scientist at The Defence Science and Technology Laboratory in the UK. He has a degree in pharmacology from the University of Manchester in Manchester, UK, and a Ph.D. from the University of Surrey, Guildford, UK. John has spent his career studying defense against and treatment of poisoning by highly toxic chemicals. He has an enduring research interest in percutaneously active chemicals, whether toxic materials or drugs, and experience in the design and testing of transdermal formulations. His current interests include the translation of *in vitro* penetration measurements to *in vivo* using mathematical modelling.

Contributors

Yuri G. Anissimov Queensland Micro- and Nanotechnology Centre, Griffith University, Brisbane, QLD, Australia

School of Biomolecular and Physical Sciences, Griffith University, Gold Coast, QLD, Australia

Eva Benfeldt Department of Dermatology, Faculty of Health Sciences, University of Copenhagen, Roskilde Hospital, Roskilde, Denmark

Nicholas Blickenstaff Department of Dermatology, University of California-San Francisco, San Francisco, CA, USA

School of Medicine, University of Utah, Salt lake City, Utah, USA

K. L. Busse Department of Dermatology, School of Medicine, University of California, San Francisco, CA, USA

Robert P. Chilcott Department of Pharmacy, University of Hertfordshire, Hatfield, Hertfordshire, UK

Garrett Coman Department of Dermatology, University of California-San Francisco, San Francisco, CA, USA

School of Medicine, University of Utah, Salt lake City, Utah, USA

Vladyslava Doktor Department of Dermatology, School of Medicine, University of California-San Francisco, San Francisco, CA, USA

Aaron Gandhi Department of Dermatology, School of Medicine, University of California, San Francisco, CA, USA

Marieke Gosau Acino AG, Miesbach, Germany

Jon R. Heylings School of Pharmacy, Keele University, Staffordshire, UK

John Jenner Biomedical Sciences Department, Dstl Porton Down, Salisbury, UK

Eui Chang Jung Department of Dermatology, University of California, San Francisco, CA, USA

Faranak Kamangar Department of Dermatology, University of California-Davis, Sacramento, CA, USA

Isadore Kanfer Faculty of Pharmacy, Rhodes University, Grahamstown, South Africa

Jürgen Lademann Departments of Dermatology, Venereology and Allergology, and Skin Physiology, Charité—Universitätsmedizin Berlin, Berlin, Germany

Majella E. Lane University College London School of Pharmacy, London, UK

Christine M. Lee Texas Oncology, The Woodlands, TX, USA

Michael S. Leo Department of Dermatology, University of California-San Francisco, San Francisco, CA, USA

Hadar A. Lev-Tov Division of Dermatology, Albert Einstein College of Medicine, Bronx, NY, USA

Guang Wei Lu Pharmaceutical Development, Research & Development, Allergan, Irvine, CA, USA

Howard I. Maibach Department of Dermatology, School of Medicine, University of California, San Francisco, CA, USA

Rashmikant Mohile C.L.A.I.M.S Private Limited, Dalia Industrial Estate, Mumbai, India

Nilab Osman Department of Dermatology, UCSF, San Francisco, CA, USA

Alexa Patzelt Departments of Dermatology, Venereology and Allergology, and Skin Physiology, Charité—Universitätsmedizin Berlin, Berlin, Germany

Michael S. Roberts Therapeutics Research Unit, School of Medicine, The University of Queensland, Princess Alexandra Hospital, Woolloongabba, QLD, Australia

School of Pharmacy & Medical Sciences, University of South Australia, Adelaide, SA, Australia

Björn Schurad Acino AG, Miesbach, Germany

Jessica Schweitzer Largo Medical Center, Largo, FL, USA

Vinod P. Shah North Potomac, MD, USA

Raja K. Sivamani Department of Dermatology, University of California-Davis, Sacramento, CA, USA

Clarence T. Ueda Department of Pharmaceutical Sciences, University of Nebraska Medical Center College of Pharmacy, Omaha, NE, USA

Fangjing Wang Pharmaceutical Development, Research & Development, Allergan, Irvine, CA, USA

Kevin S. Warner Pharmaceutical Development, Research & Development, Allergan, Irvine, CA, USA

Roger L. Williams United States Pharmacopeia, Rockville, MD, USA

Avraham Yacobi DOLE Pharma, LLC, Englewood, NJ, USA

Part I
Percutaneous Absorption

Chapter 1
Percutaneous Absorption

Michael S. Leo and Howard I. Maibach

1.1 Introduction

Skin is a primary area of body contact with the environment, and is the route by which chemicals enter the body. Introduction of chemicals in the body via skin occurs through passive contact with the environment and through direct application of chemicals on the body for the purposes of medical therapy (skin disease, transdermal drug delivery), and cosmetics. In most cases, the toxicity of chemicals is low, likely because the bioavailability (rate and amount of absorption) of the chemical is too low to cause an immediate response; however, some chemicals when applied to the skin have produced toxicity.

This chapter summarizes methodologies used to study percutaneous absorption and results from these absorption studies. The rate of percutaneous absorption can impact the bioequivalence of drugs, and thus is an important factor to consider. There are many variables involved in the study of percutaneous absorption, which can influence the final results such as study design. Interpretation of particular studies should be restricted within the limits of its study design. The methodology and supportive information discussed here are intended to help in formulating a good study design.

M. S. Leo (✉)
Department of Dermatology, University of California-San Francisco,
San Francisco, CA 94143 , USA
e-mail: mleo799@gmail.com

H. I. Maibach
Department of Dermatology, School of Medicine, University of California,
San Francisco, CA, USA
e-mail: MaibachH@derm.ucsf.edu

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_1

1.2 Powdered Human Stratum Corneum

An *in vitro* model uses the partition coefficient of the chemical contaminant between water or some other vehicle and powdered human stratum corneum. Adult foot calluses are grounded with dry ice and freeze-dried to form a powder. The portion of the powder that passes through a 40-mesh sieve but not through an 80-mesh one is used. A solution of the (radiolabeled) chemical in 1.5 mL of water or some other vehicle is mixed with 1.5 mg of powdered human stratum corneum, and the mixture is allowed to set for 30 min. The mixture is then centrifuged, and the proportion of the chemicals bound to the human stratum corneum and that remaining in water are determined by scintillation counting or by another analytical method [1].

Binding studies were performed to evaluate the capacity of skin and soil for cadmium. Cadmium chloride in water was partitioned against 1 mg of soil and 1 mg of powdered human stratum corneum. Table 1.1 shows the percentage dose in water and in matter (soil or powdered human stratum corneum); and Table 1.2 gives the partition coefficient of cadmium chloride. Soil has a relatively higher affinity for cadmium than stratum corneum. This correlates with the data indicating that skin absorbs more cadmium from water than from soil [2].

Further studies regarding arsenic prepared in a similar manner can be compared to studies for cadmium. Table 1.3 shows the percentage dose of arsenic

Table 1.1 Distribution of cadmium chloride between water and powdered human stratum corneum (SC) and between water and soil. (Adapted from [2])

	Done
	Percentage dose
Water and SC	
Water	68.6 ± 5.6
SC	3.2 ± 3.8
Total	101.8 ± 3.3
Water and soil	
Water	9.3 ± 1.4
Soil	82.5 ± 1.0
Total	91.8 ± 1.8

116 ppb Cd/1.0 mL water/1.0 mg stratum corneum or 1.0 mg soil mixed for 30 min followed by centrifugation; $n = 3$

Table 1.2 Partition coefficient of $CdCl_2$ in human powdered stratum corneum(SC)/water and soil/water. (Adapted from [2])

Material tested	Partition coefficient
SC	3.61×10^1
Soil	1.03×10^2

Partition coefficient = concentration of $CdCl_2$ in 1000 mg of HPSC (soil)/concentration of $CdCl_2$ in 1000 mg of water

Table 1.3 *In vitro* percutaneous absorption of arsenic from water and soil on human skin

Percentage dose absorbed				
Vehicle	Skin	Surface wash	Receptor fluid	Total recovery
Soil	0.33 ± 0.25	99.2 ± 27.3	0.43 ± 0.54	99.9 ± 27.5
Water	0.98 ± 0.96	69.8 ± 16.4	0.93 ± 1.1	71.7 ± 15.4

Mean ± standard deviation; $n = 9$ (three skin sources × three replicates each)

Table 1.4 Partition coefficient of arsenic in human powdered stratum corneum/water and water/soil

Test material	Partition coefficient
Stratum corneum	10,928
Soil	24,882

$$\text{Partition coefficient} = \frac{\text{Concentration of arsenic-73 in 1000 mg HPSC (soil)}}{\text{Concentration of arsenic-73 in 1000 ml water}}$$

absorption in human stratum corneum from water and soil. The *in vitro* absorption of cadmium from water is from 9 to 12 % dose and 0.08–0.2 % dose from soil; whereas the *in vitro* absorption of arsenic from water is 1.9 % dose and 0.76 % from soil [2, 3]. This difference may be due to the physical and chemical properties of the metals. The greater dose of arsenic absorbed from soil compared to cadmium absorbed from soil is explained by the small differences in the partition coefficients of arsenic chloride from water to human powdered stratum corneum, and from water to soil as seen in Table 1.4. This gives an approximate ratio of 1:2.5 for stratum corneum to soil, while cadmium chloride's ratio for stratum corneum to soil is 0.36:1000.

Another example of the use of powdered human stratum corneum is shown in Table 1.5. Here, the ability of soap and water to decontaminate the skin is shown for alachlor [4].

Table 1.5 Partitioning: alachor in lasso with powdered human stratum corneum

Test material	[^{14}C] Alachlor percentage dose
Stratum corneum	90.3 ± 1.2
Lasso supernatant	5.1 ± 1.2
Water-only wash of stratum corneum	4.6 ± 1.3
10 % soap and water wash	77.2 ± 5.7
50 % soap and water wash	90 ± 0.5

[^{14} C] Alachlor in Lasso EC formulation (1:20 dilution) was mixed with powdered human stratum corneum, and allowed to set for 30 min, then centrifuged stratum corneum was washed with: (1) water only; (2) 10 % soap and water; (3) 50 % soap and water
Details of the stratum corneum model are found in references [1–4]

1.3 *In Vitro* Percutaneous Absorption Method

The most commonly used *in vitro* technique involves placing a piece of excised skin in a diffusion chamber, applying cold or radioactive compounds to one side of the skin and then assaying for radioactivity, in the collection vessel, on the other side [4]. Excised human or animal skin may be used, and the skin can be fully intact or separated into epidermis or dermis. Artificial membranes can be used in place of skin to measure diffusion kinetics. The advantages of the standard *in vitro* technique are that the method is easy to use, and the results are obtained quickly. The disadvantage is that the fluid in the collection bath that bates the skin is saline, which may be appropriate for studying hydrophilic compounds but is not suitable for hydrophobic compounds. Table 1.6 shows that absorption of triclocarban, *in vitro*, in a standard static system was 0.13 ± 0.05 % of applied dose through human adult abdominal skin. In contrast, *in vivo*, the absorption was 7.0 ± 2.8 % in humans. The discrepancy appeared to be primarily due to the insolubility of triclocarban in the small volume of saline used in the reservoir of the static system. When a continuous flow system was employed, in which the volume of saline was greatly increased, the solubility of triclocarban was no longer the limiting factor in absorption, and the extent of absorption *in vitro*, approached that of absorption *in vivo* [5]. Studies have also employed a surfactant to increase the solubility of the tested compound, but the adequacy of this approach has not been fully examined [6].

Studies have compared this *in vitro* technique to the *in vivo* methods, particularly the *in vitro* and *in vivo* percutaneous absorption of four compounds and the different vehicles used to deliver them with the *in vivo* method generally showing better total absorption [6].

The validity of using excised skin depends on three assumptions. The first is that no living process affects the skin's impermeability. If the contribution of metabolism in maintaining cellular lipids were not counted, then this assumption would be valid for compounds that strictly follow Fick's law of diffusion. In contrast, the drug metabolizing enzyme activity in the epidermis is greatly dependent on the tissue viability. (It should be emphasized that determination of skin absorption by measurement of radioactivity in the collection vessel, when radiolabeled compounds are used, does not distinguish between the unchanged compounds and its metabolites.) It cannot be assumed that the excised skin (usually stored) will retain full enzymatic activity.

Table 1.6 Percutaneous absorption of triclocarban [5]

In vitro and *in vivo*	
System	Dose absorbed (% ± SD)
Static system (23°C)	0.13 ± 0.05
Human adult abdominal skin ($n = 8$)	
Continuous-flow system (23 °C)	
Human adult abdominal system ($n = 12$)	6.0 ± 2.0
Human, *in vivo* ($n = 5$)	7.0 ± 2.8

The second assumption is that the dermis does not affect penetration. The problem of compatibility with hydrophobic compounds was discussed above with regard to triclocarban. In addition, W. Reifenratth compared the penetrability of different thicknesses of skin and has shown the penetration of dermis to be the rate-limiting step in the penetration of DDT [7] .

The third assumption is that skin surface conditions *in vitro* are similar to those *in vivo*. In volatile compounds (mosquito repellents, perfume fragrances), the ratio of volatility to penetration is very much dependent on surface conditions. Another major unknown factor with surface conditions is the bacterial population and the role it might play in percutaneous absorption.

Despite the concern regarding the above assumptions, the excised diffusion chambers are easy to use and are capable of producing rapid, reproducible results. However, once the results are obtained, it may be necessary to verify the findings *in vivo*.

Although, the excised human skin remains the gold standard, it may be difficult to obtain at times. Porcine skin models have been proposed as a viable substitute. Particular research groups have produced a porcine ear skin culture system that was tested with benzo(a)pyrene as a model drug [8]. As the dose of the benzo(a)pyrene was increased from 25 to 800 nmol, the percentage of radioactive material that did not penetrate increased from 2.2 to 35.7 %. Porcine skin showed absorption rates from 12.3 % up to a maximum of 35.6 % depending on the dose.

1.4 *In Vitro* Individual and Regional Variation

Table 1.7 presents the results on *in vitro* percutaneous absorption of a test article in three different human skin sources. The data were summarized over five different formulations (each run on all three skin sources). Of interest is the receptor fluid accumulation in human skin source 2. It shows that the barrier properties of human skin source 2 were such that no skin absorption occurred. A formulation comparison with only that skin source would have provided negative data [9].

Table 1.8 gives the *in vitro* percutaneous absorption of pentadeconic acid from two formulations (A and B) in two human skin sources. The skin content shows that formulations were only distinguishable in one of the human skin samples. This suggests that decisions based on only one skin source may be misleading [9].

Table 1.7 Individual variation in *in vitro* percutaneous absorption

Human skin source	Percentage dose (mean ± SD)			
	Skin	Surface wash	Receptor fluid	Total recovery
1	5.0 ± 2.4	85.7 ± 7.8	2.5 ± 4.5	93.2 ± 6.0
2	3.2 ± 2.8	83.7 ± 9.5	0.3 ± 0.2	87.7 ± 8.1
3	2.6 ± 1.0	72.1 ± 12.3	4.4 ± 5.0	79.0 ± 12.5

Table 1.8 *In vitro* percutaneous absorption of pentadeconic acid

Skin source	Formulation	Skin	Surface wash	Receptor fluid	Total recovery
1	A	5.4 ± .8	80.4 ± 8.9	0.05 ± 0.04	85.9 ± 9.5
	B	6.4 ± 1.8	85.6 ± 5.2	0.15 ± 0.03	92.1 ± 4.5
2	A	5.8 ± 1.5	86.2 ± 6.6	0.03 ± 0.03	92.1 ± 5.2
	B	14.2 ± 23	80 ± 5.3	0.03 ± 0.01	94.2 ± 4.3

Percentage dose (mean ± SD)

Table 1.9 *In vitro* percutaneous absorption of taurocholic acid

Skin source	Skin content	Receptor fluid content
1	0.5 ± 0.3	8.9 ± 12.9
1 repeat	1.3 ± 1.3	8.2 ± 6.0
2	1.4 ± 1.8	16.5 ± 18.0
2 repeat	3.4 ± 6.3	11.7 ± 14.3
3	1.4 ± 1.5	0.2 ± 0.3
3 repeat	0.8 ± 0.8	0.3 ± 0.7

Table 1.10 Individual human skin source variation in *in vitro* percutaneous absorption

Human skin source	Skin content	
	DDT	Benzo[a]pyrene
1	6.7 ± 1.5	27.4 ± 10.9
2	29.6 ± 8.1	20.0 ± 8.6

Table 1.9 gives the *in vitro* percutaneous absorption of taurocholic acid. Data are summarized over five formulations and individualized for each human skin source and repeated skin absorption for each of the skin sources. Of interest is the receptor fluid content (24 h accumulation) and the totally different results in skin source 3 compared with the other two skin sources (1 and 2). Again, individual variations can be significant, but this becomes apparent only when other skin sources are also used [9].

Table 1.10 compares the *in vitro* percutaneous absorption of DDT and benzo[a] pyrene from two human skin sources. Skin content differences should be noted for both different individual skin sources and penetration of different chemicals in the human skin [9].

Table 1.11 provides an overview of the *in vivo* percutaneous absorption of multiple compounds and the ratio of the unique site's percentage of dose absorbed compared to the forearm's percentage of dose absorbed [10].

Table 1.12 compares the *in vitro* permeability of coumarin, griseofulvin, and propranolol across human abdominal skin and scalp skin. For coumarin and propranolol, the scalp showed higher permeability. Feldmann and Maibach identified

Table 1.11 Percutaneous absorption via anatomic location

Compound											
Anatomic location	Benzoic acid	4-Cyano-phenol	Cimetidine	Acetylsali-cylic acid	Benzene	Benzoic acid	Caffeine	Hydrocor-tisone	Paraquat	Parathion	Testoster-one
Forearm	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Fossa cubitalis										3.3	
Dorsal hand					1.9				0.8	2.4	
Palm								0.8		1.3	
Ankle								0.4			
Leg		0.6	0.4						1.0		
Thigh	1.1	0.7	0.5			1.3					
Abdomen	1.4	0.5	0.5	1.0		1.6	0.6			2.1	
Back	1.0	1.4	2.2			0.9		1.7			
Chest	1.4					1.2					
Foot								0.1		1.6	
Scalp								3.5		3.7	
Postauricular				1.7		2.5				3.9	
Jaw angle										3.9	
Forehead	2.0			2.1		2.9	1.8	6		4.2	
Ear canal										5.4	
Axilla								3.6		7.4	
Scrotum								42		11.8	
Vulva								2.9,5.9			1.2

Reported as rate of penetration normalized to stratum corneum depth. Values represent the ratio of the unique site's percentage of dose absorbed to the forearm's percentage of dose absorbed. Columns to the right of the bold border were measured via urinary method, and columns to the left via stripping methods

Table 1.12 *In vitro* permeability across human abdominal and scalp skin

Drug	Steady-state flux (mg/cm^2/h)	
	Abdominal skin	Scalp skin
Coumarin	130 ± 78	172 ± 64
Griseofulvin	10 ± 6	16 ± 8
Propranolol	30 ± 6	42 ± 11

Significant difference ($P < 0.05$) for abdominal versus scalp skin

the scalp as a higher absorbing area *in vivo* in humans [11]; thus the *in vitro* results of Ritschel et al. agree with the *in vivo* results [12].

Variations in percutaneous absorption are also present in animal models. The absorption rates of nicorandil, isosorbide dinitrate, and flurbiprofen were evaluated in yucatan micropig skin [13]. Both, interindividual and regional variations of skin were examined. Interindividual variations of the micropig skins were greater than the differences in absorption rates of the regional sites tested: left shoulder, right shoulder, left hip, and right hip. However, the absorption rate of the micropig skin was approximately half that of the human skin tested.

Individual and regional variations exist for *in vivo* percutaneous absorption, and as shown in this chapter, the same variability exists for *in vitro* percutaneous absorption. This can be critical for studies using human skin because of the limited availability of human skin, and the tendency to use only one human skin source to conserve supply. We recommend, if possible, the use of multiple human skin sources.

1.5 *In Vitro* Short-Term Skin Exposure

Skin exposure from drug therapy and environmental chemical contaminants usually has an extended period of a day or even longer. However, some exposures will be of a considerably shorter duration—minutes rather than days. In either case, the first step of percutaneous absorption occurs within the first 30 min. Rougier et al. have shown that *in vivo* absorption in the longer term can be predicted from the first 30 min of exposure [14–16]. With binding to powdered human stratum corneum and *in vitro* percutaneous absorption, our data reflect these early happenings. Our *in vivo* data confirm some of the potential predictions of longer exposures. The message is that even short-term chemical skin exposure can result in significant skin chemical content. This may be good for some types of drug therapy but may not be good in some instances of environmental exposure.

A study was done with human skin being exposed to cadmium in water for only 30 min, followed by skin surface wash with soap and water. One half of the replicates (nine each; three human skin sources × three replicates) were stopped at 30 min. Human skin content was 2.3 ± 3.3 %, and no cadmium was detected in the plasma

Table 1.13 Exposure of cadmium in water to human skin for 30 min followed by skin surface wash with soap and water and then a 48 h perfusion with human plasma

	Percentage dose	
Treatment	Skin content	Plasma receptor fluid
30 min exposure only	2.3 ± 3.3	0.0 ± 0.0
30 min exposure followed by 48 h perfusion	2.7 ± 2.2	0.6 ± 0.8
Statistics	$P = 0.77$	$P = 0.04$

Table 1.14 *In vivo* percutaneous absorption of p-nitroaniline in the rhesus monkey following 30 min exposure to surface water: comparison of *in vitro* binding and absorption

Phenomenon	Percentage dose absorbed/bound
In vivo percutaneous absorption, rhesus monkey	4.1 ± 2.3
In vitro percutaneous absorption, human skin	5.2 ± 1.6
In vitro, powdered human stratum corneum	2.5 ± 1.1

Table 1.15 Skin exposure time and skin content during *in vitro* percutaneous absorption

Chemical	Formulation	Percentage dose 24 h exposure	Skin content 25 min exposure
DDT	Acetone	18.1 ± 13.4	16.7 ± 13.2
	Soil	1.0 ± .7	1.8 ± 1.4
Benzo[a]pyrene	Acetone	23.7 ± 9.7	5.1 ± 2.1
	Soil	1.4 ± .8	0.14 ± 0.13

receptor fluid. The other half of the replicate was perfused for an additional 48 h. The skin content was 2.7 ± 2.2 %, which was not significantly different ($P = 0.77$). However, about 0.6 ± 0.8 % of the dose had diffused into the plasma receptor fluid ($P = 0.04$). Therefore, cadmium has the ability of binding to the human skin during a short exposure in water, and is not completely removed using soap and water wash, rather is subsequently absorbed in the body (Table 1.13).

Table 1.14 summarizes a short-term (30 min) exposure for p-nitroaniline. *In vitro* absorption, *in vivo* absorption, and binding to powdered human stratum corneum are all in agreement, and show the potential for short-term absorption. Table 1.15 gives additional data for DDT and benzo[a]pyrene. Short-term (25 min) exposure can be as extensive as that of a 24 h exposure [17].

Animal studies have also been conducted in hairless guinea pigs to test the short-term exposure to sulfur mustard ranging from 1 to 30 min. As the exposure time increased, both, wound area size as well as transepidermal water loss (TEWL) increased. A 5 min exposure to sulfur mustard led to a damaged stratum corneum 72 h after exposure, whereas 30 min of sulfur mustard exposure resulted in barrier disruption—24 h after exposure [18].

1.6 *In Vivo* Percutaneous Absorption Methods

1.6.1 Skin Stripping: Short-Term Exposure

The stripping method determines the concentration of a chemical in the stratum corneum at the end of a short application period (30 min), and by linear extrapolation predicts the percutaneous absorption of that chemical for longer application periods. The chemical is applied to the skin of animals or humans, and after a 30 min skin application time, the stratum corneum is removed by successive tape application. The tape strippings are assayed for their chemical content. Rougier and coworkers have established a linear relationship between this stratum corneum reservoir content and percutaneous absorption using the standard urinary excretion method. The main advantages are: (1) elimination of measurement of urinary (and fecal) excretion to determine absorption; (2) the applicability to non-radiolabeled determination of percutaneous absorption because the skin strippings contain adequate chemical concentrations for nonlabeled assay methodology [14–16].

There are limitations involved with the use of the skin-stripping approach. The stratum corneum is characterized by a network of furrows that lead to topical drug deposition. The tape-stripping method is unable to remove the superficial corneocytes located in the furrows, thus, using this technique, leads to some undetected drug deposition. These furrows, also, pose a problem because of their tendency to accumulate residual drug material that is difficult to remove completely by washing [19]. Furthermore, the skin-stripping method is influenced by the anatomical site, application pressure, duration, and the removal method applied [20]. The vehicle used in a topical solution can affect the amount of stratum corneum. A study has shown that twice the amount of stratum corneum was removed by the ethanol application compared to water or oil application [21]. The first tape strip is often discarded in most experiments because it represents the unabsorbed drug on the skin surface; however, this procedure has not been critically evaluated and the first tape is necessary to calculate the concentration of substance recovered. This technique can only assess the concentration of drugs in the stratum corneum, and only at a single point of time per stripping. A standardized procedure has yet to be adopted, and thus there are limitations while comparing the data obtained through this *in vivo* technique from different studies.

There are multiple methods to quantify the amount of stratum corneum removed from each strip. Weighing the tape strips before and after stripping is one of the approaches, but the sebum, topical formulation, and interstitial fluid may increase the weight difference from the initial tape. Another approach is to use a protein assay based on the amount of proteins in corneocytes. The proteins show an absorption spectrum at 278 nm that can be measured using a UV-spectrometer. However, the absorption is weak, and also there can be superpositions with stronger bands from other substances. Some approaches have been applied, such as the use of trypan

blue, to increase the absorption of the proteins in the visible light range [22]. Another relevant approach to quantify the amount of stratum corneum removed is the use of optical spectroscopy. There is a decrease in the transmission of light due to reflection, diffraction, and scattering by the corneocyte aggregates that result in an increasing absorption with a deceasing wavelength termed pseudo-absorption. The pseudo-absorption of the tape strips can be used to determine the amount of stratum corneum removed by using an UV/Vis spectrometer [23]. The colorimetric protein quantification method can also be used to determine the amount of stratum corneum removed through tape stripping. This method measures the fraction of stratum corneum proteins that is soluble in sodium hydroxide with the use of a protein assay. It has been shown to be an accurate and reproducible method as demonstrated by (Dreher et al. 1998), who measured the removed mass of stratum corneum both, gravimetrically and spectrophotometrically, as a function of shaking time [24]. These two methods produce nearly identical results with a correlation coefficient of up to 0.99, depending on the shaking time.

1.6.2 Skin Flaps

If a section of skin of an animal is surgically isolated such that the blood supply is singular, this singular source can be used to collect chemicals in blood as they absorb through the skin. The isolated skin section, while being intact on the animal, can be used for *in vivo* percutaneous absorption studies, or the skin section with its intact blood vessels can be mounted in an *in vitro* perfusion system to study percutaneous absorption.

This methodology is exemplified by the isolated perfused porcine skin flap (IPPSF) and the skin sandwich flap (SSF). The IPPSF is surgically created on a pig, and then the viable flap with an intact blood supply can be mounted in an *in vitro* perfusion system. The absorption of chemicals through the skin and metabolism within the skin can be determined by assaying the blood vessel perfusate. The IPPSF model is advantageous as it is an alternative *in vitro* animal model, and the metabolism of chemicals penetrating the skin can be determined [25, 26]. The skin sandwich flap (SSF) is an island flap that has a split thickness skin grafted to its subcutaneous surface directly under the superficial epigastric vasculature. In this setting, the dermis of the donor skin and the subcutaneous tissue of the host flap grow together, sandwiching the vessels supplying the flap, the superficial epigastric vessels. Two additional steps allow this sandwich to be converted to an island sandwich flap, which is isolated on its vasculature and transferred back to the animals using a series of surgical procedures. The juncture on the femoral vessels supplying and draining the flap can be readily visualized with an incision in the groin, and is accomplished routinely. The exposed vein draining the flap tolerates multiple venopunctures. The SSF can be constructed with either human, pig, or rat skin as the skin donor [27].

1.6.3 Systemic Bioavailability (Blood and Excreta)

Percutaneous absorption, *in vivo*, is usually determined by an indirect method based on the measurement of radioactivity in excreta following topical application of the labeled compound. In human studies, plasma levels of the compound are extremely low following topical application, often below assay detection level, so it is necessary to use the tracer methodology. The labeled compound (usually carbon-14 or tritium labeled) is applied to the skin. The total amount of radioactivity excreted in urine (or urine along with feces) is then determined. The amount of radioactivity retained in the body or excreted by some route not assayed (CO_2, sweat) is corrected for by determining the amount of radioactivity excreted following parenteral administration. This final amount of radioactivity is then expressed as the percentage of the applied dose that was absorbed.

The equation used to determine percutaneous absorption is:

$$\text{Percent} = \frac{\text{Total radioactivity following topical administration}}{\text{Total radioactivity following parenteral administration}} \times 100.$$

Determination of percutaneous absorption from urinary radioactivity does not account for metabolism by skin. The radioactivity in urine is a mixture of that of the parent compound and metabolites. Plasma radioactivity can be measured and the percutaneous absorption determined by the ratio of the areas under the plasma concentration versus time curves following topical and intravenous administration. Radioactivity in blood and excreta can include both the applied compound and metabolites. If the metabolism by skin is extensive and different from that of other systemic tissues, then this method is not valid because the pharmacokinetics of the metabolites can be different from that of the parent compound. However, in practice, this method has given results similar to those obtained from urinary excretion [28].

The only way to determine the absolute bioavailability of a topically applied compound is to measure the compound using specific assays in blood or urine followed by topical and intravenous administration. This is difficult to do, as plasma concentrations after topical administration are often very low. However, as more sensitive assays are developed, estimates of absolute topical bioavailability will become a reality. A comparison of the above methods was performed by using [^{14}C] nitroglycerin in rhesus monkeys (Table 1.16). The difference between the estimate of absolute bioavailability (56.6 %) and that of ^{14}C (72.7–77.2 %) is the percentage of the compound metabolized in the skin as the compound was being absorbed. For nitroglycerin, this is about 20 % [28].

Table 1.16 Bioavailability of topical nitroglycerin determined from plasma nitroglycerin, plasma ^{14}C, and urinary excretion of ^{14}C

Method	Mean bioavailability %
Plasma nitroglycerin AUC	56.6 ± 2.5
Plasma total radioactivity AUC	77.2 ± 6.7
Urinary total radioactivity	72.7 ± 5.8

Absolute bioavailability of nitroglycerin and 14C

Percent = (total ^{14} C excretion following topical administration)/total ^{14} C excretion following i. v. administration × 100 *AUC* Area under the curve

$$\text{Percent} = \frac{\text{AUC (ng} \times \text{h/mL)/topical dose}}{\text{AUC (AUC (ng} \times \text{h/mL))/i.v. dose}} \times 100.$$

1.6.4 Microdialysis

Another approach has been shown to be a promising *in vivo* technique for measuring percutaneous absorption. It involves the use of a fiber forming a thin hollow tube of a semipermeable membrane. The fiber allows passage of molecules with a volume smaller than the opening in the membrane. The fiber is inserted into the dermis parallel to the skin and a physiological solution is pumped through the probe from the afferent side of the tube to the efferent side. Microdialysis allows the continuous monitoring, assessment, and metabolism of substances directly in the target tissue. However, microdialysis does not allow for systemic measurements of percutaneous absorption. The insertion of the probe causes an increase in blood flow, and thus requires equilibration time. Finally, the depth of the insertion of the probe in the dermis can be inconsistent [29, 30]. Details are found in references [29, 30]. See also Chap. 10 for a more complete analysis.

1.6.5 Surface Disappearance

Another approach used to determine *in vivo* percutaneous absorption is to measure the loss of radioactive material from the surface as it penetrates the skin. Recovery of an ointment or solution following skin application is difficult because total recovery from the skin is never assured. Using topical application of a transdermal delivery device, the total unit can be removed from the skin and the residual amount of drug in the device can be determined. The difference between the applied and the residual dose is assumed to be the amount of drug absorbed. One must be aware that the skin may act as a reservoir for unabsorbed material.

1.6.6 Suction Blister

Interstitial fluid and serum can be extracted from a fluid-filled blister formed by applying a partial negative pressure to the epidermal–dermal junction. This allows the sampling of any previously applied compounds with the use of a hypodermic needle and a concentration–time profile for the compound can be made. However, very low compound levels may be present due to the potential binding of the compound to skin tissue, and more so for lipophilic species. This indirect technique is used to assess skin drug levels following systemic administrations, and is rather an invasive technique that can cause scarring [29].

1.6.7 Biological Response (Pharmacodynamics)

Another *in vivo* method of estimating absorption is to use a biological/pharmacological response. Here, a biological assay is substituted for a chemical assay, and absorption is estimated. An obvious disadvantage of using biological response is that it is good only for compounds that will elicit an easily measurable response. An example of a biological response would be the vasoconstrictor assay when the balancing effect of one compound is compared with that of a known compound. This method is perhaps more qualitative than quantitative. More details are given in reference [31]. The physiologically based pharmokinetic model (PBPK) was designed to provide a more quantitative measurement of absorption of drugs. PBPK relies on a system of equations derived from previous knowledge of human anatomy, physiology, and biochemistry to facilitate interspecies extrapolations or relate data from different administration methods. A novel PBPK model based on rats injected with ^{14C}patupilone has been utilized to predict concentrations of ^{14C}patupilone in cancer patients [32].

Other qualitative methods of estimating *in vivo* percutaneous absorption include whole-body autoradiography and fluorescence. Whole-body autoradiography will give and overall picture of dermal absorption followed by the involvement of other body tissues with the absorbed compound.

1.6.8 Raman Spectroscopy

Another novel *in vivo* approach uses a monochromatic laser light that transfers energy from photons to molecules in the skin to cause distinct excited vibrational modes in those molecules. Using this knowledge and the fact that varying amounts of energy is needed to excite molecules of various sizes and the types of bonds in those molecules, Raman Spectroscopy can be used as a highly molecule-specific tool to analyze percutaneous absorption of drugs. Confocal Raman Spectroscopy is a type of Raman Spectroscopy that can filter the sample's analysis volume in 3D. It has

been used to determine the water concentration profile in the stratum corneum as a function of distance to the skin surface. However, this technique not only requires the levels of the compound under investigation to be in significant concentrations but also determines relative and not absolute levels of percutaneous absorption [33]. Details are found in [33].

1.7 Other Methods to Examine Maturity of Stratum Corneum (The Major Barrier to Water Loss)

1.7.1 Quantitative Structure Activity Relationship (QSAR)

Using the relationship between chemical structure and biological activity of a compound, QSARs can be used to predict the percutaneous absorption rates of various compounds. Variables that affect the penetration of the skin barrier, such as the lipophilicity, size, solubility, etc. can be used to create a QSAR model to determine the absorption rate of a particular compound. Details are found in [34].

1.7.2 Transepidermal Water Loss

Transepidermal water loss, TEWL, is a physiological phenomenon that demonstrates the integrity of the barrier function of stratum corneum that can be used to test the maturity of the skin. A high TEWL value is associated with a poor epidermal barrier, thereby, increasing the absorption of topical formulations. An inverse relationship exists between the corneocyte size, measured through tape stripping, and TEWL in the human cheek [35].

1.7.3 Skin Impedance

Skin impedance is another method used to measure the integrity of the stratum corneum barrier. A pair of electrodes is placed on the skin, and current is applied to test the electrical resistance of the epidermis between the two electrodes. There is a sharp decrease in impedance once the outer layers of the epidermis are removed. TEWL, also, shows a significant increase once the outer layer is removed. However, impedance is more strongly affected by the removal of the outer layer than TEWL [36].

1.8 Concluding Remarks

It is likely that human neonatal skin is different from adult skin; however, there has been limited research on this topic. For more details regarding neonatal skin consult references [37]. Altogether, the significant advances in penetration methodology have developed since World War II. For additional details, textbooks by references [38–40] should be valuable. It is likely that many surprises and advances will follow.

References

1. Wester RC, Mobayen M, Maibach HI. In vivo and in vitro absorption and binding to powered stratum corneum as methods to evaluate skin absorption of environmental chemical contaminants from ground and surface water. J Toxicol Environ Health. 1987;21(3):367–74.
2. Wester RC, et al. In vitro percutaneous absorption of cadmium from water and soil into human skin. Fundam Appl Toxicol. 1992;19(1):1–5.
3. Wester RC, et al. In vivo and in vitro percutaneous absorption and skin decontamination of arsenic from water and soil. Fundam Appl Toxicol. 1993;20(3):336–40.
4. Bucks DA, et al. In vitro percutaneous absorption and stratum corneum binding of alachlor: effect of formulation dilution with water. Toxicol Appl Pharmacol. 1989;100(3):417–23.
5. Wester RC, Maibach HI, Surinchak J, Bucks DAW. Predictability of in vitro diffusion systems: effect of skin types and ages on percutaneous absorption of triclocarban. In: Bronaugh RL, Maibach HI, Editor. Percutaneous penetration. Marcel Dekker: New York; 1985. 223–6.
6. Franz TJ, Lehman PA, Raney SG. Use of excised human skin to assess the bioequivalence of topical products. Skin Pharmacol Physiol. 2009;22(5):276–86.
7. Reifenrath WG, Hawkins GS, Kurtz MS. Percutaneous penetration and skin retention of topically applied compounds: an in vitro-in vivo study. J Pharm Sci. 1991;80(6):526–32.
8. Jacques C, et al. Disposition and biotransformation of ^{14}C-Benzo(a)pyrene in a pig ear skin model: ex vivo and in vitro approaches. Toxicol Lett. 2010;199(1):22–33.
9. Wester RC, Maibach H, Individual and regional variation with in vitro percutaneous absorption. In: Bronaugh RL, Maibach HI, Editor. In vitro percutaneous absorption: principles, fundamentals, and applications. CRC: Boca Raton; 1991. S 25–30.
10. Lev-Tov H, Maibach HI. Regional variations in percutaneous absorption. J Drugs Dermatol. 2012;11(10):48–51.
11. Feldmann RJ, Maibach HI. Regional variation in percutaneous penetration of ^{14}C cortisol in man. J Invest Dermatol. 1967;48(2):181–3.
12. Ritschel WA, Sabouni A, Hussain AS. Percutaneous absorption of coumarin, griseofulvin and propranolol across human scalp and abdominal skin. Methods Find Exp Clin Pharmacol. 1989;11(10):643–6.
13. Takeuchi H, et al. Variation assessment for in vitro permeabilities through Yucatan micropig skin. Biol Pharm Bull. 2011;34(4):555–61.
14. Dupuis D, et al. In vivo relationship between horny layer reservoir effect and percutaneous absorption in human and rat. J Invest Dermatol. 1984;82(4):353–6.
15. Rougier A, et al. In vivo correlation between stratum corneum reservoir function and percutaneous absorption. J Invest Dermatol. 1983;81(3):275–8.
16. Rougier A, et al. Regional variation in percutaneous absorption in man: measurement by the stripping method. Arch Dermatol Res. 1986;278(6):465–9.
17. Wester RC, et al. Percutaneous absorption of [^{14}C]DDT and [^{14}C]benzo[a]pyrene from soil. Fundam Appl Toxicol. 1990;15(3):510–6.
18. Dachir S, et al. Characterization of acute and long-term sulfur mustard-induced skin injuries in hairless guinea-pigs using non-invasive methods. Skin Res Technol. 2010;16(1):114–24.

19. VanderMolen RG, et al. Tape stripping of human stratum corneum yields cell layers that originate from various depths because of furrows in the skin. Arch Dermatol Res. 1997;289(9):514–8.
20. Loffler H, Dreher F, Maibach HI. Stratum corneum adhesive tape stripping: influence of anatomical site, application pressure, duration and removal. Br J Dermatol. 2004;151(4):746–52.
21. Jacobi U, et al. Effect of the vehicle on the amount of stratum corneum removed by tape stripping. J Dtsch Dermatol Ges. 2003;1(11):884–9.
22. Lademann J, et al. The tape stripping procedure—evaluation of some critical parameters. Eur J Pharm Biopharm. 2009;72(2):317–23.
23. Weigmann H, et al. Determination of the horny layer profile by tape stripping in combination with optical spectroscopy in the visible range as a prerequisite to quantify percutaneous absorption. Skin Pharmacol Appl Skin Physiol. 1999;12(1–2):34–45.
24. Dreher F, et al. Colorimetric method for quantifying human stratum corneum removed by adhesive-tape stripping. Acta Derm Venereol. 1998;78(3):186–9.
25. Riviere JE, et al. The isolated perfused porcine skin flap (IPPSF). I. A novel in vitro model for percutaneous absorption and cutaneous toxicology studies. Fundam Appl Toxicol. 1986;7(3):444–53.
26. Riviere JE, Bowman KF, Monteiro-Riviere NA. On the definition of viability in isolated perfused skin preparations. Br J Dermatol. 1987;116(5):739–41.
27. Pershing LK, Krueger GG. Human skin sandwich flap model for percutaneous absorption. In: Bronaugh RL, Maibach HI, Editor. Percutaneous Absorption. 2nd ed. New York: Marcel Dekker; 1989. S. 397–414.
28. Wester RC, et al. Pharmacokinetics and bioavailability of intravenous and topical nitroglycerin in the rhesus monkey: estimate of percutaneous first-pass metabolism. J Pharm Sci. 1983;72(7):745–8.
29. Herkenne C, et al. In vivo methods for the assessment of topical drug bioavailability. Pharm Res. 2008;25(1):87–103.
30. Kreilgaard M. Assessment of cutaneous drug delivery using microdialysis. Adv Drug Deliv Rev. 2002;54(Suppl 1):99–121.
31. Andersen P, Milioni K, Maibach H. The cutaneous corticosteroid vasoconstriction assay: a reflectance spectroscopic and laser-Doppler flowmetric study. Br J Dermatol. 1993;128(6):660–5.
32. Xia B, et al. Novel physiologically based pharmacokinetic modeling of patupilone for human pharmacokinetic predictions. Cancer Chemother Pharmacol. 2012;69(6):1567–82.
33. Caspers PJ, et al. In vivo confocal raman microspectroscopy of the skin: noninvasive determination of molecular concentration profiles. J Invest Dermatol. 2001;116(3):434–42.
34. Walker JD, Rodford R, Patlewicz G. Quantitative structure-activity relationships for predicting percutaneous absorption rates. Environ Toxicol Chem. 2003;22(8):1870–84.
35. Gorcea M, et al. In vivo barrier challenge and long-term recovery in human facial skin. Int J Cosmet Sci. 2013;35(3):250–6.
36. Kalia YN, F Pirot, RH. Guy homogeneous transport in a heterogeneous membrane: water diffusion across human stratum corneum in vivo. Biophys J. 1996;71(5):2692–700.
37. Maibach, Howard I, Edward KB. Neonatal skin: structure and function. New York: Marcel Dekker; 2003.
38. Bronaugh, Robert L, Howard IM. Percutaneous absorption: drugs, cosmetics, mechanisms, methodology. Boca Raton: Taylor & Francis; 2005.
39. Smith EW, Howard IM. Percutaneous penetration enhancers. CRC/Taylor & Francis: Boca Raton; 2006.
40. Murthy S, Narasimha, Howard IM. Topical nail products and ungual drug delivery. CRC: Boca Raton; 2013.

Chapter 2
Animal Models for Percutaneous Absorption

Eui Chang Jung and Howard I. Maibach

2.1 Introduction

A most relevant way to determine the percutaneous penetration rate or absorption rate of chemicals in humans is *in vivo* studies. However, it has been increasingly complex to perform *in vivo* human studies because of regulation such as US EPA's human research rule [1]. An alternative way is *in vitro* human skin absorption study not banned by the current human research guidelines. However, it does not have an intact physiologic and metabolic system present in *in vivo* models, and is associated with limited tissue durability, and subject to practical issues of obtaining human tissue. Therefore, animals remain practical models because they are easier to obtain, less subject to regulation, have less intersubject variability due to inbred animals, and there is a large body of valuable data not only on percutaneous absorption/penetration but also on related toxicokinetic and toxicodynamic parameters [2]. However, animal skin is generally more permeable than human skin. To develop most predictive data of the human skin penetration or absorption, animal model's physiology, biochemistry, and anatomy of skin should be similar to humans [3]. Thus, animals phylogenetically close to humans would be good models, but it is not absolutely required for an animal to be genetically close to humans to be a good animal because an animal that is not genetically close to human can have skin characteristics similar to humans. Two basic criteria help judge whether an animal is relevant; the animal model should give percutaneous absorption similar to that in humans; if it is not possible, then percutaneous absorption in the animal model should be constantly different from that in humans.

E. C. Jung (✉)
Department of Dermatology, University of California, 90 Medical Center Way, Surge 110, San Francisco, CA 94143-0989, USA
e-mail: cutis2004@gmail.com

H. I. Maibach
Department of Dermatology, School of Medicine, University of California, San Francisco, CA, USA
e-mail: MaibachH@derm.ucsf.edu

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_2

In 1972, Bartek et al. [4] challenged the world of comparative cutaneous biology to begin to understand relative percutaneous penetration in several species. Subsequently, extensive observations have extended Bartek's investigation—and here we evaluate the subsequent four decades—in hopes of aiding dermatopharmacology and dermatotoxicology studies.

This chapter describes monkeys, pigs, rats, rabbits, guinea pigs, and hairless rodents such as hairless rats, hairless mice, and hairless guinea pigs (HGPs), and then some alternative models such as a human skin grafted onto the nude mouse model (HuSki model).

2.2 Monkeys: Rhesus/Squirrel

The monkey is a most relevant animal model for percutaneous absorption because it is phylogenetically close to humans; therefore, its skin resembles human skin and areas such as the inner arm, legs, and trunk are relatively hairless like human skin. Its regional variation in percutaneous absorption resembles human skin; therefore, the same anatomical site can be used in comparative study. It is sufficiently large for serial blood sampling. However, the use of monkeys in experiments is somewhat limited by cost and restricted availability. Also, they are difficult to handle and require expertise with special facilities. There are some differences in skin anatomy between monkeys and humans; monkeys are covered with a dense coat of pelage not hair; their epidermis has little undersculpture; they have numerous apocrine glands over nearly the entire hairy skin; monkeys have fewer sebaceous glands that directly open to the skin surface [5].

We found three studies for four chemicals, which described the permeability of both monkey and human skin and were published after 1993. 2, 4-dichlorophenoxyacetic acid penetrated similar to human skin [6]. However, acitretin was 0.3 times as permeable as in human skin [7]. In Panchangnula et al.'s study [8], water and 7-hydroxycoumarin were 2.3 and 3.8 times more permeable, respectively, than human skin even if the thickness of full-thickness and stratum corneum (SC) as well as hair density of monkey skin were similar to those of humans. Thus, percutaneous absorption across monkey skin often, but not always, resembles human skin.

2.3 Pigs

Another appropriate animal model for human skin absorption is the pig, both *in vivo* and *in vitro* [2]. Porcine skin is easily obtainable; the pig is large enough for collection of multiple samples (body fluids, biopsies) over extended periods, while at the same time not too large to be inconvenient to handle in standard laboratory animal facilities. There are similarities between porcine and human skin; the skin is characterized by a spare hair coat, a thick epidermis that has a well-differentiated

Table 2.1 Thickness of skin layers of different species. (Modified from [11])

Species, anatomic site	SC (μm)	Epidermis (μm)	Whole skin (mm)
Human, forearm	17	36	1.5
Pig, back	26	66	3.4
Pig, ear	10	50	1.3
Mouse, back	5	13	0.8

Table 2.2 Thickness of human and animal skin. (Modified from [12])

Species	SC (μm)	Epidermis (μm)	Whole skin (mm)
Human	16.8	46.9	2.97
Pig	26.4	65.8	3.43
Rat	18	32	2.09
Mouse	9	29	0.70
Hairless mouse	8.9	28.6	0.70

undersculpture, a dermis that has a well-differentiated papillary body and a large content of elastic tissue [3]. The follicular structure of pig skin also resembles that of humans. The average of 20 hairs/cm^2 of porcine ear skin is similar to 14–32 hairs/cm^2 in humans [9].

The histological appearance of the epidermis is similar in humans and pigs [3]. Porcine and human epidermis appear similar in tissue turnover time and the characterization of keratinous proteins. Porcine SC contains protein fractions grossly similar to humans. It has similar variable filament density and areas of cell overlapping with human skin SC. The epidermal–dermal junction of pigs resembles that of humans. The number, size, distribution, and communications of the dermal blood vessels of the pig were remarkably similar to those of human skin. The architecture of collagen fibers and fiber bundles as wells as the thickness of collagen fibrils in the dermis of the pig is generally similar to those of human skin. In immunohistochemical study with 93 monoclonal or polyclonal antibodies, many antibodies showed similar immunoreactivity on porcine and human skin [10].

Biochemical similarities were found while studying glycosphingolipids and ceramides in human and pig epidermis. The enzyme patterns of the skin of the domestic pig revealed by enzyme histochemical investigations mirror that in man [3]. The thickness of skin layers in porcine skin resembles that of human skin (Tables 2.1 and 2.2) [11, 12].

However, dissimilarities also exist: vascularization is rich in man but poor in pigs; humans have mostly eccrine sweat glands, whereas pigs have mostly apocrine glands. As there is high fat component in pigs, lipid soluble compounds concentrate in the fatty area of pigs rather than the central compartment (blood sampling) [3].

Barbero and Frasch [13] extensively reviewed porcine skin as surrogates for human *in vitro* penetration studies. In 18 studies which reported permeability coefficient of 26 chemicals, correlation efficient (r) between pig and human skin is 0.88 ($p < 0.0001$). It supports a strong positive correlation between two skins.

In another 20 studies of 50 measurements on 40 chemicals that did not report permeability and factors of difference (FODs) calculated from permeability studies, 80% fell within the range of ±1/2 log interval; that is $0.3 < FOD < 3.0$. Average intraspecies coefficient of variation for pig skin is 21% and for human skin 35%. Smaller variation in pig skin than human skin means that fewer experiments would be required to attain sufficient statistic power to confirm subtle differences. In lag-time data (13 measurements from 9 studies on 10 compounds), there is no significant correlation between lag-time in pig skin compared with the human skin.

As the first edition of this book was published in 1993, we reviewed the original papers published after 1993 that described permeability of both pig skin and human skin. These included 46 studies, which measured permeability of 77 chemicals. For 38 chemicals in 26 studies, percutaneous permeability of porcine skin is close to that of human skin ($0.625 < FOD < 1.6$). For 25 chemicals in 15 studies, percutaneous permeability of pigs is higher than that of humans. In this group, nine chemicals were absorbed in porcine skin in a much higher rate than human skin ($FOD > 3$). For 16 chemicals in six studies, human skin permeability is higher than that of pigs. However, only three chemicals showed higher difference ($FOD > 3$). In conclusion, 86% (65 chemicals of 76) fell within the range of ±1/2 log interval.

As seen above, experiments with many chemicals showed similar permeability through pig skin and human skin. But, the degree of resemblance varies with groups of compounds of different chemical characteristics.

2.4 Rats

Rodents are readily available, small and easy to handle, inexpensive, have considerable cumulated data about them; so, they are most commonly used in permeation studies as well as regulatory toxicity studies. However, rodent skin generally shows higher permeation rates compared to human skin. Among rodents, rat skin has more structural similarities to human skin (Table 2.2). Therefore, permeation kinetic parameters of rat skin are frequently comparable with human skin [14]. However, differences between rat skin and human skin are large. In rat skin, epidermis and SC are thinner, appendage number is higher, intercellular lipid composition of the SC is different, and corneocyte surface is lower than in human skin [15].

We reviewed the original papers published after 1993 that described permeability of both rat and human skin. These included 79 studies, which measured absorption of 110 chemicals. For 23 chemicals in 21 studies, permeability of rat skin resembled that of human skin ($0.625 < FOD < 1.6$). For 83 chemicals in 54 studies, rat skin is more permeable than human skin. Only four chemicals are less permeable through rat than through human skin. In the group of chemicals ($n = 83$) that were more permeable in the rat than human skin, twenty-eight chemicals show FOD within the range of 3–10, twenty-four chemicals show FOD within the range of 11–99, and five chemicals show FOD within the range of 100–500. In conclusion, 48% (53 chemicals of 110) fell within the range of ±1/2 log interval and rat skin is generally more permeable than human skin.

van Razenzwaay and Leibold [16, 17] compared *in vivo* rate of penetration of 14 pesticides with a wide range of lipophilicities and molecular weights with *in vitro* rate of penetration in rat as well as *in vitro* rate of penetration in humans. In *in vitro* studies, rat skin was always more permeable for all tested substances than human skin (FOD ranged from 2.3 to 36.5, mean: 13.4 ± 11.1-fold). *In vivo* rat skin is always less permeable than *in vitro* rat skin, but, in most cases (9/12), it was more permeable than *in vitro* human skin. No constant factor of difference was identified. Factor of difference would not appear to be determined by molecular weight, lipophilicity, or aqueous solubility. Because of inconsistent difference in permeability between rat and human skin, it is not possible to derive a general adjustment factor for estimation of human skin permeability. Thus, the systemic exposure of humans may be significantly overestimated if risk assessment is based only on the results of an *in vitro* or an *in vivo* rat study.

To overcome this problem, several research groups (US EPA 1992; Thongsinthusak et al. 1993; van Ravenzwaay and Leibold 2004; WHO 2005) [17–20] suggested a method, the so-called parallelogram, to estimate dermal penetration through human skin from the combined use of *in vivo* and *in vitro* rat data and *in vitro* human data, using the following equation: add reference

$$\%\ \text{human dermal penetration} = \frac{[\%\ \text{dermal penetration in rat in vivo}] \times [\text{rate of dermal penetration in human in vitro}]}{[\text{rate of dermal penetration in rat in vitro}]}$$

Ross et al. [21] examined the predictive worth of this method as outlined in Table 2.3 for five other compounds with widely varying log K_{ow} (log P varies from −0.1 for caffeine to 6.1 for permethrin). Agreement between estimated and measured values is remarkable. More importantly, the predicted dermal absorption estimate ≤1.7-fold of the actual human *in vivo* measured value for each compound except fluazifop-butyl and *o*-phenylphenol.

The parallelogram method to estimate human dermal absorption can also be utilized with other test animal data besides rat. Shown in Table 2.4 are the values predicted using pig data, which also show a good agreement between estimated and measured values [21]. While the ratio of animal to human absorption varies with the compound, this approach is only valid if the ratio of *in vivo* to *in vitro* absorption for a given compound remains the same in both human and animal species. It is also desirable if three study types (*in vitro* human, *in vitro* rat, *in vivo* rat) were conducted concurrently under the same condition by the same laboratory [21].

Table 2.3 Comparison of measured human absorptions and new predictions of human dermal absorption using the parallelogram method. (Modified from Table 4 in [21])

Compound	$\frac{\text{Rat}_{in\ vivo}}{\text{Rat}_{in\ vivo}}$	$\text{Human}_{in\ vivo}$ (%)	$\text{Human}_{in\ vivo}M$ (predicted %)	$\text{Human}_{in\ vivo}M$ (measured %)	$\frac{\text{Human}_{in\ vivo}p}{\text{Human}_{in\ vivo}M}$
Benzoic acid	1.3	46.5	60.5	60.6	1.0
Caffeine	1.0	40.6	40.6	40.6	1.0
Fluazifop-butyl	0.9	2.2	2.0	8.0	0.25
o-Phenyl phenol	3.5	16.3	56.7	24.2	2.4
Permethrin	1.3	1.3	1.7	1.2	1.4
PBO	1.2	7.4	8.9	5.3	1.7
Propoxur	0.6	25.9	14.5	14.5	1.0

Table 2.4 Estimated human dermal absorption using parallelogram method with pig data. (Modified from Table 9 in [21])

Compound	$\frac{\text{Pig}_{in\ vivo}}{\text{Pig}_{in\ vivo}}$	$\text{Human}_{in\ vivo}$ (%)	$\text{Human}_{in\ vivo}P$ (predicted %)	$\text{Human}_{in\ vivo}M$ (measured %)	$\frac{\text{Human}_{in\ vivo}p}{\text{Human}_{in\ vivo}M}$
Benzoic acid	1.9	46.5	88.4	60.6	1.5
Caffeine	1.2	40.6	40.6	48.7	1.2
Lindane	1.3	7.5	9.8	9.0	1.1
Malathion	0.4	17.0	6.8	8.0	0.9
Testosterone	0.5	39.4	19.7	49.5	0.4

2.5 Rabbits

Similar to rat, rabbit skin is generally more permeable than human skin and the difference in percutaneous absorption between rabbit skin and human skin is not consistent. In 2008, Nicoli et al. [22] performed an experiment to compare rabbit ear skin with pig ear skin on histology, lipid composition, and permeability of skin (Tables 2.5 and 2.6). Rabbit ear skin is characterized by the density of hair follicles (80/cm^2) much lower than that of the skin of the rabbit back and of other rodents (rat 8000/cm^2). Rabbit ear skin also showed comparable permeability in some molecules (lidocaine, triptorelin, thiocolchicoside). One study demonstrated that rabbit ear skin is a reasonable model for studying the iontophoretic transport of drugs *in vitro* since the relative electro-osmotic and electrorepulsive contributions were almost similar for human skin and rabbit skin [23].

As seen in Tables 2.5 and 2.6, rabbit ear skin has SC thickness similar to pig ear and human skin. However, the lipid composition of rabbit SC was substantially different from that of the pig, which showed a higher content of nonpolar lipids. And viable epidermis of rabbit ear was much thinner than that of pig ear skin. Hair

Table 2.5 Rabbit ear skin as a skin model for *in vitro* transdermal permeation experiments. (Summarized from [22])

	Rabbit ear skin	Pig ear skin (control)
SC thickness	11.7 μm	9.1 μm
Lipid amount in SC	6%	5%
Lipid composition in SC	More lipophilic	Less lipophilic
Ceramide *(polar)*	35%	43%
Cholesterol *(polar)*	11%	32%
Cholesterol esters *(nonpolar)*	32%	1%
Triglycerides *(nonpolar)*	5%	1%
Epidermis thickness	17 μm	62 μm
Hair density	80/cm^2	11–30/cm^2
Permeation		
Hydrophilic (caffeine, nicotinamide)	4–7 times less permeable than pig skin	
Lipophilic (progesterone)	Comparable with isolated pig epidermis	

Table 2.6 Mean thickness of different layers of rabbit, pig, human, and mouse skins. (Modified from Table 2 in [22])

Species	SC (μm)	Epidermis (μm)	Whole skin (mm)
Human	12.5	53.5	–
Pig, outer ear	9.1	61.7	1.1771
Rabbit, inner ear	11.7	17.0	0.276
Mouse	6.7	9.6	–

follicle density is also still higher than pigs and humans (human back and abdominal skin are 29–93/cm^2 and 6/cm^2, respectively) though it is much lower than other hairy rodents. In permeation studies, hydrophilic chemicals (caffeine, nicotinamide) were 4–7 times less permeable through rabbit ear than through pig skin, probably because of the higher lipophilicity of its SC while lipophilic chemical, progesterone showed permeability similar to pig ear skin [22].

We reviewed the original papers published after 1993 that described permeability of rabbit skin and human skin, including 16 studies, which measured 19 chemicals. Only 2 chemicals showed similar permeability in both and 16 chemicals higher permeability through rabbit skin than through human skin. Among 14 chemicals, di-n-butylphthalate is 24 times and terbutaline is 14 times more permeable through rabbit skin that through human skin. In conclusion, rabbit skin is generally more permeable than human skin and 10 chemicals of 19 (53%) fell within the range of ±1/2 log interval.

2.6 Guinea Pigs

Guinea pig skin is also generally more permeable than human skin like other rodents. Barbero and Frasch [13] performed an extensive quantitative review on guinea pig skin, including HGP skin as well as porcine skin as surrogates for human *in vitro* penetration studies. These included data from 14 *in vitro* studies consisting 15 measurements of 13 chemicals on permeability through both human and guinea pig skin. Their review showed an excellent correlation exists between guinea pig skin and human skin; the linear correlation of the log transformed data gave an r^2 of 0.90 with a slope very close to 1.0 (0.96 ± 0.10), and an intercept not distinguishable from 1 (0.11 ± 0.3). But, for those where FOD only is measured (17 studies, 25 measurements, 21 chemicals), 65% fell within the range $0.3 < \text{FOD} < 3.0$. These FOD studies generally exhibit less agreement between guinea pig and human permeation.

Average intraspecies coefficient of variation for guinea pig skin is 19%, which is less than for human skin (24%). Twelve lag-time measurements of 12 chemicals taken from 11 studies comparing human and guinea pig skins have a Pearson correlation coefficient of 0.90 ($p < 0.0001$). Linear correlation slope was 1.07 with an intercept of -0.22 h, and r^2 of 0.82. Thus, time-lag correlations between guinea pig and human skins were significant. From these results they concluded that, in general, the guinea pig is a good model for human skin *in vitro* permeability measurements. For chemicals with substantial disagreement they suggest that higher hair density in guinea pigs may contribute to the high permeability of guinea pig skin for those chemicals, particularly hydrophilic ones (e.g., paraquat dichloride, sodium chloride).

We reviewed the original papers published after 1993 that described permeability of both guinea pig and human skins. These included 10 studies, which measured absorption of 10 chemicals. Six chemicals showed higher permeability through guinea pig skin than through human skin. Three chemicals were less permeable through guinea pig skin than human skin. In conclusion, five chemicals of ten fell within the range of $\pm 1/2$ log interval. This result differs from Barbero and Frasch's result. This may be due to the small number of studies reviewed and that they also included HGP that showed much more comparable results to human skin as well as the haired guinea pig skin in their review.

2.7 Hairless Rats/Hairless Mice/Hairless Guinea Pigs

Hairy rodents have the disadvantage of an extremely high density of hair follicles and require hair removal before permeation experiment. As both issues can affect percutaneous absorption of chemicals, hairless rodents have been gaining more ground in permeation studies.

2.7.1 Hairless Rats

Earlier there were *in vivo* studies in which chemicals showed permeability through hairless rat skin similar to human skin. Therefore, Shah et al. [24] stated in 1991 that, together with pigs and rhesus monkeys, hairless rats are the only animals in which permeation data are consistently, qualitatively, and quantitatively similar to human permeation data.

We reviewed original papers published after 1993 that described permeability of hairless rat skin and human skin. These included 13 studies, which measured absorption of 21 chemicals. For four chemicals from three studies, absorption was similar in hairless rat and human skin. For 14 chemicals from seven studies absorption through hairless rat skin is higher than human skin. Most (12 of 14) were more than three times permeable than human skin and seven chemicals showed more than ten times permeability than human skin. Three chemicals from three studies are less permeable through hairless rat skin than through human skin. In conclusion, 33 % (7 chemicals of 21) fell within the range of ± 1/2 log interval. Thus, hairless rat skin seems to be generally more permeable than human skin.

2.7.2 Hairless Mice

Chantasart et al. [25] described the advantage of hairless mouse skin. Hairless mouse skin SC has relatively constant lipid content whereas human skin lipid content varies considerably, thus making the interpretation of the partition experiment data difficult. Hairless mouse SC lipid composition resembles that of human skin. The large body of hairless mouse skin data available allows direct comparisons of the present results with those in previous studies. Hairless mouse skin has been found to be an adequate, quantitative model for human skin in the investigation of chemical permeation enhancers when defined protocols are employed.

Simon and Maibach [26] reviewed the relevance of the hairless mouse as an experimental model for human skin penetration. Regarding histology, SC of the hairless mouse is less than half as thick as that of the human tissue and accordingly with lower barrier properties. It is more susceptible to chemical perturbations than human skin. Their conclusion was that statistically significant correlations were not obtained between the hairless mouse skin and human skin and the *in vivo* hairless mouse data is not usefully predictive for human skin *in vitro* permeability. For *in vitro* studies, hairless mouse skin needs to be hydrated thoroughly to be a model for human skin penetration. Some compounds penetrated in an almost similar manner, but many differed in at least one logarithmic order, human skin being the less permeable. Relative effect of each enhancer formulation on the two skins was not consistent and therefore the hairless mouse model should not be used to predict the effects of penetration enhances in human skin.

We reviewed the original papers published after 1993 that described permeability of hairless rat skin and human skin. These included 16 studies, which measured ab-

sorption of 17 chemicals. Five chemicals penetrated through the hairless mouse skin at a rate similar to human skin. Twelve chemicals penetrated through the hairless mouse more than through human skin, seven of them showing more than a threefold difference between hairless mouse skin and human skin. These results support that the hairless mouse is not a good model to predict human skin absorption.

2.7.3 Hairless Guinea Pigs (HGPs)

The skin of HGP has some structural similarities with human skin that the skin of the haired guinea pig does not have [27]. The HGP epidermis is as thick as human skin and has distinct layers (5–10 layers) similar to human epidermis and SC thickness and the number of blood vessels in the dermis is similar as well.

Skin permeability values in HGP were similar to those of humans.Frasch and Barbero [28] performed an experiment to compare HGP skin permeability and lag-time measurements for six chemicals with a wide range of lipophilicity (log K_{ow} 0.90–3.40) with those of human skin. They found an excellent correlation between HGP and human skin in terms of permeability (*Kp*) and lag-time. The data of permeability (*Kp*) for six chemicals through HGP skin are mostly slightly more permeable, but close to those of humans. Thus, they concluded that HGP is a good substitute for human skin.

We reviewed the original papers published after 1993 that described permeability of both HGP and human skin. These included 20 studies, which measured absorption of 28 chemicals. Eighteen chemicals from 11 studies showed a close absorption rate through HGP to human skin. Only one chemical was less permeable through HGP than human skin and 11 chemicals from eight studies showed higher permeability through HGP skin than human skin. Overall, 89 % (25 of 28) chemicals are within the range of $0.3 < \text{FOD} < 3$. These results support that HGP skin is a good model for human skin absorption.

2.8 *In Vitro* Species Comparison and *In Vitro*/*In Vivo* Correlation

Compared to *in vivo* animal study, *in vitro* animal models are more easily available, easy to perform, and can provide results in a shorter period. They provide important tools for screening a series of drug formulations, evaluation of skin permeation enhancing properties and mechanism of action of the carrier systems, and estimation of rank of skin transport for a series of drug molecules [14].

There are numerous *in vitro* and *in vivo* animal studies, but fewer *in vitro–in vivo* comparative studies. This makes it difficult to interpret *in vitro* animal data. van de Sandt et al. [29, 30] compared *in vitro* absorption of the pesticide propoxur (log *P* 1.56) and the fungicide *o*-phenylphenol (log *P* 3.28) with *in vivo* absorption in

Table 2.7 Comparison of *in vivo* and *in vitro* potential absorbed dose (PAD, μg/cm^2) of propoxur and *o*-phenylphenol in rat and human

Compounds	Rat *in vitro*	Rat *in vivo*	Human *in vitro*	Human *in vivo*
Propoxur	29	48	17	23
o-Phenylphenol	108	67	110	105

Table 2.8 Comparison of *in vivo* and *in vitro* dermal absorption values measured in pigs. (Modified from Table 5 in [21])

Compound	Pig *In vitro*	Pig *In vivo*	Ratio, *in vivo*/*in vitro*
Benzoic acid	15	28	1.9
Caffeine	20	23	1.2
DEET	6	9	1.5
Fluocinolone acetonide	4	6	1.5
Lindane	6	8	1.3
Malathion	10	4.4	0.4
Mean			1.2

human and rat skin. For direct comparison, experimental conditions were standardized with respect to dose, vehicle, exposure duration, temperature, and humidity. In *in vitro* studies, samples of receptor fluid were collected at various time intervals after application in static diffusion cells. In *in vivo* studies, blood sampling was performed and urine (human, rat) and feces (rat) were collected at predetermined time intervals after application. They found that *in vitro* absorption in most cases overestimated the *in vivo* situation. Most close agreement between *in vitro* and *in vivo* results could be obtained on the basis of potentially absorbed dose (amount applied minus dislodged) for both rat and human (Table 2.7).

In an *in vivo* and *in vitro* comparative study in rats, van Ravenzwaay and Leibold [16, 17] determined the rates of penetration for 14 chemicals. *In vitro* results were always higher, irrespective of the compound tested and the duration of exposure, as compared to *in vivo* values. *In vitro* methods provided a more accurate prediction of *in vivo* dermal absorption for water-soluble molecules than lipophilic molecules with a log *P* greater than 3. However, considerable difference between *in vitro* and *in vivo* values for highly lipophilic compounds was reported for lindane (log *P* 3.5) showing the 40-fold overprediction. Ross et al. [21] reviewed comparison of *in vivo* and *in vitro* dermal absorption values measured in pigs (Table 2.8), humans (Table 2.9), and rats (Table 2.10). This showed that *in vitro* studies agree with *in vivo* studies for most compounds tested. This suggests that properly conducted *in vitro* study is generally accurate in predicting *in vivo* skin absorption and one may avoid *in vivo* studies.

However, the majority of studies reported that *in vitro* percutaneous absorption overestimated *in vivo* percutaneous absorption. *In vitro* studies showed considerable

Table 2.9 Comparison of *in vivo* and *in vitro* dermal absorption values measured in humans. (Modified from Table 6 in [21])

Compound	*In vitro* human	*In vivo* human	Ratio, *in vivo*/*in vitro*
Benzoic acid	46.5	60.6	1.3
Caffeine	40.6	40.6	1.0
Fluazifopbutyl	2.2	8.0	3.6
Lindane	7.5	9	1.2
Malathion	17	8	0.47
Ortho phenyl phenol	16.3	24.2	1.5
Permethrin	1.3	1.2	0.95
PBO	7.4	5.3	0.72
Propoxur	25.9	14.5	0.56
Testosterone	39.4	49.5	1.3
Mean			1.0

Table 2.10 Comparison of *in vivo* and *in vitro* dermal absorption values measured in rats. (Modified from Table 8 in [21])

Compound	*In vitro* rat	*In vivo* rat	Ratio, *in vivo*/*in vitro*
Acetyl salicylic acid	29.0	24.8	0.86
Benzoic acid	49.1	37.0	0.75
Caffeine	48	57	1.2
DEET	34	38	1.1
Fluazifopbutyl	80	74.3	0.93
Ortho phenyl phenol	10.3	35.8	3.5
Permethrin	20.7	35	1.7
PBO	35	42	1.2
Propoxur	31	20.8	0.67
Urea	7.2	8.1	1.1
Mean			1.1

variation depending on the experimental details or conditions. Predictive value *in vitro* assays showed to be influenced by factors such as skin type and thickness and choice of receptor fluid (RF). Agreement between *in vitro* and *in vivo* is better for hydrophilic than for lipophilic compounds. *In vivo*, the capillary bed acts as a sink, removing chemicals as they diffuse into the epidermis and dermis. *In vitro* methods rely on receptor fluid as sink, but the thermodynamics of partitioning the skin into the RF is highly dependent on the chemicals' lipophilicity, the physiochemical attributes of the RF, and the chemicals solubility into the RF [8]. Most studies showed that the use of full-thickness skin resulted in a lower absorption of lipophilic chemicals into the receptor fluid when compared with results obtained with split thickness skin, indicating a reservoir effect of these compounds. There is

also a point of debate in the way the amount of a chemical is retained in the skin at the end of exposure [2].

In conclusion, more comparative studies are needed to determine the factors that influence the predictive value of the *in vitro* and animal models.

2.9 Alternative *In Vitro* Test Methods

New regulatory guidelines increasingly demand the reduction of tests using laboratory animals in research as well as in drug, chemical, and cosmetic screening. Thus, alternative *in vitro* test methods are gaining in importance to avoid excessive animal use.

2.9.1 Isolated Perfused Porcine Skin Flap (IPPSF)

Among the 45 studies done on pigs in our review only four are *in vivo*. To overcome some of the limitations of *in vitro* study while still taking advantages of *in vitro* study, Riviere et al. [31] developed isolated perfused porcine skin flap (IPPSE) model. It provided an anatomically intact, viable, isolated, perfused tube-like preparation in which epidermis and dermis are viable with functional microcirculation, which can be used for collecting blood containing chemicals absorbed through skin. Wester et al. [32] compared percutaneous absorption of five chemicals through human skin and IPPSF model. Percutaneous absorption values of IPPSF model were comparable to those of human skin (correlation coefficient = 0.78; $p < 0.04$) (Fig. 2.1). However, they concluded that though their results and other studies are promising, this model needs more studies with a broader group of diverse chemicals.

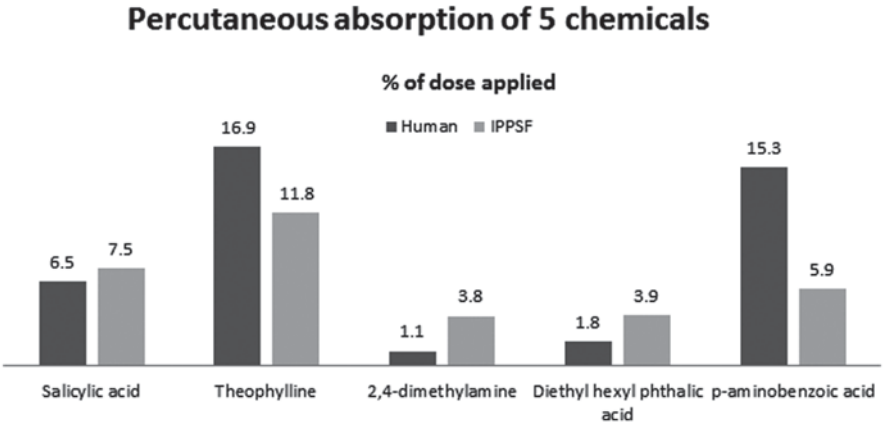


Fig. 2.1 Comparison of percutaneous absorption of five chemicals through human skin and IPPSF model

These *ex vivo* model systems allow cutaneous toxicology and pharmacology studies to be conducted on viable skin that has a normal structure and intact microvasculature. However, surgical procedures and perfusion techniques for these models are complex and time consuming.

2.9.2 Isolated Blood-Perfused Pig Ear

There are two major advantages of the perfused pig ear model over *in vitro* models. In the pig ear model, blood is used as the recipient medium instead of a buffer containing organic solvents, and the model does not ignore the effect of chemicals on the dermal vascular system (dose-dependent increase in perfusion pressure after noradrenaline and reversal of the same by isoxsuprine) [3].

In van de Sandt et al.'s comparative *in vitro–in vivo* percutaneous absorption studies for propoxur and *ortho*-phenylphenol, data generated in the perfused pig ear model were generally intermediate between full-thickness skin and epidermal membrane of human *in vitro* studies and 5–10 times overestimated the human *in vivo* data for both chemicals [29, 30]. The practical perfusion period is limited to about 6 h [33].

2.9.3 Isolated Normothermic Hemoperfused Porcine Forelimb

Wagner et al. [34] developed this model to replace animal testing with maintaining the characteristics of porcine skin as close to physiologic conditions as possible. The perfusion of the isolated porcine forelimb in several respects meets the requirements of an *in vitro* assay model. However, it was not predictive of *in vivo* absorption in humans. Maximal nitroglycerin concentration as determined in their study was considerably higher than that in humans from the literature studies (Table 2.11) [35, 36].

They explained the difference as follows: the perfusion of the porcine is run in a recirculating mode, which leads to continuous accumulation of nitroglycerin in the perfusion medium and the isolated porcine limb is deprived of the metaboliz-

Table 2.11 Comparison of maximal blood nitroglycerin concentration in isolated normothermic hemoperfused porcine forelimb and humans

	Maximal blood nitroglycerin concentration	
Patch	Wagner SM et al.'s study	Human *in vivo* (published data)
TTS 5[a]	Up to 3.86 ng/ml	0.27 ng/ml[35]
TTS 10**b**	4.54 ng/ml	1.1 ng/ml[36]

[a] Patches containing nitroglycerin 25 mg
[b] Patches containing nitroglycerin 50 mg

ing and eliminating mechanisms of the liver and kidney, which are present *in vivo*. They concluded that comparing nitroglycerin penetration rates in the porcine limb model with *in vivo* in humans is difficult. So, further improvement of the perfusion setup is necessary. Like isolated blood-perfused pig ear model, its vitality of model maintained for 5–6 h.

2.9.4 Mouse Dorsal Skin Fold Chamber Model

Eros et al. [37] used mouse dorsal skin fold chamber model, which permits precise determination of drug quantity penetrating living full-thickness skin with a functioning microcirculation. A skin fold in the dorsal region of a nude mouse was fixed with two fenestrated titanium plates. A circular wound was made on one side of the skin fold. A metal cylinder with phosphate buffer was fixed in the window of the titanium plate. Concentration of penetrated drug was measured in the buffer. It is an *in vitro* study under *in vivo* condition, so repeated measurements can be performed in the same animal to determine the kinetics of penetration, which can reduce the number of animals required for study. However, the continuous presence of an investigator is required for assessment of the animals and for the maintenance of anesthesia. And observation period was only 6 h.

Eros et al. [37] measured ibuprofen permeation through dorsal skin fold chamber model. A flux of 11.57 μg/cm2/h of ibuprofen reported by them was not comparable with the data of human skin in published data; 20–30 μg/cm^2/h or higher in human skin *in vitro* study [38, 39]. So, it may replace *in vivo* mouse study but is not comparable with human studies.

2.9.5 Isolated Bovine Udder

Netzlaff et al. [40] compared bovine udder skin with human and porcine skin in percutaneous permeation experiments. Bovine udder skin exhibited a weaker but less variable barrier against caffeine, benzoic acid, testosterone, and flufenamic acid while pig skin and human skin were found to be equally permeable.

2.10 Human Skin Grafted onto Nude Mouse Model (HuSki Model)

This model allows evaluation of chemicals using a system consisting of a viable human skin and SC with a physiological capillary circulation of the nude mouse. Reifenrath et al. [41] investigated skin absorption in several *in vivo* models, including the HuSki model and demonstrated a significant correlation between the skin

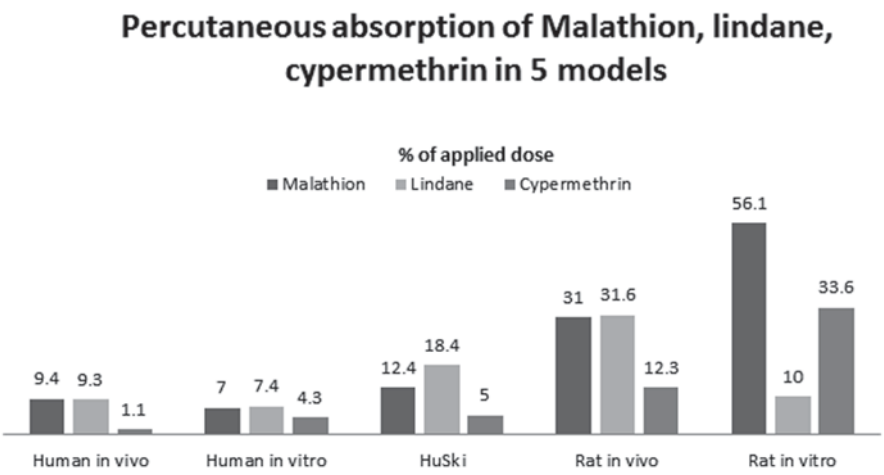


Fig. 2.2 Distribution of malathion, lindane, and cypermethrin in five models at 24 h. (Modified from [15])

penetration values obtained for nine chemicals with the HuSki model and the human volunteer values ($r = 0.74$; $p = 0.05$).

Capt et al. [15] compared skin penetration for three reference insecticides (malathion, lindane, and cypermethrin) using two *in vivo* (rat and HuSki) and two *in vitro* (rat and human) models. Then they compared data obtained from these models to human volunteer data [42–44] for their ability to predict the human skin absorption (Fig. 2.2). Human *in vitro* model was the most predictive of human *in vivo* absorption, but it could not be used for more than 24 h as the skin samples in the diffusion cells may not maintain their vitality beyond 24 h. The HuSki model was similar to the human *in vitro* model in predicting human *in vivo* absorption for the three compounds. Rat *in vivo* model overestimated human *in vivo* skin absorption much more than human *in vitro* and the HuSki model. As HuSki is a vivo model and allows absorption experiment for longer period (at least 11 days), it was also suitable to study the fate of chemicals in the skin and SC over prolonged periods.

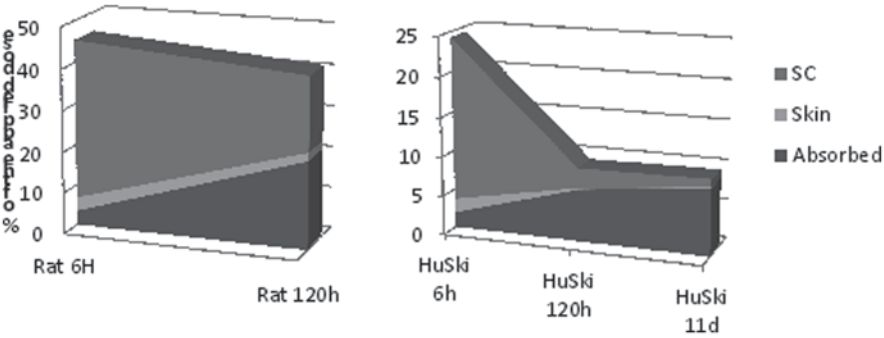


Fig. 2.3 Evolution of the distribution of cypermethrin over time in rat *in vivo* and the HuSki model. (Modified from [15])

Evolution of the distribution of cypermethrin between 6 and 120 h was different in the rat model compared to the HuSki model (Fig. 2.3). In the rat model, about 1/3 of cypermethrin present in the SC at 6 h was further absorbed at 120 h. On the other hand, in the HuSki model, only a small fraction of the cypermethrin present in the SC at 6 h was further absorbed at 120 h and 3/4 of cypermethrin present in the SC at 6 h was eliminated in the wash-off compartment at 120 h.

2.11 Animal Skin: Physical and Chemical Parameters

Many physical and chemical differences exist between the skin of animals and between animal skins and human skin; skin (especially SC) thickness; composition of SC lipids; hair density and thickness of hair follicles; density and distribution of sweat glands and sebaceous glands; epidermal–dermal junction; architecture of vasculature, collagen fibers and fiber bundles in the dermis; and distribution of fat. In addition to these, dosing variables (concentration, surface area, formulation, time, etc.) affect percutaneous absorption in animal models.

Among the many factors mentioned above, Godin et al. [14] suggested that skin thickness, composition of intercellular SC lipids, and hair shaft number are the most important factors causing lack of correlation in percutaneous absorption of chemicals across species or from different application sites in the same animal model. Netzlaff et al. [40] showed that the amount of free fatty acids and triglycerides and the density of hair follicles are important factors causing differences between the skin barriers among species.

2.12 Dose Response

In vivo percutaneous absorption can vary depending upon skin concentration. Therefore, a topical dose response can give additional information about the relevance of an animal model. Rhesus monkeys showed the same dose response with human *in vivo* [45]. Hairless rat also showed the same dose response to human *in vivo*, which showed a linear increase in absorption with increased dose [46]. However, it was different in the absolute amount absorbed between two species. In hairless Rat, 80–90 % of applied dose was absorbed, however, much less was absorbed in rhesus monkeys.

2.13 Regional Variation in Animals

Regional variation in animal models may affect prediction of human skin absorption. In rhesus monkeys, regional variation resembles humans; thus, it is a most relevant animal model for human regional variation [47]. In rat and hairless rat, skin

thickness is much less than human skin, which causes percutaneous absorption to be higher.

2.14 Summary

For critical studies, percutaneous absorption in human remains a key option. However, it may sometimes be difficult or impossible to perform human study. Thus, animal models have been introduced to predict percutaneous absorption in human. However, it is complex to correlate absorption data from animal studies with humans because there are differences in percutaneous absorption between human and animal species, which comes from either the species themselves or methods or technologies used in the study. Thus, the experimental method and conditions that affect absorption should be controlled by investigator. Some are easy to control, i.e., application site, occlusion, dose concentration, surface area, and vehicle. However, others may be difficult to control, i.e., skin metabolism, skin age, and skin condition.

We also need familiarity with limitation of animal models and experimental methods. Absorption in common laboratory animals (i.e., rat or rabbit) is generally higher than humans, but absorption in pigs, monkeys (squirrel, rhesus) is more predictive of humans *in vivo*. In *in vivo–in vitro* comparative studies, absorption data appeared to be comparable in many chemicals. For those chemicals, *in vitro* study may replace *in vivo* study. And to overcome limitations of *in vitro* or animal models, new alternative models such as isolated perfused porcine skin flap or HuSki models were introduced.

The most efficient animal model depends on scientific question, availability, regulatory issues, and cost. Data, as noted in this chapter, remains incomplete. At the moment, monkeys and pigs appear closest to humans. And the hairless guinea pig and the HuSki model seem to be promising; however, they need more studies to validate their usefulness in predicting human absorption. Last, further studies focusing on 15 factors of percutaneous absorption of human would suggest future development on clinically relevant model [48].

References

1. Environmental Protection Agency (EPA). Protection for subjects in human research. Final rule. Fed Regist. 2006;71(24):6137–76.
2. Jakasa I, Kezic S. Evaluation of in-vivo animal and in-vitro models for prediction of dermal absorption in man. Hum Exp Toxicol. 2008;27(4):281–8. doi:10.1177/0960327107085826.
3. Simon GA, Maibach HI. The pig as an experimental animal model of percutaneous permeation in man: qualitative and quantitative observations–an overview. Skin Pharmacol Appl Skin Physiol. 2000;13(5):229–34.
4. Bartek MJ, LaBudde JA, Maibach HI. Skin permeability in vivo: comparison in rat, rabbit, pig and man. J Invest Dermatol. 1972;58(3):114–23.
5. Montagna W. The skin of nonhuman primates. Am Zoologist. 1972;12:109–24.

6. Wester RC, Melendres J, Logan F, Hui X, Maiback HI, Wade M, Huang KC. Percutaneous absorption of 2,4-dichlorophenoxyacetic acid from soil with respect to soil load and skin contact time: in vivo absorption in rhesus monkey and in vitro absorption in human skin. J Toxicol Environ Health. 1996;47(4):335–44.
7. Surber C, Wilhelm KP, Maibach HI. In vivo percutaneous absorption of [14C] acitretin in the hairless guinea pig and in the rhesus monkey. Arzneimittelforschung. 1993;43(9):1001–4.
8. Panchagnula R, Stemmer K, Ritschel WA. Animal models for transdermal drug delivery. Methods Find Exp Clin Pharmacol. 1997;19(5):335–41.
9. Jacobi U, Kaiser M, Toll R, Mangelsdorf S, Audring H, Otberg N, Sterry W, Lademann J. Porcine ear skin: an in vitro model for human skin. Skin Res Technol. 2007;13(1):19–24.
10. Debeer S, Le Luduec JB, Kaiserlian D, Laurent P, Nicolas JF, Dubois B, Kanitakis J. Comparative histology and immunohistochemistry of porcine versus human skin. Eur J Dermatol. 2013;23(4):456–66.
11. Boudry I, Trescos Y, Vallet V, Cruz C, Lallement G. Methods and models for percutaneous absorption studies of organophosphates. Pathol Biol (Paris). 2008;56(5):292–9. doi:10.1016/j.patbio.2007.09.024.
12. Zendzian RP. Dermal absorption of pesticides in the rat. AIHAJ. 2000;61(4):473–83.
13. Barbero AM, Frasch HF. Pig and guinea pig skin as surrogates for human in vitro penetration studies: a quantitative review. Toxicol In Vitro. 2009;23(1):1–13. doi:10.1016/j.tiv.2008.10.008.
14. Godin B, Touitou E. Transdermal skin delivery: predictions for humans from in vivo, ex vivo and animal models. Adv Drug Deliv Rev. 2007;59(11):1152–61.
15. Capt A, Luzy AP, Esdaile D, Blanck O. Comparison of the human skin grafted onto *nude mouse model* with in vivo and in vitro models in the prediction of percutaneous penetration of three lipophilic pesticides. Regul Toxicol Pharmacol. 2007;47(3):274–87.
16. van Ravenzwaay B, Leibold E. The significance of in vitro rat skin absorption studies to human risk assessment. Toxicol In Vitro. 2004;18(2):219–25.
17. van Ravenzwaay B, Leibold E. A comparison between in vitro rat and human and in vivo rat skin absorption studies. Hum Exp Toxicol. 2004;23(9):421–30.
18. US Environmental Protection Agency. Dermal exposure assessment: principles and applications. EPA/600/8–91/011B. Washington, DC: U.S. EPA, Office of Health and Environmental Assessment; 1992.
19. Thongsinthusak T, Ross J, Meinders D. Guidance for the preparation of human pesticide exposure assessment documents. Report No. HS-1612. Workers Health & Safety Branch, Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA; 2003.
20. World Health Organization, International Programme on Chemical Safety. Dermal absorption. Environmental health criteria 235. Geneva: WHO Press; 2005.
21. Ross JH, Reifenrath WG, Driver JH. Estimation of the percutaneous absorption of permethrin in humans using the parallelogram method. J Toxicol Environ Health A. 2011;74(6):351–63. doi:10.1080/15287394.2011.534425.
22. Nicoli S, Padula C, Aversa V, Vietti B, Wertz PW, Millet A, Falson F, Govoni P, Santi P. Characterization of rabbit ear skin as a skin model for in vitro transdermal permeation experiments: histology, lipid composition and permeability. Skin Pharmacol Physiol. 2008;21(4):218–26. doi:10.1159/000135638.
23. Nicoli S, Cappellazzi M, Colombo P, Santi P. Characterization of the permselective properties of rabbit skin during transdermal iontophoresis. J Pharm Sci. 2003;92(7):1482–8.
24. Shah VP, Flynn GL, Guy RH, Maibach HI, Schaefer H, Skelly JP, Wester RC, Yacobi A. Workshop report on in vivo percutaneous penetration/absorption. Washington DC, 1989. Skin Pharmacol. 1991;4(3):220–8.
25. Chantasart D, Li SK, He N, Warner KS, Prakongpan S, Higuchi WI. Mechanistic studies of branched-chain alkanols as skin permeation enhancers. J Pharm Sci. 2004;93(3):762–79.
26. Simon GA, Maibach HI. Relevance of hairless mouse as an experimental model of percutaneous penetration in man. Skin Pharmacol Appl Skin Physiol. 1998;11(2):80–6.
27. Sueki H, Gammal C, Kudoh K, Kligman AM. Hairless guinea pig skin: anatomical basis for studies of cutaneous biology. Eur J Dermatol. 2000;10(5):357–64.

28. Frasch HF, Barbero AM. A paired comparison between human skin and hairless guinea pig skin in vitro permeability and lag time measurements for 6 industrial chemicals. Cutan Ocul Toxicol. 2009;28(3):107–13. doi:10.1080/15569520902950474.
29. van de Sandt JJ, Meuling WJ, Elliott GR, Cnubben NH, Hakkert BC. Comparative in vitro-in vivo percutaneous absorption of the pesticide propoxur. Toxicol Sci. 2000;58(1):15–22.
30. Cnubben NH, Elliott GR, Hakkert BC, Meuling WJ, van de Sandt JJ. Comparative in vitro–in vivo percutaneous penetration of the fungicide ortho-phenylphenol. Regul Toxicol Pharmacol. 2002; 35(2 Pt 1):198–208.
31. Riviere JE, Bowman KF, Monteiro-Riviere NA, Dix LP, Carver MP. The isolated perfused porcine skin flap (IPPSF). I. A novel in vitro model for percutaneous absorption and cutaneous toxicology studies. Fundam Appl Toxicol. 1986;7(3):444–53.
32. Wester RC, Melendres J, Sedik L, Maibach H, Riviere JE. Percutaneous absorption of salicylic acid, theophylline, 2,4-dimethylamine, diethyl hexyl phthalic acid, and p-aminobenzoic acid in the isolated perfused porcine skin flap compared to man in vivo. Toxicol Appl Pharmacol. 1998;151(1):159–65.
33. de Lange J, van Eck P, Bruijnzeel PL, Elliott GR. The rate of percutaneous permeation of xylene, measured using the "perfused pig ear" model, is dependent on the effective protein concentration in the perfusing medium. Toxicol Appl Pharmacol. 1994;127(2):298–305.
34. Wagner SM, Nogueira AC, Paul M, Heydeck D, Klug S, Christ B. The isolated normothermic hemoperfused porcine forelimb as a test system for transdermal absorption studies. J Artif Organs. 2003;6(3):183–91.
35. Heidemann R, Menke G, Letzel H, Rietbrock N. Serum concentration of glyceryl trinitrate (GTN) in transdermal application of GTN dressings of various origins. Dtsch Med Wochenschr. 1985;110(41):1568–72.
36. Muller P, Imhof PR, Burkart F, Chu LC, Gerardin A. Human pharmacological studies of a new transdermal m containing nitroglycerin. Eur J Clin Pharmacol. 1982;22(6):473–80.
37. Eros G, Hartmann P, Berkó S, Csizmazia E, Csányi E, Sztojkov-Ivanov A, Németh I, Szabó-Révész P, Zupkó I, Kemény L. A novel murine model for the in vivo study of transdermal drug penetration. ScientificWorldJournal. 2012;2012:543536. doi:10.1100/2012/543536.
38. Iervolino M, Cappello B, Raghavan SL, Hadgraft J. Penetration enhancement of ibuprofen from supersaturated solutions through human skin. Int J Pharm. 2001;212(1):131–41.
39. Swart H, Breytenbach JC, Hadgraft J, du Plessis J. Synthesis and transdermal penetration of NSAID glycoside esters. Int J Pharm. 2005;301(1–2):71–9.
40. Netzlaff F, Schaefer UF, Lehr CM, Meiers P, Stahl J, Kietzmann M, Niedorf F. Comparison of bovine udder skin with human and porcine skin in percutaneous permeation experiments. Altern Lab Anim. 2006;34(5):499–513.
41. Reifenrath WG, Chellquist EM, Shipwash EA, Jederberg WW, Krueger GG. Percutaneous penetration in the hairless dog, weanling pig and grafted athymic nude mouse: evaluation of models for predicting skin penetration in man. Br. J. Dermatol. 1984;111(Suppl. 27):123–35.
42. Maibach HI, Feldman RJ, Milby TH, Serat WF. Regional variation in percutaneous penetration in man. Pesticides. Arch Environ Health. 1971;23(3):208–11.
43. Feldmann RJ, Maibach HI. Percutaneous penetration of some pesticides and herbicides in man. Toxicol Appl Pharmacol. 1974;28(1):126–32.
44. Woollen BH, Marsh JR, Laird WJ, Lesser JE. The metabolism of cypermethrin in man: differences in urinary metabolite profiles following oral and dermal administration. Xenobiotica. 1992;22(8):983–91.
45. Wester RC, Maibach HI. Relationship of topical dose and percutaneous absorption in rhesus monkey and man. J Invest Dermatol. 1976;67(4):518–20.
46. Dupuis D, Rougier A, Roguet R, Lotte C, Kalopissis G. In vivo relationship between horny layer reservoir effect and percutaneous absorption in human and rat. J Invest Dermatol. 1984;82(4):353–6.
47. Wester RC, Noonan PK, Maibach HI. Variations in percutaneous absorption of testosterone in the rhesus monkey due to anatomic site of application and frequency of application. Arch Dermatol Res. 1980;267(3):229–35.
48. Ngo M, Maibach HI. 15 Principles of percutaneous penetration. In: Parameters for Pesticide QSAR and PBPK/PD Models for Human Risk Assessment, ACS Symposium Series, Vol. 1099, Washington DC: American Chemical Society; 2012. pp. 67–86. doi:10.1021/bk-2012-1099.

Chapter 3
Mitigating Dermal Exposure to Agrochemicals

Robert P. Chilcott

3.1 Introduction

Dermal absorption may represent the predominant route of entry for agrochemicals such as pesticides and herbicides [1–3]. Whilst the adoption of good working practices can limit exposure within the occupational setting [4], dermal contamination is difficult to eliminate in practice and so effective methods for skin cleansing are integral to maintaining the health of those involved in the production and use of agrochemicals. However, hygiene associated with the use of protective clothing such as overalls and gloves is also an important aspect for mitigating dermal exposure.

3.2 Agrochemicals

The term 'agrochemical' can encompass a wide variety of substances which, at a most empirical level, can be broadly categorised into pesticides (e.g. insecticides, herbicides and fungicides) and chemicals which promote plant growth (e.g. fertilisers and hormones). This encompasses a diverse range of chemicals and corresponding mechanisms of toxicity (Table 3.1). It is important to note that commercial formulations may also contain excipients such as solvents, emulsifiers and preservatives, in addition to the active ingredients—an important factor which may complicate exposure assessments when extrapolating measurements of percutaneous absorption of active compounds from different vehicles [5].

An additional hazard within certain agricultural occupations is contact with phytogenic substances, classic examples being allergic contact dermatitis from poison ivy [6] and green tobacco sickness resulting from dermal absorption of nicotine [7]. Interestingly, the dermal absorption (and thus toxicity) of nicotine has traditionally

R. P. Chilcott (✉)
Department of Pharmacy, University of Hertfordshire, Hatfield AL10 9AB, Hertfordshire, UK
e-mail: r.chilcott@herts.ac.uk

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_3

Table 3.1 Examples of agrochemicals used for pesticide [13] or herbicide applications [14]

Application	Chemical derivative	Examples	Toxic mechanism
Pesticide	Carbamate	Aldicarb, carbofuran, carbaryl, ethienocarb, fenobucarb	Inhibition of cholinesterase
	Neonicotinoid	Acetamiprid, clothianidin, imidacloprid, thiacloprid, thiamethoxam	Nicotine receptor agonist
	Organophosphate	Azamethiphos, chlorpyrifos, diazinon, dichlorvos, fenitrothion, malathion, methyl parathion, parathion, phosmet	Inhibition of cholinesterase
	Pyrethroid	Bifenthrin, cyfluthrin, deltamethrin, imiprothrin, permethrin, resmethrin, tetramethrin, tralomethrin	Voltage-dependent sodium channel blockade
Herbicide	Imidazolinone	Imazamox, imazapic, imazaquin, imazethapyr	Inhibition of acetolactate synthase or acetohydroxyacid synthase
	Sulphonylurea	Chloriumuron, chlorsulfuron, foramsulfuron, halosulfuron, iodosulfuron, mesosulfuron, metsulfuron, nicosulfuron, primisulfuron, prosulfuron, rimsulfuron, sulfosulfuron, thifensulfuron, triasulfuron, tribenuron	
	Triazolopyrimidine	Cloransulam-methyl, diclosulam, flumetsulam, pyroxsulam	
	Sulfonylamino-carbonyltriazolinones	Flucarbazone, propoxycarbazone	
	Arloxyphenoxypropionates	Diclofop, fenoxaprop, fluazifop, Quizalofop	Inhibition of acetyl coenzyme A carboxylase
	Cyclohexanediones	Clethodim, sethoxydim	
	Phenylpyrazolines	Pinoxaden	
	Glycine	Glyphosate	Inhibition of aromatic amino acid synthesis
	Bipyridilium	Diquat, paraquat	Inhibition of photosystem I
	Triazine	Atrazine, prometryn, simazine	Inhibition of photosystem II
	Triazinone	Hexazinone, metribuzin	
	Other (uracil, nitrile, urea and benzothiadiazinone)	Bentazon, bromoxynil, diuron, linuron, terbacil	
	Phenoxy-carboxylic acid	2,4-D, 2,4-DB	Synthetic auxins
	Benzoic acid	Dicamba	
	Carboxylic acids	Clopyralid, fluroxypyr, picloram, quinclorac	

been reduced by tobacco harvesters through the practice of crushing tomatoes or citrus fruits in the hand to acidify the skin surface [8]. This relatively simple method of decontamination is due to the pH-dependent change from nicotine freebase to a charged salt, the skin being relatively impermeable to the latter [9].

Of particular relevance to human health are the organophosphorus (OP) pesticides. Indeed, early research into the development of these compounds resulted in the synthesis of compounds with extremely high mammalian toxicity. These were recognised as having military potential and resulted in the development of chemical warfare agents such as tabun (ethyl dimethylphosphoramidocyanidate) and VX (S-[2-(diisopropylamino)ethyl] O-ethyl methylphosphonothioate) [10]. The potential for dermal absorption arising from occupational exposure to OP pesticides is particularly great due to their desirable properties, namely, relatively low molecular weight, generally low volatility and moderate lipophilicity, all of which contribute to enhancing absorption by the target species and prolonging persistency in the environment. Unfortunately, these very characteristics are those which tend to facilitate percutaneous absorption [11]. The global incidence of poisoning caused by dermal exposure to pesticides is difficult to establish, but has reportedly accounted for around 40 % of pesticide-related incidents in Finland [12].

3.3 Dermal Absorption of Agrochemicals

The dermal absorption of chemicals can be affected by a diverse array of factors which commonly affect percutaneous absorption [15]. Those which seem most pertinent to agrochemicals include environmental factors, skin condition and adoption of safe working practice.

3.3.1 Environmental Factors

It has been recognised for some time that the presence of water on the surface of the skin can affect the percutaneous absorption of chemicals, a classic example being the enhanced dermal toxicity of sulphur mustard [16]. Thus, the percutaneous toxicity of agrochemicals which are susceptible to the effects of water may be augmented under wet conditions such as rain or heavy dew [17]. The effect of water on skin permeability also has potential implications for decontamination (see the later section on factors influencing decontamination).

3.3.2 Skin Condition

Where available, risk assessments of dermal exposure to agrochemicals may be based on evidence derived from laboratory or human volunteer studies. However,

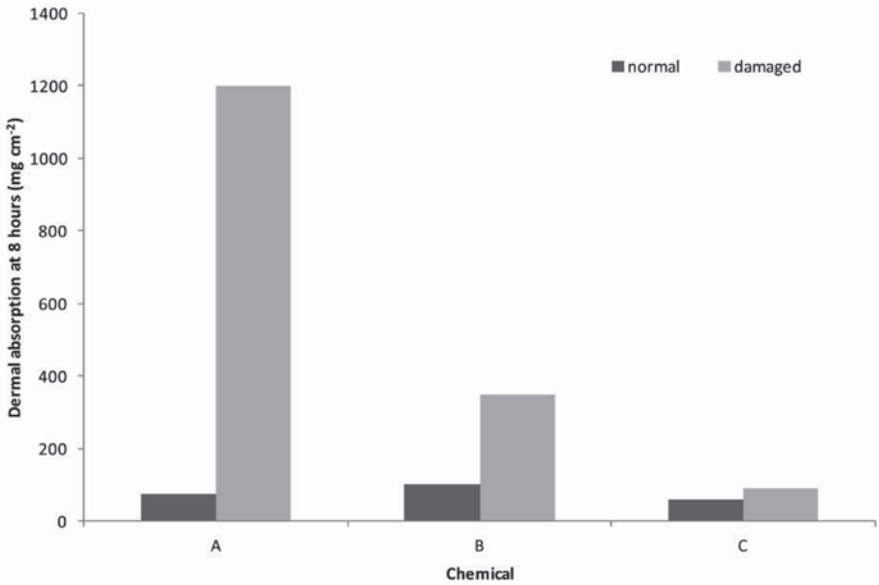


Fig. 3.1 Variation in the amount of chemical (*A, B* and *C*) penetrating normal and damaged pig skin over an 8 h period (equivalent to a normal working shift). Chemicals *A* and *B* were organophosphorous (OP) compounds. Chemical *C* is an organochlorine compound. Skin was damaged by mechanical removal of the *upper* 100 μm of epidermis [23–25]

a factor which is frequently overlooked is absorption through damaged skin [18, 19]. Mild damage to the skin caused by exposure to detergents, punctures or partial removal of the stratum corneum (SC) by tape stripping can cause a two- to tenfold increase in skin permeability [20–22]. More substantial damage, such as total loss of the SC, may enhance percutaneous absorption by more than tenfold depending on the lipophilicity of the penetrant (Fig. 3.1).

3.3.3 Adherence to Safe Working Practices

Controlling dermal exposure in the workplace is, in principle, relatively straightforward: prevent or avoid contact and cleanse contaminated skin promptly if accidental exposure occurs. However, this oversimplifies what is effectively a very complex issue involving a multitude of behavioural, psychological and environmental factors such as training, education, availability and use of personal protective equipment (PPE), perceptions on exposure to toxic materials and working conditions [26]. Non-adherence to good practices can often arise through lack of education. For example, farm workers often consider dry (powder) pesticides to be nontoxic (via percutaneous absorption) compared to liquid formulations [27]. Such misconceptions, as well as financial constraints, social factors and lack of training

are major causes of noncompliance with good practices and may result in clinically relevant exposure to pesticides and herbicides [28, 29].

3.3.4 Exposure Scenarios and Environmental Decontamination

An obvious cause of dermal exposure is direct contact during the preparation and use of agrochemical formulations [30]. However, clothing can also represent a substantial source of contamination and can extend the duration of exposure (and associated adverse health effects) past the time of initial contact [31]. Other direct contact hazards include contaminated soil and water [32, 33] and recreational (outdoor) furniture [34].

A variety of methods have been assessed for remediating environmental contamination caused by OP pesticides (Table 3.2). In principle, some methods of chemical neutralisation have potential for incorporation into skin cleansing (or decontamination) products. However, there are presently few commercially available products for topical application that actively sequester or neutralise agrochemical contaminants.

3.4 Skin Decontamination

3.4.1 Definition and Practice

Decontamination can be defined as 'the process of removing hazardous material(s), both on or available to the external surfaces of the body, in order to reduce local or systemic exposure to a contaminant and thus minimise the risk of subsequent adverse health effects' [75]. Inherent to this definition is the process of removing clothing (disrobing), which can be an order of magnitude more effective than skin decontamination for preventing dermal exposure.

In practical terms, the decontamination of agrochemicals can be considered a facet of personal hygiene, attained through hand-washing during or after potential exposure combined with a change of clothing at the end of a work shift [76]. Water, either alone or in combination with soap, is the most commonly advocated means of skin decontamination for chemicals such as pesticides and herbicides [77, 78].

3.4.2 Factors Affecting Decontamination

There are a variety of factors which influence the effectiveness of decontamination such as the physicochemical properties of the contaminant, total dose, timeliness of decontamination, duration of decontamination and anatomical site [79]. Other factors include hydrodynamics (pressure, temperature and flow rate of water), selection

Table 3.2 Summary of methods investigated for neutralising environmental organophosphorus (OP) residues

Category	Method	Examples	References
Chemical	Catalysis	Cu(II) complexes	[35]
		Copper bipyridyl	[36]
		Polyoxometallates (e.g. Fe, Co, Ca, Cu)	[37, 38]
	Inactivation	Hydrolysis (e.g. sodium perborate)	[39]
		Nucleophilic substitution	[40]
		Oxidation (peroxyacids, nitric oxide)	[41–44]
Biological	Catalysis	Phosphotriesterases	[45, 46]
		Organophosphorus hydrolase (OPH)	[47]
		Cholineterases (ChE)	[48]
		Carboxyl esterase (CaE B1)	[49]
		Methyl parathion hydrolase (MPH)	[50]
		Mixture of recombinant enzymes	[51]
	Bioremediation	*Specific bacteria (e.g. Pseudomonas dimunuta)*	[52]
		Plant sequestration (e.g. marsh plant)	[53]
		Bio-filters (e.g. 'urban waste compost')	[54]
Physical	Passive absorbance	Plant derivates (e.g. husks, pods, etc.)	[55]
		Clays	[56]
		Cyclodextrins	[57–59]
	Ionising radiation	*Gamma radiolysis*	[60, 61]
Physico-chemical	*Photochemical*	*Solar (in combination with photocatalytic agents)*	[62]
		Photolysis (e.g. H_2O_2)	[63]
		Photo-Fenton reaction	[64]
		Photo-catalytic agents (TiO_2, ZnO, CeO_2, ZrO_2, SnO_2, CdS, ZnS, polyoxometallates)	[62, 64–70]
	Electrochemical	*Electro-Fenton reactions ($OH^{\bullet}$)*	[64]
	Vehicle-mediated	*Supercritical fluid extraction (e.g. CO_2)*	[71, 72]
		Micelles	[41, 73]
		Micro-emulsions	[37, 42, 73]
		Nanofibres	[59, 74]

The text in italic indicate that technology is not suitable for personal decontamination

Table 3.3 Fundamental aspects of skin decontamination as reviewed by Wester and Maibach [22]

Parameter investigated	Effect on dermal absorption	Primary reference
Occlusion	Up to tenfold enhancement	[89]
Anatomical site	Up to tenfold difference	
Skin condition	Up to tenfold enhancement	
Delay before decontamination	Inverse relationship between decontamination efficiency and time after exposure	
Skin partitioning	Can be immediate and thus resistant to decontamination	

of appropriate excipients such as detergents, use of physical washing aids, spread of contaminant across the skin surface, toxicokinetics and the presence of clothing on the skin surface [80]. Indeed, whilst the principle of skin decontamination appears to be a simple process, it belies a complex interaction of biological, physical and chemical processes [81, 82].

In terms of skin decontamination, the most extensively studied group of agrochemicals to date are pesticides. A review [22] of seminal work in this area highlighted a number of challenges associated with the skin absorption and decontamination of such chemicals (Table 3.3). Moreover, subsequent studies have questioned the long-standing belief that water is a safe and effective medium for skin decontamination due to the 'rinse-in' or 'wash-in' effect [83–87]. In a comprehensive review, Moody and Maibach (2006) tentatively suggested five mechanisms for the wash-in effect, viz., the effects of surfactants (detergents), skin hydration, change in pH, friction or experimental artefact. Recent work with the hydrophobic compound sulphur mustard has identified a sixth mechanism: the presence of a film of water on the skin surface (distinct from the effects of skin hydration) which may alter the thermodynamic activity of lipophilic substances to favour enhanced dermal absorption [88]. The effect of water on sulphur mustard absorption is a good example of the clinical manifestation of the wash-in effect [16].

3.4.3 Experimental Methods to Assess Skin Decontamination

The assessment of skin decontamination procedures and products can be approached through a variety of methodologies which vary in their level of technical sophistication from simple, physical test systems through to full human field trials (Table 3.4). With increasing technical refinement comes increasing confidence in the test results and, correspondingly, increased cost. Thus, the availability of multiple tests provides the basis for a tiered strategy for the rational development of new decontamination products.

The most basic method of assessing a product is to apply a contaminant to a flat, inert surface, transiently apply the test product and then recover and quantify any residual contaminant. This empirical approach can allow a large number of decon-

Table 3.4 Summary of test methods used to determine the effectiveness of skin decontamination products

Test model	Description
Inert surface	Contaminant is applied to a flat surface such as a steel plate, which is subsequently treated with decontamination product. Effectiveness is quantified by measuring surface recovery of contaminant. The method can be adapted to incorporate biological end-points such as residual insecticidal activity [90]
Powdered stratum corneum	Ground callous (stratum corneum; SC) is preloaded with contaminant and then subjected to immersion in decontamination product. Mixture is centrifuged to separate out the SC pellet from the decontamination matrix. The amount of contaminant in each compartment is then quantified to provide a measure of decontamination effectiveness [91, 92]. The method may not be appropriate model for assessment of nonliquid decontamination products, but is relatively inexpensive and rapid
3D Cell culture	Three-dimensional cell models can quantify biological end-points of decontamination such as cell viability [93]. Such models currently have deficient skin barrier function and so may underestimate decontamination effectiveness
In vitro—artificial membrane	Generally use standard skin diffusion cell apparatus but substitute excised skin sample with homogenous, artificial membrane such as cellulose acetate, acetophane and nylon [94]. May predict ranking of decontamination products but may underestimate decontamination effectiveness due to higher permeability than mammalian skin. Lacks skin–contaminant–decontaminant interactions but does not require access to skin tissue
In vitro—skin	A standard and well-characterised system for assessing skin decontaminants based on standard diffusion cell apparatus with *ex vivo* skin samples. Takes into account biophysical interactions between skin, contaminants and decontamination product and can detect rinse-in effect. Relatively inexpensive and contributes to reduction and replacement of animals in scientific research. Lacks systemic circulation and normal physiological responses (such as erythema and inflammatory response). Inappropriate use of aqueous receptor chamber fluids may limit apparent absorption of lipophilic compounds. Can be difficult to source human skin (which may also require ethical approval in some countries)
Animal studies	Provide organism-level responses to contaminants which can be used to quantify the biological effectiveness of decontamination products. Incorporate most physical, biological and chemical interactions which can affect performance of decontamination. Relatively expensive, resource intensive and require ethical approval
Human studies	The "gold standard" for assessment of decontamination products and may take the form of controlled clinical studies or field trials under more realistic conditions. Use limited to relatively nontoxic contaminants, although historical data may be available for more toxic substances. Can be expensive, resource intensive and requires ethical approval

tamination products to be tested rapidly and economically [90] and is likely to be of use in eliminating products which demonstrate little or no efficacy. However, the lack of interaction between the decontaminant and skin severely limits this approach.

The use of powdered SC is a simple technique which permits some interaction between the decontaminant test product, the contaminant and the outer (barrier) layer of the skin (SC). In such studies, a sample of powdered SC is exposed to the contaminant prior to mixing with a solution of decontamination test product. The SC is then separated by centrifugation and the amount of contaminant in the decontaminant product and SC can be quantified to provide a measure of decontaminant effectiveness. Despite the empirical nature of the test system, powdered SC may provide a good prediction of the rank order of effectiveness of decontaminants [91, 92]. Overall, the method is relatively quick and inexpensive but may be better suited to liquid decontamination products such as soapy water, acetone, ethanol and polyethylene glycol.

Over the past decade or so, three-dimensional skin models have become established techniques for assessing skin irritancy [95]. The presence of a coherent SC provides a biologically relevant surface for the application of contaminants and test products, with decontamination effectiveness being quantified by cell viability assays of the supporting tissue [93]. However, such 3D models tend to have a deficient barrier layer [96] and so a future approach may be to use a hybrid system comprising mature *ex vivo* SC (to provide a more realistic barrier layer) combined with epidermal cell culture bioassays [97]. The use of a 3D model with impaired barrier function is likely to underestimate decontamination effectiveness, since the applied contaminant may partition into or diffuse through the SC more rapidly and so be less available for decontamination.

The most common method for assessing skin decontamination products is with standard skin diffusion cell systems containing excised human or animal skin [32, 83, 84, 91, 92, 94, 98–106]. Human skin is generally accepted to be the most appropriate model (for human decontamination) but can be difficult to source in appropriate quantities and is subject to ethical approval in many countries. Alternatives include rat- and pig skin, although artificial membranes such as silicone rubber, cellulose and nylon have been used as alternatives [94, 107]. Skin diffusion cells are a popular choice, as they are an established technology and provide data which is generally predictive of *in vivo* decontamination effectiveness, although some disparity between *in vitro* and *in vivo* data has been observed [32, 104]. The isolated nature of the skin sample within a diffusion cell provides an ideal environment for controlled, mechanistic studies and assessment of decontamination effectiveness. However, the corresponding disadvantages are that such *in vitro* models do not incorporate an immune response, systemic circulation or target organ toxicity, which may be important factors when considering the holistic effects of exposure and decontamination.

Animal studies provide a more extensive evaluation of decontamination products than *in vitro* studies and may provide final confirmation of efficacy if the contaminant is too toxic for use in human volunteer studies. Animal species most com-

monly used include the pig [108, 109], rat [110–112], guinea pig [108, 113] and rhesus monkey [32, 104, 114]. Two primary considerations for animal studies are ethical (i.e. is the research justifiable?) and economical (*in vivo* studies are expensive to perform). Moreover, animal studies cannot reproduce the effects of human behaviour such as compliance and variability in self-washing.

Human studies provide the gold standard for assessing decontamination products and can be broadly categorised into two types: field trials and clinical studies[1]. Field trials provide the ultimate assessment of decontamination products (or procedures) and incorporate 'real-world' factors such as the influence of human behaviour which cannot be replicated with *in vitro* or animal studies [115]. In contrast, clinical studies provide a more controlled environment which allows for the systematic evaluation of individual parameters of decontamination such as water temperature, different detergents and time between exposure and decontamination [116–119].

3.4.4 Consideration of Experimental Factors for Design of Decontamination Studies

In addition to the various factors discussed above, there are specific aspects of decontamination which require attention when designing decontamination studies. For example, spray-application of agrochemicals will contaminate hair as well as exposed skin surfaces and very little research has been performed to assess the effectiveness of decontamination protocols on scalp skin. Indeed, human scalp skin was recently demonstrated to be twice as permeable as human abdominal skin [101]. One explanation for this difference could be the expected doubling of exposed skin surface area due to additional SC associated with the lining of hair follicles [120].

The period elapsing between exposure and onset of decontamination is a critical factor [22, 92, 93, 98, 99, 104–106, 109, 113, 118, 121]. It has long been recognised that decontamination is most effective when performed immediately after exposure [122]. However, this is generally incongruous with agricultural practices where workers may be exposed for 8 h or more before having the opportunity to wash.

For studies with human volunteers, the method of exposure needs to closely model conditions of use. Thus, consideration should be given to substituting topical application of contaminants using accurate methods (such as pipetting) with more practical (but more variable) techniques such as skin contact with contaminated surfaces [117]. It should also be noted that dermal exposure to contaminated surfaces can result in the accumulation of the contaminant. For example, the concentration of nicotine on the skin surface of harvest workers can be ten times greater than the concentration of nicotine on tobacco leaves [115].

The design of exposure scenarios for assessing decontamination products or procedures needs to incorporate the possibility that more dilute solutions of a

[1] Clinical study here refers to a controlled, laboratory study using human volunteers and is distinct from clinical studies which support conformance with the licensing of drugs or medical devices.

contaminant may result in greater dermal absorption than from more concentrated solutions [91, 116, 123]. This seemingly paradoxical situation is likely the result of thermodynamic effects caused by the vehicle and so also emphasises the need to ensure that the vehicle used for topical application of a contaminant is the same as under "in use" conditions.

An unexpected but relatively frequent observation is the effect of pre-exposure skin washing on the outcome of decontamination. In general, decontamination is more effective at removing hydrophilic compounds when the skin is prewashed before exposure. Conversely, the effectiveness of decontamination for hydrophobic compounds appears to be reduced following prewashing [117, 123].

3.4.5 Current Recommendations for Skin Decontamination of Agrochemicals

At present, the general advice for those working with agrochemicals is to use warm soapy water for skin decontamination [77, 78]. This generic approach is mainly based on perceived best practice, although a number of studies have supported the efficacy of this simple approach [92, 98–100, 103, 108, 109, 111, 119, 121, 123]. However, the veracity of this advice has been questioned [81–85, 100, 103]. In assessing the available evidence, a clear conclusion is that more research is required to identify robust recommendations on skin decontamination which are likely to be chemical specific rather than a single, generic procedure.

3.5 Summary

Dermal absorption is a major route of entry for agrochemicals. OP pesticides represent an important class of potentially toxic agrochemical which have properties favouring dermal absorption. Decontamination is a practical method for reducing dermal exposure and subsequent local or systemic absorption of agrochemicals. There are many factors which affect the effectiveness of decontamination, some of which are paradoxical or counterintuitive in nature (such as enhancement of absorption by washing with water). There are a variety of methods available to assess different decontamination products and protocols, judicious use of which can provide a tiered and rational approach to the development of new and effective methods of decontamination. At present, the generic recommendation is to wash contaminated skin with warm soapy water. However, it is clear that this advice should be reviewed with the possibility of introducing chemical-specific decontamination procedures.

References

1. Johnson PD, et al. Operator exposure when applying amenity herbicides by all-terrain vehicles and controlled droplet applicators. Ann Occup Hyg. 2005;49(1):25–32.
2. Kangas J, et al. Exposure of sprayers and plant handlers to mevinphos in Finnish greenhouses. Am Ind Hyg Assoc J. 1993;54(4):150–7.
3. Semple S. Dermal exposure to chemicals in the workplace: just how important is skin absorption? Occup Environ Med. 2004;61(4):376–82.
4. Packham C. Prevention of occupational skin disease. In: Chilcott RP, Price S, eds. Principles and practice of skin toxicology. Chichester: Wiley. pp. 279–95.
5. Otto AJ, du Plessis J, Wiechers JW. Formulation effects of topical emulsions on transdermal and dermal delivery. Int J Cosmet Sci. 2009;31(1):1–19.
6. Gladman AC. Toxicodendron dermatitis: poison ivy, oak, and sumac. Wilderness Environ Med. 2006;17(2):120–8.
7. McBride JS, et al. Green tobacco sickness. Tob Control. 1998;7(3):294–8.
8. Klingner TD. Fundamentals of percutaneous chemical absorption and decontamination. J Chem Health Saf. 2007;14(4):11–15.
9. Travell J. Absorption of nicotine from various sites. Ann N Y Acad Sci. 1960;90:13–30.
10. Tucker JB, ed. War of nerves. New York: Pantheon Books; 2006.
11. Brain KR, Chilcott RP. Physicochemical factors affecting skin absorption. In: Chilcott RP, Price S, eds. Principles and practice of skin toxicology. Chichester: Wiley; 2008. pp. 83–92.
12. Lamminpaa A, Riihimaki V. Pesticide-related incidents treated in Finnish hospitals—a review of cases registered over a 5-year period. Hum Exp Toxicol. 1992;11(6):473–9.
13. Simpson WM Jr, Schuman SH. Recognition and management of acute pesticide poisoning. Am Fam Physician. 2002;65(8):1599–604.
14. Armstrong J. PSS-2778 Herbicide How-to: Understanding herbicide mode of action. Oklahoma Cooperative Extension Service Fact Sheet. http://pods.dasnr.okstate.edu/docushare/dsweb/Get/Document-6656/Pss2778web.pdf. Accessed 28 Oct 2013.
15. Schaefer H, Redelmeier TE. Factors effecting percutaneous absorption, in skin barrier. Basel: Karger; 1996. pp. 153–212.
16. Renshaw B. Observations on the role of water in the susceptibility of human skin to injury by vesicant vapors. J Invest Dermatol. 1947;9(2):75–85.
17. Gehlbach SH, Williams WA, Freeman JI. Protective clothing as a means of reducing nicotine absorption in tobacco harvesters. Arch Environ Health. 1979;34(2):111–4.
18. Kezic S, Nielsen JB. Absorption of chemicals through compromised skin. Int Arch Occup Environ Health. 2009;82(6):677–88.
19. Nielsen JB, Nielsen F, Sorensen JA. Defense against dermal exposures is only skin deep: significantly increased penetration through slightly damaged skin. Arch Dermatol Res. 2007;299(9):423–31.
20. Chilcott RP, et al. Transepidermal water loss does not correlate with skin barrier function in vitro. J Invest Dermatol. 2002;118(5):871–5.
21. Nielsen JB. Percutaneous penetration through slightly damaged skin. Arch Dermatol Res. 2005;296(12):560–7.
22. Wester RC, Maibach HI. In vivo percutaneous absorption and decontamination of pesticides in humans. J Toxicol Environ Health. 1985;16(1):25–37.
23. Hall CA. Haemostatic products as a potential therapy for vesicant-contaminated wounds. In: School of biosciences. Birmingham: University of Birmingham; 2012.
24. Dalton CH. Removal of soman from injured skin by haemostatic materials. In: School of biosciences. Birmingham: University of Birmingham; 2013.
25. Lydon HL. Investigations of a possible novel decontamination role for a haemostatic product: studies with S-[2(diisopropylamino)ethyl-o-ethyl methylphosphonothioate (VX). In: School of biosciences. Birmingham: University of Birmingham; 2012.
26. Levesque DL, Arif AA, Shen J. Association between workplace and housing conditions and use of pesticide safety practices and personal protective equipment among North Carolina farmworkers in 2010. Int J Occup Environ Med. 2012;3(2):53–67.

27. Snipes SA, et al. “Pesticides protect the fruit, but not the people”: using community-based ethnography to understand farmworker pesticide-exposure risks. Am J Public Health. 2009;99(Suppl 3) 616–21.
28. Macfarlane E, et al. Training and other predictors of personal protective equipment use in Australian grain farmers using pesticides. Occup Environ Med. 2008;65(2):141–6.
29. Macfarlane E, et al. Dermal exposure associated with occupational end use of pesticides and the role of protective measures. Saf Health Work. 2013;4(3):136–41.
30. Krieger R, et al. Gauging pesticide exposure of handlers (mixer/loaders/applicators) and harvesters in California agriculture. Med Lav. 1990;81(6):474–9.
31. Clifford NJ, Nies AS. Organophosphate poisoning from wearing a laundered uniform previously contaminated with parathion. JAMA. 1989;262(21):3035–6.
32. Wester RC, et al. In vivo and in vitro percutaneous absorption and skin decontamination of arsenic from water and soil. Fundam Appl Toxicol. 1993;20(3):336–40.
33. Wester RC, et al. Percutaneous absorption of 2, 4-dichlorophenoxyacetic acid from soil with respect to soil load and skin contact time: in vivo absorption in rhesus monkey and in vitro absorption in human skin. J Toxicol Environ Health. 1996;47(4):335–44.
34. Edwards JW, et al. Worker exposure and a risk assessment of malathion and fenthion used in the control of mediterranean fruit fly in South Australia. Environ Res. 2007;103(1):38–45.
35. Blacker NC, Findlay PH, Sherrington DC. Synthesis of CuII-complexed polymers and use as catalysts in the hydrolytic decontamination of sarin nerve agent. Polym Adv Technol. 2001;12(3–4):183–96.
36. Tafesse F, Eguzozie K. Efficient hydrolysis of 4-nitrophenylphosphate catalyzed by copper bipyridyl in microemulsions. Ecotoxicol Environ Saf. 2009;72(3):954–9.
37. Tafesse F. Hydrolysis of 4-nitrophenyl phosphate in microemulsions assisted by polymetallic complexes: a bio-mimetic model for decontamination of organophosphate pollutants. Synth React Inorg Met Org Nano Met Chem. 2005;35(8):645–50.
38. Tafesse F, Deppa NC. Polymetallic complexes in microemulsions for the hydrolysis of 4-nitrophenyl phosphate: a bio-mimetic model for decontamination of organophosphates in the environment. Ecotoxicol Environ Saf. 2004;58(2):260–6.
39. David MD, Seiber JN. Accelerated hydrolysis of industrial organophosphates in water and soil using sodium perborate. Environ Pollut. 1999;105(1):121–8.
40. Dai QY, Huang QB, Deng YD. Synthesis and reaction of a powerful nucleophilic long-chain imidazole-2-aldoxime. Chin J Org Chem. 2002;22(2):130–4.
41. Dubey DK, et al. Micellar effects on hypochlorite catalyzed decontamination of toxic phosphorus esters. Langmuir. 2002;18(26):10489–92.
42. Gonzaga F, et al. New microemulsions for oxidative decontamination of mustard gas analogues and polymer-thickened half-mustard. New J Chem. 2001;25(1):151–5.
43. Lion C, Da Conceicao L, Sayag H. Décontamination chimique. Destruction rapide, douce, totale de toxiques organophosphorés et organosoufrés par une nouvelle série de ω-phtalimidoperacides. C R Acad Sci II C Chem. 1999;2(1):57–62.
44. Tafesse F, Enemchukwu M. Nitric oxide assisted hydrolysis of nitrophenylphosphate. Nitric Oxide Biol Chem. 2008;18(4):274–8.
45. Ghanem E, Raushel FM. Detoxification of organophosphate nerve agents by bacterial phosphotriesterase. Toxicol Appl Pharmacol. 2005;207(Suppl 2):459–70.
46. Porzio E, et al. A new phosphotriesterase from *Sulfolobus acidocaldarius* and its comparison with the homologue from *Sulfolobus solfataricus*. Biochimie. 2007;89(5):625–36.
47. Grimsley JK, et al. A novel, enzyme-based method for the wound-surface removal and decontamination of organophosphorus nerve agents. In: Edwards JV and Vigo TL, eds. ACS Symposium Series. 2001;792:35–49.
48. Gordon RK, et al. Organophosphate skin decontamination using immobilized enzymes. Chem Biol Interact. 1999;119–120:463–70.
49. Zheng YZ, et al. Decontamination of vegetables sprayed with organophosphate pesticides by organophosphorus hydrolase and carboxylesterase (B1). Appl Biochem Biotechnol. 2007;136(3):233–41.
50. Yang J, et al. Overexpression of methyl parathion hydrolase and its application in detoxification of organophosphates. Biodegradation. 2008;19:831–39.

51. Cheng TC, et al. Non-corrosive, non-caustic, non-flammable, catalyst-based decontaminant formulation. United States patent and trademark office granted patent. 2007.
52. Martin M, et al. Propachlor removal by Pseudomonas strain GCH1 in an immobilized-cell system. Appl Environ Microbiol. 2000;66(3):1190–4.
53. McKinlay RG, Kasperek K. Observations on decontamination of herbicide-polluted water by marsh plant systems. Water Res. 1999;33(2):505–11.
54. Vischetti C, et al. Biochemical parameter changes in urban-waste compost used as biofilter for pesticide decontamination. Int J Environ Anal Chem. 2006;86(3–4):195–205.
55. Akhtar M, et al. Low cost sorbents for the removal of methyl parathion pesticide from aqueous solutions. Chemosphere. 2007;66(10):1829–38.
56. Celis R, et al. Triadimefon interactions with organoclays and organohydrotalcites. Soil Sci Soc Am J. 2000;64(1):36–43.
57. Cabal J, et al. Cyclodextrines as functional agents for decontamination of the skin contaminated by nerve agents. Facultas Medica Hradec Kralove. 2004;47(2):115–8.
58. Lee Y, Singh A. Poly-β-Cyclodextrins: multifunctional polymer support for the hydrolysis and removal of methyl parathion. In: Materials Research Society Symposium—Proceedings. 2003. San Francisco, CA.
59. Ramaseshan R, et al. Functionalized polymer nanofibre membranes for protection from chemical warfare stimulants. Nanotechnol. 2006;17(12):2947–53.
60. Basfar AA, et al. Degradation of diazinon contaminated waters by ionizing radiation. Radiat Phys Chem. 2007;76(8–9):1474–9.
61. De Campos SX, Vieira EM. Estudo da degradação do herbicida ácido 2, 4-diclorofenoxiacético (2,4-D) por meio da radiação gama do cobalto-60 em solução aquosa contendo ácido húmico. Quím. Nova 2002;25(4):529–32.
62. Gelover S, Mondragón P, Jiménez A. Titanium dioxide sol-gel deposited over glass and its application as a photocatalyst for water decontamination. J Photochem Photobiol A Chem. 2004;165(1–3):241–6.
63. Kruithof JC, Kamp PC, Belosevic M. UV/H2O2-treatment: the ultimate solution for pesticide control and disinfection. In: Water science and technology: water supply. 2002. pp. 113–22.
64. Aaron JJ, Oturan MA. New photochemical and electrochemical methods for the degradation of pesticides in aqueous media. Environmental applications. Turkish J Chem. 2001;25(4):509–20.
65. Gkika E, et al. Polyoxometallates as effective photocatalysts in water purification from pesticides. Int J Photoenergy. 2004;6(4):227–31.
66. Herrmann JM. Heterogeneous photocatalysis: state of the art and present applications. Top Catal. 2005;34(1–4):49–65.
67. Herrmann JM, et al. Environmental green chemistry as defined by photocatalysis. J Hazard Mater. 2007;146(3):624–9.
68. Hiskia A, et al. Polyoxometallate photocatalysis for decontaminating the aquatic environment from organic and inorganic pollutants. Int J Environ Anal Chem. 2006;86(3–4):233–42.
69. Malato S, et al. Solar photocatalytic decontamination of wastewater from the rinsing of pesticide containers. J De Phys. IV: JP. 1999;9(3).
70. Rabindranathan S, Devipriya S, Yesodharan S. Photocatalytic degradation of phosphamidon on semiconductor oxides. J Hazard Mater. 2003;102(2–3):217–29.
71. Crespi M, Palet D, Brach J. Decontamination of sludge from washing wool by means of supercritical fluids. Descontaminacion de lodos de lavado de lanas mediante fluidos supercriticos. 1998;(140):14–22.
72. Zhao C, et al. Decontamination of organochlorine pesticides in *Radix codonopsis* by supercritical fluid extractions and determination by gas chromatography. Biomed Chromatogr. 2006;20(9):857–63.
73. Costas-Costas U, et al. Solvolysis of Tris-p-nitrophenyl-phosphate in aqueous and reverse micelles. Colloids Surf A Physicochem Eng Asp. 2004;250(1–3):385–394. (Special Issue).
74. Hussain MM, Subbiah T, Ramkumar SS. Nanofibers for defense and value added applications. In: International nonwovens technical conference. Houston: INTC 2006.

75. Chilcott RP. Initial management of mass casualty incidents. In: Arora R, Arora P, eds. Disaster management: medical preparedness, response and homeland security. Oxford: CABI Press; 2013. pp. 311–24.
76. Anon. WHO-UNEP sound management of pesticides and diagnosis and treatment of pesticide poisoning: a resource tool; 2006. World Health Organisation.
77. Brent J. Water-based solutions are the best decontaminating fluids for dermal corrosive exposures: a mini review. Clin Toxicol (Phila). 2013;51(8):731–6.
78. Houston M, Hendrickson RG. Decontamination. Crit Care Clin. 2005;21(4):653–72v.
79. Chan HP, et al. Skin decontamination: principles and perspectives. Toxicol Ind Health. 2013;29:955–68.
80. Matar H, et al. Design and characterisation of a novel in vitro skin diffusion cell system for assessing mass casualty decontamination systems. Toxicol In Vitro. 2014;28(4):492–501.
81. Hui X, et al. Isolated human and animal stratum corneum as a partial model for the 15 steps of percutaneous absorption: emphasizing decontamination, part II. J Appl Toxicol. 2013;33(3):173–82.
82. Hui X, et al. Isolated human/animal stratum corneum as a partial model for 15 steps in percutaneous absorption: emphasizing decontamination, Part I. J Appl Toxicol. 2013;33(3):157–72.
83. Filon FL, et al. Skin absorption of inorganic lead (PbO) and the effect of skin cleansers. J Occup Environ Med. 2006;48(7):692–9.
84. Misik J, et al. In vitro skin permeation and decontamination of the organophosphorus pesticide paraoxon under various physical conditions—evidence for a wash-in effect. Toxicol Mech Methods. 2012;22(7):520–5.
85. Moody RP, Maibach HI. Skin decontamination: importance of the wash-in effect. Food Chem Toxicol. 2006;44(11):1783–8.
86. Jones DR. *Optimisation of aqueous decontamination methods following exposure to organophosphorus nerve agents*, PhD Thesis, 2012. University of Surrey: Guildford.
87. Moody RP, Nadeau B. In vitro dermal absorption of two commercial formulations of 2, 4-dichlorophenoxyacetic acid dimethylamine (2, 4-D amine) in rat, guinea pig and human skin. Toxicol In Vitro. 1997;11(3):251–62.
88. Larner J. *Optimisation of mass casualty decontamination: studies with sulphur mustard and simulants*. PhD Thesis, 2012. University of Surrey: Guildford.
89. Maibach HI, Feldmann RJ. Systemic absorption of pesticides through the skin of man. Report to the Federal Working Group on Pest Management from the Task Group on Occupational Exposure to Pesticides. Appendix B. Washington DC: US Government Printing Office; 1975. pp. 120–7.
90. Claborn DM, et al. A rapid and inexpensive bioassay to evaluate the decontamination of organophosphates. US Army Med Dep J. 2012. pp. 36–42.
91. Bucks DA, et al. In vitro percutaneous absorption and stratum corneum binding of alachlor: effect of formulation dilution with water. Toxicol Appl Pharmacol. 1989;100(3):417–23.
92. Wester RC, et al. Glyphosate skin binding, absorption, residual tissue distribution, and skin decontamination. Fundam Appl Toxicol. 1991;16(4):725–32.
93. Fosse C, et al. Decontamination of tetramethylammonium hydroxide (TMAH) splashes: promising results with diphoterine in vitro. Cutan Ocul Toxicol. 2010;29(2):110–5.
94. Mircioiu C, et al. Evaluation of in vitro absorption, decontamination and desorption of organophosphorous compounds from skin and synthetic membranes. Toxicol Lett. 2013;219(2):99–106.
95. Kolle SN, et al. Applicability of in vitro tests for skin irritation and corrosion to regulatory classification schemes: substantiating test strategies with data from routine studies. Regul Toxicol Pharmacol. 2012;64(3):402–14.
96. Netzlaff F, et al. Permeability of the reconstructed human epidermis model episkin in comparison to various human skin preparations. Eur J Pharm Biopharm. 2007;66(1):127–34.

97. Stewart CA, et al. Classification of irritant/non-irritant and corrosive chemicals using excised human skin in vitro. In: Chilcott RP, Brain KR, eds. Advances in dermatological sciences. Royal Society of Chemistry, Chichester; 2013. pp. 308–20.
98. Hewitt PG, Hotchkiss SA, Caldwell J. Decontamination procedures after in vitro topical exposure of human and rat skin to 4, 4'-methylenebis[2-chloroaniline] and 4, 4'-methylenedianiline. Fundam Appl Toxicol. 1995;26(1):91–8.
99. Hui X, Domoradzki JY, Maibach HC. In vitro study to determine decontamination of 3, 5-dichloro-2, 4, 6-trifluoropyridine (DCTFP) from human skin. Food Chem Toxicol. 2012;50(7):2496–502.
100. Loke WK, et al. Wet decontamination-induced stratum corneum hydration—Effects on the skin barrier function to diethylmalonate. J Appl Toxicol. 1999;19(4):285–90.
101. Rolland P, et al. Human scalp permeability to the chemical warfare agent VX. Toxicol In Vitro. 2011;25(8):1974–80.
102. Rolland P, et al. Hairy skin exposure to VX in vitro: effectiveness of delayed decontamination. Toxicol In Vitro. 2013;27(1):358–66.
103. Weber LW, Zesch A, Rozman K. Decontamination of human skin exposed to 2, 3, 7, 8-tetrachlorodibenzo-p-Dioxin (TCDD) in vitro. Arch Environ Health. 1992;47(4):302–8.
104. Wester RC, et al. Percutaneous absorption and skin decontamination of PCBs: in vitro studies with human skin and in vivo studies in the rhesus monkey. J Toxicol Environ Health. 1990;31(4):235–46.
105. Zhai H, et al. In vitro model for decontamination of human skin: formaldehyde. Food Chem Toxicol. 2007;45(4):618–21.
106. Zhai H, et al. Skin decontamination of glyphosate from human skin in vitro. Food Chem Toxicol. 2008;46(6):2258–60.
107. Chilcott RP, et al. Inter- and intralaboratory variation of in vitro diffusion cell measurements: an international multicenter study using quasi-standardized methods and materials. J Pharm Sci. 2005;94(3):632–8.
108. Fairhurst S, et al. Skin effects of trichothecenes and their amelioration by decontamination. Toxicology. 1987;46(3):307–19.
109. Monteiro-Riviere NA, et al. Efficacy of topical phenol decontamination strategies on severity of acute phenol chemical burns and dermal absorption: in vitro and in vivo studies in pig skin. Toxicol Ind Health. 2001;17(4):95–104.
110. Cabal J, Kassa J, Severa J. A comparison of the decontamination efficacy of foam-making blends based on cationic and nonionic tensides against organophosphorus compounds determined in vitro and in vivo. Hum Exp Toxicol. 2003;22(9):507–14.
111. Pelletier O, Ritter L, Caron J. Effects of skin preapplication treatments and postapplication cleansing agents on dermal absorption of 2, 4-dichlorophenoxyacetic acid dimethylamine by Fischer 344 rats. J Toxicol Environ Health. 1990;31(4):247–60.
112. Phieffer LS, et al. Evaluation of a cleanser for petroleum-contaminated skin. J Trauma. 2003;55(6):1148–51.
113. Joosen MJ, et al. Timing of decontamination and treatment in case of percutaneous VX poisoning: a mini review. Chem Biol Interact. 2013;203(1):149–53.
114. Wester RC, et al. In vivo skin decontamination of methylene bisphenyl isocyanate (MDI): soap and water ineffective compared to polypropylene glycol, polyglycol-based cleanser, and corn oil. Toxicol Sci. 199948(1):1–4.
115. Curwin BD, et al. Acephate exposure and decontamination on tobacco harvesters' hands. J Expo Anal Environ Epidemiol. 2003;13(3):203–10.
116. Dick IP, Blain PG, Williams FM. The percutaneous absorption and skin distribution of lindane in man. I. In vivo studies. Hum Exp Toxicol. 1997;16(11):645–51.
117. Fenske RA, Lu C. Determination of handwash removal efficiency: incomplete removal of the pesticide chlorpyrifos from skin by standard handwash techniques. Am Ind Hyg Assoc J. 1994;55(5):425–32.
118. Fredriksson T. Percutaneous absorption of parathion and paraoxon. IV. Decontamination of human skin from parathion. Arch Environ Health. 1961;3:185–8.

119. Moffett PM, et al. Evaluation of time required for water-only decontamination of an oil-based agent. Mil Med. 2010;175(3):185–7.
120. Chilcott RP. Cutaneous anatomy and function. In: Chilcott RP, Price S, eds. Principles and practice of skin toxicology. Chichester: Wiley; 2008. pp. 4–16.
121. Wester RC, Melendres J, Maibach HI. In vivo percutaneous absorption and skin decontamination of alachlor in rhesus monkey. J Toxicol Environ Health. 1992;36(1):1–12.
122. Chilcott RP. Dermal aspects of chemical warfare agents. In: Marrs TC, Maynard RL, Sidell FR, eds. Chemical warfare agents: toxicology and treatment. Chichester: Wiley; 2008. pp. 409–22.
123. Campbell JL, et al. Comparison of solvents for removing pesticides from skin using an in vitro porcine model. AIHAJ. 2000;61(1):82–8.

Part II
In Vitro Drug Release

Chapter 4
Importance of *In Vitro* Drug Release

Vinod P. Shah and Roger L. Williams

4.1 Introduction

The dissolution or drug release test is an important and time-honored test to assure stability in product performance over many years once bioavailability (BA) or bioequivalence (BE) has been established. It is applicable to nonsolution orally administered drugs. Typically, the dissolution test procedure and acceptance criteria are developed following the understanding of the *in vivo* performance. While the dissolution procedure has many limitations and has been challenged on many occasions, it remains widely used and has continued to be improved over the years. It might well be argued that understanding of dissolution, particularly for immediate release oral drug products, had a substantial impact on the success of the US multisource system, given some of the inherent limitations in the primary documentation of BA and BE [1].

For topically applied drug products, drug release from the formulation is also considered important. They fall into two major categories: (1) transdermal systems (TDS) that are designed to allow entry of the drug substance into the systemic circulation and (2) topical dermatological drug products such as creams, ointments, gels, and lotions that are designed to treat local skin disorders with minimal systemic exposure. Thus far, the only known exception for the latter classification is nitroglycerin ointment applied topically yet used to achieve systemic effects in the treatment of angina pectoris. Recently, over-the-counter patches have been introduced for analgesic and antiarthritic activity.

The US Food and Drug Administration's (FDA) approval of topical and transdermal dosage forms requires documentation of *in vivo* and, where appropriate, *in vitro* data.

V. P. Shah (✉)
11309 Dunleith Place, North Potomac, MD 20878, USA
e-mail: Dr.vpshah@comcast.net

R. L. Williams
United States Pharmacopeia, Rockville, MD, USA

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_4

The former include clinical safety and efficacy, local irritation, systemic toxicity, BA, and chemistry, manufacturing, and controls (CMC) information. For control, studies should cover critical quality attributes, including identity, description, strength (assay), purity, content uniformity, and drug release characteristics, where appropriate. These control approaches are well considered in available ICH documents, which are taken up in FDA guidances that are reflective of applicable laws, regulation, and policy. They are also pertinent to the identity, strength, quality, and purity requirements stated in the United States Pharmacopeia (USP). The various terms speak about the overarching term *quality*, which is part of the triad that includes safety and efficacy.

Recently, two types of *in vitro* tests have been identified to better assess the critical quality attributes of many drug products: (1) product quality tests and (2) product performance tests [2]. Product quality tests are intended to assess attributes such as identity, strength, quality, and purity, using basic language in the Food, Drug, and Cosmetic Act that speak about adulteration and misbranding [3]. Product performance tests assess the *in vitro* release of a drug substance from a dosage form in ways that help assure or, through *in vitro*/*in vivo* correlations, document *in vivo* performance. Quality and performance tests speak about the overall quality of pharmaceutical dosage form as a means of assuring its continuing equivalence—both pharmaceutical and biopharmaceutical—relative to the clinical trial material on which safety and efficacy data were based.

A simple, reliable, and reproducible *in vitro* drug release test is desired for most nonsolution dosage forms, irrespective of the route of administration. For most oral products, an appropriate *in vitro* dissolution test has proved to be the optimum test method for this purpose. The sequence of steps leading to pharmacological activity after oral administration is shown in Fig. 4.1, which also compares the sequence of steps that occur after topical drug application. Thus, for topical products, the

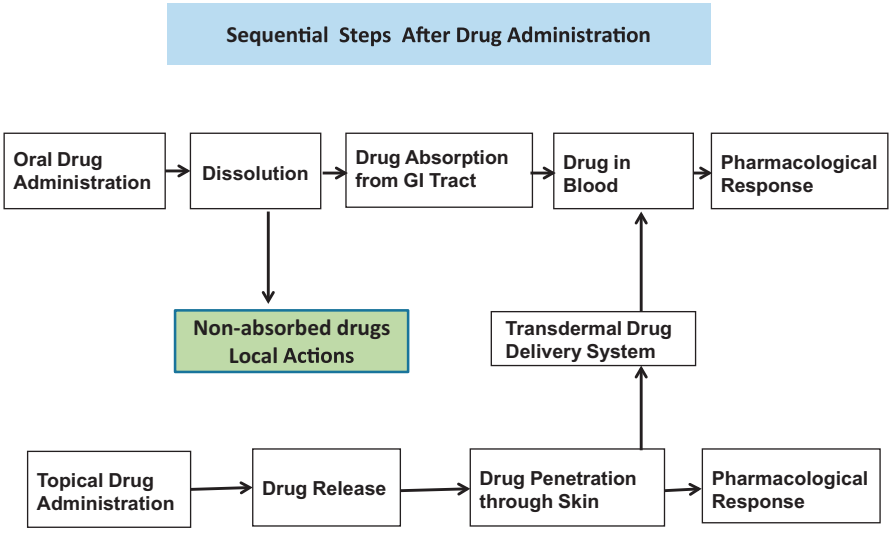


Fig. 4.1 Sequential steps after dosage form administration/application

understanding of safety and efficacy rests on the release of the active drug from its dosage form to the skin surface [4]. Once the drug is applied to the skin surface, it penetrates through the stratum corneum to achieve its pharmacological action. Release rate determination through *in vitro* studies, then, is an important parameter for controlling the quality of a topical product just as release testing is important for nonsolution orally administered dosage forms.

The following points should be considered when developing an *in vitro* release procedure for dermatological products:

- Assurance of batch-to-batch uniformity
- Applicability to all products that are (and will be) marketed
- Ability to discriminate manufacturing changes that (may) influence product performance
- Relevance to *in vivo* product performance
- Simplicity, reliability, reproducibility, and capability for automation

The two major categories of topical products: (i) transdermal patches and (ii) topical dermatological creams, ointments, and gels pose different issues for the development of an *in vitro* release test. These are discussed separately in the remaining sections of the chapter.

4.2 *In Vitro* Release Methods for Transdermal Systems

The current USP identifies apparatus 5, 6, and 7 for the *in vitro* release of transdermal systems such as patches [5]. Developed with FDA, USP apparatus 5 is a simple, reproducible method that employs a watch glass-patch-Teflon mesh sandwich assembly coupled with the USP paddle method to determine the release rate of a drug substance from a transdermal patch. The *in vitro* release profile generated by using apparatus 5 provides a release profile comparable to that obtained by more cumbersome methods needed for USP apparatus 6 and 7 and is thus preferred [6, 7]. Procedures utilizing apparatus 5 can be easily automated.

The nitroglycerine patch is an example of a multisource product available from several manufacturers. All brands of nitroglycerin patches exhibit different release characteristics in apparatus 5 because of the different manufacturing technologies employed [6]. This suggests that it is difficult to have a single *in vitro* release test for all brands of nitroglycerin patches. This is analogous to the situation for oral extended-release preparations (and some products as well), where different manufacturers have different release specifications for the same drug product, given that adequate BA and BE studies have been performed. Similarly, transdermal products such as controlled/extended-release preparations, require product-specific release requirements in order to assure batch-to-batch uniformity and BE. Similarity of *in vitro* release in transdermal patches is also used as a confirmation of equivalence in product performance under scale-up and post-approval changes (SUPAC) of level 1 [8]. The absence of a common test for all products, as frequently happens for many

immediate release formulations administered orally is not necessarily a negative finding. Rather it means that each manufacturer must develop a test using general methodology found in the USP. With this methodology, continuing equivalence in product performance can be assured batch-to-batch and, as noted, some changes in manufacturing can occur with reliance on the product performance test as a means of assuring continuing BE.

4.3 *In Vitro* Release Method for Semisolid Dosage Forms

Semisolid dosage forms include creams, ointment, and gels. As the efficacy of a topically applied dosage form is dependent on drug release, the *in vitro* release rate (profile) theoretically constitutes an important and valuable product quality control parameter. Despite some debate, a recent workshop entitled "Principles and Practices of *In vitro* Percutaneous Penetration Studies: Relevance to Bioavailability and Bioequivalence" emphasized the importance of developing a methodology to assure batch-to-batch drug release equivalency for topical dosage forms [9]. The report also indicated that the use of a skin membrane (human cadaver or animal) in diffusion studies is subject to great variability (source, preparation, skin history, etc.). Based on these and prior reports, these types of membranes are now considered unsuitable for use in routine quality control testing.

A simple, reliable, and reproducible method to determine the drug release of corticosteroids from creams, ointments, and gels, has been developed [10]. It uses a commercially available static vertical diffusion cell (VDC) system and a synthetic membrane. Use of a commercially available synthetic membrane obviates the problems associated with the preparation and variability of skin membranes. The semisolid dosage form is placed in the donor chamber on the synthetic membrane and aqueous buffer is used as the receptor medium. The release of the drug substance is determined over a 6 h interval and plotted as amount released versus square root of time. This will typically result in a straight line, with the slope representing the release rate (flux) of the drug substance from the drug product [11]. The method is capable of being automated.

With creams containing sparingly soluble drugs, the use of a hydroalcoholic medium as a receptor phase is essential to increase drug solubility for detection and to maintain sink conditions. Detection can usually be made by high-pressure liquid chromatographic (HPLC) testing without further extraction. When employing a hydroalcoholic medium as a receptor phase, a challenge arises from the possibility of back diffusion of alcohol through the synthetic membrane and alteration of the integrity of the cream preparation. Suitable studies can detect or refute this occurrence. For example, in the case of betamethasone valerate cream, where 60% ethanol/water was used as the receptor phase, only 0.032% of alcohol was detected in the cream (donor chamber) at the end of 6 h of study. The microscopic examination of the cream before and after the *in vitro* release experiment showed no difference. Thus, the concentration of ethanol had a negligible effect on cream integrity [12].

Hydrocortisone ointment showed a much slower rate of release compared with a cream formulation [9]. This does not necessarily reflect a difference in pharmacological activity between two formulations, but emphasizes instead that release rates between the pharmaceutically inequivalent dosage forms may differ substantially. Because of the complexity of any specific topical dosage form, it is difficult to set a release rate specification for a particular class of products. Yet, each manufacturer can have its release rate requirement to assure batch-tobatch uniformity and BE, just as the case with transdermal systems.

Equivalence of *in vitro* release rates can be a measure of continuing *in vivo* performance in topical products. For this reason, FDA has allowed *in vitro* release studies for Level 2 SUPAC changes [13]. The drug release rate should be within defined acceptability criterion before and after the proposed change [13]. The general approach considered in an AAPS workshop entitled "Assessment of Value and Application of *In vitro* Testing of Topical Dermatological Drug Products" reasserted the importance of *in vitro* testing to assure batch-to-batch uniformity and for some SUPAC changes [14].

The USP has carried out a collaborative study using a hydrocortisone cream (as a performance verification approach), betamethasone dipropionate cream, ointment, and gel in four laboratories using a VDC system. The results indicated that the *in vitro* release procedure is simple and reproducible [15]. A general chapter, <1724> in the current USP/NF 36/31 Supplement 1, 2013 describes product performance tests for semisolid dosage forms. Along with the use of VDC, other methods are also discussed for *in vitro* release measurements.

Recently, the FDA published a draft guidance for BE determination of acyclovir ointment 5% with an option of using *in vitro* drug release data for drug approval purposes [16]. This includes the requirement that the generic product must have qualitatively (Q1) and quantitatively (Q2) same inactive ingredients and must have the same microstructure arrangement (Q3) as the innovator product. The FDA has also suggested that *in vitro* drug release studies are a first step in advancing QbD approaches for topically applied products [17].

4.4 Importance of *In Vitro* Drug Release

Just as dissolution is used as a quality procedure for orally administered nonsolution dosage forms, so is *in vitro* release testing for a topical drug product. The *in vitro* release test for topical products (also termed semisolids) has been recognized in US FDA's SUPAC-SS guidance as a test for equivalence tests for product performance after certain manufacturing-related changes [13]. Recently, the *in vitro* release test is also recognized as a reasonable and useful test to consider for product release and stability testing [18]. When the generic product is Q1 and Q2 for excipients and has the same release characteristics as the innovator product in an *in vitro* system (specified as Q3), the test is considered to be suitable for biowaiver of acyclovir ointment 5% [16]. Can the principle of "Q1, Q2 and Q3" be applied to other semisolid dos-

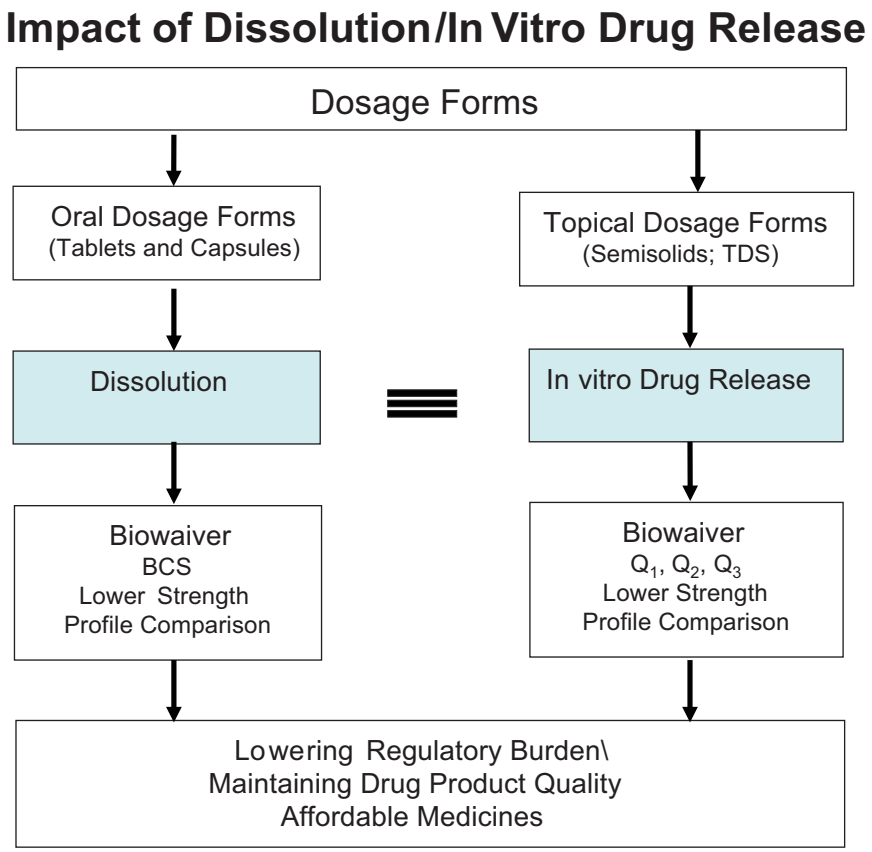


Fig. 4.2 Impact of dissolution/drug release

age forms? The answer is probably yes. In the case of nonsolution orally administered dosage forms such as tablets and capsules, lower dosage strengths may have BA and BE requirement waivers as long as they are formulation proportional and employ the same drug releasing ingredients as the higher strength. Can this also be applied to topical dosage forms? Also, the answer is probably yes. The general analogy between oral and topical dosage forms is shown in Fig. 4.2.

4.5 Conclusion

Two simple methods to determine the *in vitro* drug release profile for topical drug products have been discussed. USP apparatus 5 provides a watch glass-patch-Teflon mesh assembly with the paddle method and an appropriate dissolution medium is suggested for TDS products. A simple static VDC system with a synthetic membrane and an appropriate receptor medium has been proposed for topically applied

drug products. Both methods have been shown to be simple, reliable, and reproducible and are suitable for automation. While the resulting procedures are applicable to specific products, the overall value as a product performance test to assure batch-to-batch consistency is increasingly evident. The application to assure BE is also beginning to be better understood and utilized. These approaches have the possibility of reducing manufacturer and regulatory resources, as well as moving away from costly clinical trials as a means of assuring continuing equivalence over time for both TDS and topically applied medicines. Manufacturers, regulators, practitioners, and patients are likely to be the beneficiaries of these advances in *in vitro* product release testing.

References

1. US 21 Code of Federal Regulations, Section 320.
2. Ueda CT, Shah VP, Derdzinski K, et.al. Performance test for topical and transdermal dosage forms. Pharmacop Forum. 2006;32(5):1586–9.
3. US Food, Drug and Cosmetic Act, Sec 301; 21 USC 331.
4. Guy RH, Guy AH, Maibach HI, Shah VP. The bioavailability of dermatological and other topically administered drugs. Pharm Res. 1986;3:253–62.
5. United States Pharmacopeial Convention, USP/NF 36/31, General chapters <3>, <724> and <1724>.
6. Shah VP, Tymes NW, Skelly JP. Comparative in vitro release profile of marketed nitroglycerin patches by different dissolution methods. J Control Release. 1988;7:79–86.
7. Shah VP, Tymes NW, Skelly JP. In vitro release profile of clonidine transdermal therapeutic systems and scopolamine transdermal patches. Pharm Res. 1989;6:346–51.
8. Van Buskirk GA, Gonzalez M, Shah VP, et.al. Workshop report: scale-up of adhesive transdermal drug delivery systems. Pharm Res. 1997;14:848–52.
9. Skelly JP, Shah VP, Maibach HI, et al. FDA and AAPS report of the workshop on principles and practices of in vitro percutaneous studies: relevance to bioavailability and bioequivalence. Pharm Res. 1987;4:265–7.
10. Shah VP, Elkins J, Hanus J, Noorizadeh C, Skelly JP. In vitro release of hydrocortisone from topical preparations and automated procedures. Pharm Res. 1991;8:55–9.
11. Higuchi WI. Analysis of data on the medicament release from ointments. J Pharm Sci. 1962; 51:802–4.
12. Shah VP, Elkins J, Skelly JP. Relationship between in vivo skin blanching and in vitro release rate for betamethasone valerate creams. J Pharm Sci. 1992;81:55–9.
13. FDA Guidance for Industry. SUPAC-SS nonsterile semisolid dosage forms. Scale-up and postapproval changes: chemistry, manufacturing and controls; in vitro release testing and in vivo bioequivalence documentation. Guidance. 1997.
14. Flynn GL, Shah VP, Tanjarla S, et al. Assessment of value and application of in vitro testing of topical dermatological drug products. Pharm Res. 1999;16(9):1325–30.
15. Hauck WW, Shah VP, Shaw SW, Ueda CT. Reliability and reproducibility of vertical diffusion cells for determining release rates from semisolid dosage forms. Pharm Res. 2007;24:2018–24.
16. FDA draft guidance, Acyclovir ointment 0.5%, March 2012.
17. Chang RK, Raw A, Lionberger R, Yu L. Generic development of topical dermatologic products, Part II: quality by design for topical semisolid products. AAPS J. 2013;15:674–83.
18. Chang RK, Raw A, Lionberger R, Yu L. Generic development of topical dermatologic products: formulation development, process development, and testing of topical dermatologic products. AAPS J. 2013;15(1):41–52.

Chapter 5
Diffusion Cell Design

Jon R. Heylings

5.1 Introduction

This chapter will cover aspects of skin diffusion cell design and how various types of devices have evolved over the years for the purpose of measuring the absorption of drugs and chemicals across the skin. Much of this relates to the author's own experience, but to do justice to this topic, the historical aspects of flux chamber devices and adaptations to conventional designs, including a new generation of automated diffusion cell equipment will be covered. Recognising that the rest of this book covers the dermal absorption of compounds and formulated products used in the pharmaceutical sector where dermal exposure is intentional, the author has focused mainly on this area. However, much of the published work that utilizes skin diffusion cells and how they have evolved over the last 50 years actually comes from the area of human safety assessment of industrial chemicals, where *in vitro* methodology is used extensively for predicting dermal absorption in humans. Absorption data generated in these models is a key element of human risk assessment for a broad range of chemical products that come in contact with human skin, either for cosmetic purposes [30] or in the case of pesticides [7, 9] and industrial chemicals [40], during occupational exposure that may occur during manufacture or intended use. The basic chamber design is similar for the approved methods used across the different industrial sectors including the pharmaceutical industry.

J. R. Heylings (✉)
School of Pharmacy, Keele University, Staffordshire, UK
e-mail: j.heylings@dermaltechnology.com

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration*, DOI 10.1007/978-1-4939-1289-6_5

5.2 Historical Perspective

Diffusion cell devices that use resected skin for estimating dermal absorption in man have been around for many decades. Of all the portals of entry to the human body, the skin is a relatively accessible organ to study in the laboratory. The skin from many animal species has been investigated *in vitro* using chamber approaches with the pig being probably the most extensively studied animal species as well as a widely accepted surrogate model for human skin [5, 32].

As far as the use of flux chambers as devices for measuring the transport of chemicals from external (environmental) compartments to internal (systemic) compartments goes, they have been used to provide the rigid structure to support a functional epithelial layer or some other form of biological barrier, such as the skin, in a situation where the exposed surface is fixed and of known engineered area, and each side of the biological specimen faces a different environmental or biological condition. The most commonly used chambers are designed to replicate the polarized nature of the skin, where the external surface faces an air environment and the internal surface faces the systemic circulation. However, much of the history of chamber devices actually comes from *in vitro* experiments using isolated mucosal sheets from the gastrointestinal tract, where ion transport and gut absorption (not dermal absorption) have been investigated in great detail for many decades. One of the classical chamber approaches with isolated gut was developed primarily by Hans Ussing in Denmark for the study of gastric ion transport using amphibian mucosa [41]. Much of the understanding on how the gastric mucosa transports hydrogen ions into the lumen of the stomach in order to create a pH gradient between the serosal side of the gastric mucosa and the luminal side came from the classical 'Ussing' chamber studies. These chamber systems were constructed using Lucite or Perspex, and usually had discs of gut mounted on pins to stretch and flatten the tissue over the circular exposed aperture of the chamber. This allowed a fixed surface area of tissue to be bathed with separate circulating media in the two halves of the chamber. Such chamber devices were used mainly in the pharmaceutical industry to study the effect of drugs on both mucosal secretion and absorption. These gut mucosal chambers were also used to characterize mucosal protection. One such device that the author developed in the 1980s was a dual mucosal chamber. This device incorporated two discs of gut orientated such that their blood sides were in contact with a common bathing solution. The luminal sides of the membranes were bathed with separate oxygenated solutions. This chamber device demonstrated that the control of bicarbonate secretion, the protective mechanism against acid autodigestion, was via a humoral mechanism, as acidification of one mucosa caused a protective secretory response of the other mucosa, despite the physical separation of the two tissue discs [16].

A major difference between gut chambers and skin chambers is the requirement for tissue viability. The isolated gut requires a constant oxygen flow to the media bathing the tissue as even transient anoxia will arrest the active transport processes fundamental to the function of the tissue. In contrast, the skin surface, the *stratum corneum* is a dead cornified layer, which can still serve its main function of a barrier

to the passage of substance through it, in the absence of an oxygenated media. As such, the design of a typical gut and skin chambers is similar in several respects such as a fixed surface area for application of a measured dose of test substance and expression of flux or other parameter relating to the proportion of the substance that has moved to the alternate compartment over a measured time period. However, the most obvious difference is the orientation of the device. Generally, a gut chamber is horizontal with a tissue held vertically. In contrast, a skin chamber is vertical with the tissue held horizontally. The gut mucosa is held between two aqueous physiological environments to measure the transport between the compartments, whereas the skin has an upper air-exposed surface and a lower aqueous-exposed dermal side bathed by a receptor fluid. There are, of course, some exceptions where some skin chambers were designed horizontally, but controlling the receptor temperature and mixing makes this system inherently more suitable to a vertical or upright design. In fact, these horizontal skin chambers can only be used for infinite dose type studies, where the chambers on both sides of the skin are filled with donor and receptor fluids [33]. A conventional skin diffusion cell uses a dose application that is typical of human exposure to a chemical or drug treatment. This normally involves a low (finite) volume of the product containing the active ingredient. This covers the skin surface in a vertical designed system [13, 33]. There are now a considerable number of publications on studies with skin diffusion cells across all industrial sectors [21]. These mainly stemmed from the original work in the area of drug absorption and skin disposition of pharmaceutical products. With the publication of OECD test guidelines in 2004, there are now increasing numbers of papers with literature on industrial chemicals, particularly pesticides, where the method is used extensively as part of the prediction of human exposure during the manufacture, handling and use of such chemicals [9]. The *in vitro* method, as used for chemicals and drug intermediates, has a guideline status and is used throughout the world as part of the human risk assessment process [25, 26]. This has now almost completely replaced the corresponding *in vivo* test method that used rats for such studies for pesticides only [24, 39]. In addition to the formal OECD test guidelines, the *in vitro* method using glass diffusion chambers has the endorsement of many different European industry bodies, many of whom have published their own specific guidance over the last two decades [7–9, 27, 30]. There are also several key publications detailing the methodology in the area of dermal absorption and how the *in vitro* approach fits into the overall risk assessment process for chemicals [17, 20, 21].

Probably the most comprehensive earlier publications comparing different permeants using diffusion chambers and resected human skin come from investigators such as Tom Franz, certainly on the static cells [13] and others such Bob Bronaugh on the flow-through type systems [1]. In order to gain credibility as a reliable model, the *in vitro* chamber system must be able to predict dermal absorption in humans with an acceptable degree of accuracy. A systematic evaluation of 12 organic compounds that all had *in vivo* data [10] was investigated in some of the earlier systematic evaluations of the glass chamber system. The static diffusion cell system was shown to predict the dermal absorption observed in humans very well [13]. Indeed, the author of this particular investigation, Tom Franz, did a lot of work developing

the chamber system over the years, so much so that these devices are often referred to as 'Franz' chambers. Although skin chamber type studies were performed many years prior by investigators such as Burch and Winsor [2] and Malkinson [23], it was the work in the next two decades by others such as Scheuplein [31], Flynn and Yalkowsky [12], and Feldmann and Maibach [11] that investigated specific areas of both medicinal and industrial chemicals using chamber devices and identified the key methodological aspects that are important to the emerging field of *in vitro* dermal absorption at that time. A general understanding on how the physicochemical properties of the penetrant and the vehicle in which it was applied influenced the kinetics of skin penetration began to emerge. No formal validation of the chamber method was in place for the approach to be used as stand-alone in risk assessment, but many investigators from their own areas of industry, particularly for pesticides, were demonstrating the usefulness of the *in vitro* approach as a predictive model for humans. [28, 29, 34, 35].

5.3 Static Diffusion Cell

Practically all the earlier studies on diffusion chambers that utilized human or animal skin were of the 'static' design [6, 14, 33]. These devices were simple and based on a two-part glass cell, the donor and the receptor compartments engineered to accommodate a fixed circular area of skin tissue between the compartments. Some of the designs had water-jacketed outer glass compartment in order to maintain the receptor fluid at skin temperature (32 °C). Other systems used open water baths in which the static cell was semi immersed so that the receptor compartment was maintained at this skin temperature but the donor compartment was placed above the waterline. A key design feature that is often overlooked is the thorough mixing of the receptor fluid during the experiment. A typical dermal absorption study normally involves a 24 h time period, whether for a pharmaceutical investigation, such as drug delivery, or for a safety investigation such as the systemic exposure to a potentially toxic agent. Indeed, a number of years ago these experiments often involved leaving a chemical on the skin for up to 3 days [39]. This is no longer a guideline requirement where the maximum duration of a correctly designed *in vitro* study is now 24 h [9, 25, 26]. The experiment relies on sampling of the receptor fluid at regular (or prescribed test guideline) time periods from the time of application of the test material to the skin till the end of the study. The small sample taken at each time point, which may be as low as 50 μl from a 4.5 ml receptor chamber volume, must be representative of the bulk concentration in the whole receptor volume of the test substance being evaluated. Therefore, rapid stirring of the receptor fluid to ensure the concentration of the test compound immediately below the dermal side of the tissue is the same as the point of entry into the collection device is essential. There are several interlinking aspects here such as the solubility of the test substance in the receptor fluid, the binding of the test compound to any component of the system (such as the glass wall of the chamber or sampling tubing) and the type of skin preparation (epidermal sheet versus split thickness skin). These can

all affect the measured concentration of test substance that has permeated into the receptor fluid at any given time. These aspects are discussed in the respective test guidelines and guidance documents for *in vitro* percutaneous absorption [9, 25–27, 30], and form the basis for inclusion or otherwise of the so-called skin dose or mass of test substance that has crossed the *stratum corneum*, but not reached the receptor fluid, as being potentially systemically available.

The conventional type static diffusion cell must also accommodate the resected skin sample so that the seal between the glass donor and receptor chambers is effective. Most cells have an engineered ground glass joint so that even the thinnest heat-separated epidermal can be supported by a nickel (or other inert) grid and effectively sealed, and no lateral migration of the test application from its site of application can occur. A strong clamp with a steel edge in contact with the full circumference on either side of the joint ensures that this donor–receptor chamber seal is maintained until the chamber is dismantled. This same clamp is engineered with a wheel-locking device and base supports under the glass chamber, so that it sits perfectly horizontal in a water bath [33].

The surface area of exposed skin is a key element of diffusion cell design, and in the author's experience the most fundamental aspect of a successful OECD 428 mass balance recovery *in vitro* percutaneous absorption study. The signal-to-noise ratio or that of skin surface-to-glass chamber should be as high as possible, to allow good surface spreading of a finite low volume dose of a formulated product, but without bowing or distortion of the skin sample and, of course, without utilizing too much precious biological material. With static cells, the exposed cell area is usually between 1 and 5 cm^2. The smaller 'mini' cells around 1 cm^2 are often used for nonregulatory investigations, or for other functional end points, including skin irritation assessment [18, 19]. The larger 'standard' static cells, around 2–3 cm^2, are most common in regulatory dermal absorption studies. Figure 5.1 shows the diffusion cell designed and used by the Dermal Technology Laboratory (DTL). It has a relatively large surface area of skin to the size of the glassware. A key design feature is the broad rim of ground glass joint that evokes a good seal preventing lateral compound migration, and importantly any transfer round the rim of the specimen. The device is clamped with a spring holder and the skin sits on a thin nickel porous support to prevent any bowing or stretching of the skin. The design also allows easy access to the skin, post exposure, to facilitate sponge washing and tape stripping of the *stratum corneum*.

The static cell design has not changed much over the years. Some laboratories use cells that are slightly smaller than the one described above. This conserves the precious commodity, human skin. However, it has been shown that the interlaboratory concordance for a variety of drugs is very good when the studies are performed correctly using different size static diffusion cells. One example of this was a published interlaboratory investigation of caffeine, benzoic acid, and testosterone. The dermal absorption of these three reference chemicals in terms of both, the maximum absorption rate and the percentage absorption after 24 h, was shown to be very similar for each drug, despite the fact that the human skin was sourced, stored and prepared in different laboratories and by different technicians [15]. The most important aspect here to achieve this concordance was the measurement of the skin integ-

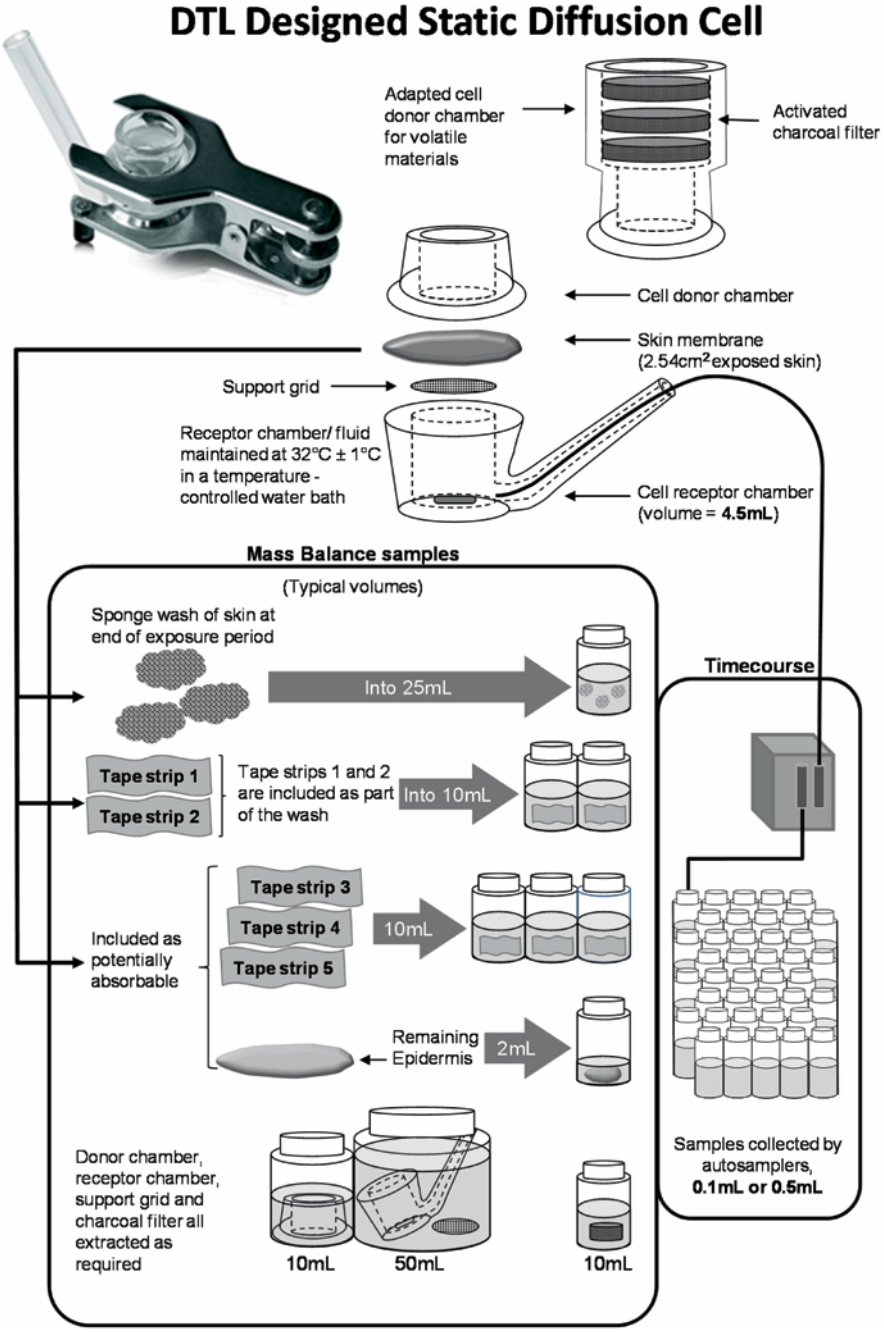


Fig. 5.1 Schematic of the DTL glass diffusion cell and the various compartments that are collected and analysed in the mass balance recovery of a test substance 24 h after application to the surface of the skin

rity for each skin diffusion cell. The laboratories used the same rejection criteria for tritiated water flux and electrical resistance measurement to assess the skin integrity [4]. Only skin samples that were deemed to have normal barrier function were used for drug application. This should apply, of course, to all *in vitro* dermal absorption studies, as specified by the OECD test guidelines [25].

5.4 Flow-Through Diffusion Cell

By the late 1970s, many laboratories throughout the world were using resected skin mounted in static diffusion cells. As more and more papers on the predictive ability of these models came to press, further refinements in this approach were investigated. The drivers for moving towards a continuous flow-through receptor design included a general move towards physiological receptors and moved away from solvent-type systems in an attempt to replicate the normal physiological conditions of the skin and its draining blood supply that carries any absorbed material away from the skin [1]. This, of course, makes solubility in the receptor more of a challenge as it is important that sink conditions are maintained and the concentration of test substance in the receptor never reaches a point where back diffusion can occur. Recent guidance indicates that the maximum concentration of the test substance observed in the receptor fluid should not exceed 10% of its saturated solubility in the receptor fluid selected [9]. The flow-through design with physiological receptor fluid means that for many studies the flow rate and replacement of fresh receptor needs careful attention. Another issue with the flow-through design relates to the analytical sensitivity. For non-radiolabelled investigations, the extraction and analysis of the test compound in a mass balance type investigation may be more challenging due to the greater volumes of receptor fluid compared to static cell designs.

Static diffusion cells are more commonly used than their flow-through counterparts. One distinct advantage of the static cell is that it runs independently from the other diffusion cells in the group. It is also much easier to undertake the mass balance washing and tape stripping procedures that form a part of the dermal absorption protocol [9, 30] than in a flow-through diffusion cell system.

Most flow-through type systems utilize a dry heated-block type design or water-jacketed approach to maintain a constant skin temperature of 32 °C. There is an electric pump to move the receptor under the dermal side of the skin at a constant flow rate into some form of collection device or autosampler vials. A typical flow rate is around 1.5 ml/h for such devices [1, 3]. Although most of the systems are quite reliable, one disadvantage of the flow-through system is that if the receptor flow system fails or the pump speed varies then all the diffusion cells in the group may be lost. In a group of static cells if there is a single blockage, say in the receptor collection line and the other samples are running independently then, the autosampler will continue to take accurate receptor volumes. As these *in vitro* experiments run for 24 h to obtain daily exposure data, and taking samples of receptor every 1–2 h through the night, it is important that the system is reliable.

5.5 Special Devices

5.5.1 Volatile Compounds

Diffusion cells can be used to study chemicals that are volatile at room temperature, or likely to be lost from the skin via evaporation during their normal use. However, care must be taken in the design of such studies to assess the likely proportion of an applied dose that is lost to the atmosphere, and this often requires the use of radiolabelled compounds due to the complexity of extraction and quantification of the compound in the matrices used to trap the chemical. Indeed, it is a regulatory requirement for many classes of chemicals under OECD test guidelines to fully define the distribution of the volatile compounds above the skin, within the skin, and in the receptor fluid [25]. This is now routinely undertaken in regulatory dermal absorption studies and devices have been developed for *in vitro* diffusion cells (Fig. 5.1). This is a key element of the mass balance and in situations where this has not been quantified by trapping the test substance in the void above the skin surface, the study is compromised and the 'missing' fraction of the dose would therefore be assumed to have been absorbed, under a precautionary principle. The usual procedure for studies with volatile test substances is to place a charcoal filter above the donor chamber (*in vitro*) or skin device (*in vivo*), and to extract the compound from the matrix at the end of the exposure period. The use of such filters allows the skin surface to remain essentially unoccluded because an occluding trapping device would not allow the evaporation of the vehicle thus, ultimately enhancing skin penetration. An adaptation of the standard static diffusion cell to incorporate charcoal filters is shown in the Fig. 5.1.

5.5.2 Solids and Powders

Glass diffusion cells are very useful for studying the dermal absorption of actives from solid applications. For example, studies in humans using solids or powders containing an active ingredient are challenging and are actually best undertaken *in vitro* using chamber devices. A distinct advantage here is that the application stays where it was on a measured area of the skin. In humans, the application is prone to move or even come away from the site of application. In animals, the skin site also needs to be protected from grooming by the animal and also oral ingestion. Therefore, this is one area where the diffusion chamber could be perceived as a better model compared with the *in vivo* situation. Indeed, the large static type cell allows good spreading of material, which can be applied to a fixed area of skin.

In the pharmaceutical sector, there are few drugs that are applied in solid form to the skin as spreading and skin contact are important aspects for delivering the intended dose. Hence, gels, ointments, lotions and creams are the conventional types of platforms used in dermatological products. There are some obscure dusting treatments for foot infections, but use of powders poses other safety issues such

as inhalation exposure and also the fact that effective dermal penetration normally relies on other adjuvants present in the product. The fact that the dermal absorption of test substances (or formulated products containing the test substance) that are in a solid form at the temperature of the skin is poor and normally requires a vehicle or carrier to allow it to penetrate into the *stratum corneum*; and has been exploited by manufacturers of pesticides and other potentially toxic chemicals as dermal exposure is generally lower for the same compounds in solid form.

One area that does impact on the pharmaceutical industry when it comes to solids is during the manufacture of the drug active and any intermediates in the synthetic process that are solid at room temperature. Another scenario is the dermal exposure during veterinary use of powders containing an active drug. Studies on the dermal absorption for solid or powder materials using *in vitro* chamber systems can therefore be useful in measuring absorption and setting exposure limits and indicating the correct protective equipment to be used. Dry solid large particles or granules are generally very poor platforms for dermal absorption. Fine particles and dusts (depending on the actual particle size) will absorb any surrounding moisture on the skin surface and will therefore have more direct surface contact and a greater ability to penetrate into the skin. When *in vitro* or *in vivo* dermal absorption studies are designed to assess the risk from contact with solids, it is important to examine the actual real life exposure scenario. This includes a number of aspects such as the particle size and the potential exposure period. For example, if the material at the top of a container is a lumpy granule but is a fine dust at the bottom of the same container due to settling, it is prudent to test the finer dust material as it is likely to have a greater opportunity to release and deliver the active onto and into the skin. The OECD test guidelines suggest that moistening of the solid should be undertaken for *in vitro* and *in vivo* dermal absorption studies [25]. In order to better simulate typical exposure, it would be more useful and relevant to compare the neat material with a simulated sweat type of application. This would involve preparing a ground down version of the solid in this sweat medium that is then applied to the skin as a paste. This would provide a conservative assessment of dermal absorption relative to the neat solid, and represent a worst case scenario that may occur during handling of the chemical or product.

5.6 Bioequivalence Testing

An important area that utilizes the *in vitro* chamber approach is the area of dermatological product development. The chamber method provides a rapid and consistent method of selection and optimization of the release, and ultimately the dermal absorption and skin distribution of drugs. With so many generic products containing the same active (but often different adjuvants and other ingredients) the diffusion cell method provides data to confirm equivalence, or otherwise, for newly introduced products. There are several approaches here, depending on the specific question being addressed. In some cases, it is the release of a drug from its formulation

that is important. Here, there is a guidance for the type of artificial membranes and the protocols that are acceptable in diffusion chamber experiments [36]. Standard static diffusion cells can normally be used with these artificial membranes. In other situations, where the question relates to predicting systemic exposure of the drug, human skin is used in conventional chamber systems to study the flux or proportion of a drug that is absorbed over a specific time course. An example of this in the literature is the bioequivalence of the drug aciclovir in a study using a wide range of dermatological preparations that were compared using the static diffusion cell chamber system and human dermatomed skin [38]. This is a key area in pharmaceutics that is not only ethically acceptable, as it minimizes the number of *in vivo* investigations, but it also allows a systematic assessment of the impact of even minor formulation components in addition to the effect of changing the drug loading concentration. The *in vitro* chamber method can also provide important information on the time course kinetics for release into and through the skin, and also the stability of the drug under simulated physiological conditions.

During the early stages of drug development of a new dermatological product, various adaptations of the *in vitro* chamber model have been utilized. For example, pig skin is used as a surrogate for human skin, and is recognized as the closest animal species to human skin for dermal absorption investigation. The key element is the barrier properties of pig skin either used as heat-separated epidermal membranes prepared from pig ears, or as flank skin prepared with a dermatome. The functional properties of the barrier, as assessed by chemical permeation, electrical resistance or transepidermal water loss, are all quite similar between pig and human [4], making pig skin a very useful model to compare the dermal absorption of drugs from different formulations. Another advantage of pig skin over, say rodent skin, is the density and depth of hair follicles. Porcine skin is very similar to human skin and this therefore allows the *stratum corneum* to be tape-stripped using an *in vitro* procedure with resected human skin that was developed and validated with human volunteers in a side-by-side investigation [37]. Such a procedure allows the profiling of a drug through individual strips of *the stratum corneum*, thus providing key information on the penetration into the skin barrier. This technique, another adaptation of the glass chamber methodology, is now widely used to study drug delivery and is particularly powerful when the tape stripping investigation is substantiated by visualization of the drug in the different layers of the *stratum corneum* using techniques such as time-of-flight mass spectrometry [22].

References

1. Bronaugh RL, Stewart RF. Methods for in vitro percutaneous absorption studies IV: the flow-through diffusion cell. J Pharm Sci. 1985;74:64–7.
2. Burch CE, Winsor T. Diffusion of water through dead plantar, palmar and dorsal human skin and through toe nails. Arch Dermatol Syphilol. 1942;53:39–41.
3. Clowes HM, Scott RC, Heylings JR. Skin absorption: flow-through or static diffusion cells. Toxicol In Vitro. 1994;8:827–30.

4. Davies DJ, Ward RJ, Heylings JR. Multi-species assessment of electrical resistance as a skin integrity marker for in vitro percutaneous absorption studies. Toxicol In Vitro. 2004;18:351–8.
5. Dick IP, Scott RC. Pig ear skin as an in vitro model for human skin permeability. J Pharm Pharmacol. 1992;44:640–5.
6. Dugard PH. Absorption through skin: theory, in vitro techniques and their applications. Food Chem Toxicol. 1986;24:749–53.
7. E.C: Guidance document on dermal absorption. Eur Comm, SANCO/222/2000 rev. 2004;7:1–15.
8. ECETOC: Percutaneous absorption. European centre for ecotoxicology and toxicology of chemicals monograph no. 20, 1993; Brussels.
9. EFSA: European food safety authority. Guidance on dermal absorption. EFSA J. 2012;10(4):2665.
10. Feldmann RJ, Maibach HI. Absorption of some organic compounds through the skin in man. J Invest Dermatol. 1970;54:399–404.
11. Feldmann RJ, Maibach HI. Percutaneous penetration of some pesticides and herbicides in man. Toxicol Appl Pharmacol. 1974;28:399–404.
12. Flynn GL, Yalkowsky SH. Correlation and prediction of mass transport across membranes. I. Influence of alkyl chain length on flux-determining properties for barrier and diffusant. J Pharm Sci. 1972;61:838–52.
13. Franz TJ. Percutaneous absorption. On the relevance of in vitro data. J Invest Dermatol. 1975;64:190–5.
14. Franz TJ. The finite dose technique as a valid in vitro model for the study of percutaneous absorption in man. Curr Problems Dermatol. 1978;7:58–68.
15. Heylings JR. Risk assessment. In Benson HAE, Watkinson AC, editors. Transdermal and topical drug delivery: principles and practice. 1st. ed. Hoboken: Wiley; 2012. pp. 183–99.
16. Heylings JR, Garner A. Regulation of HCO_3^- transport by luminal acid in the frog in vitro. Am J Physiol. 1984;246:G235–42.
17. Heylings JR, Esdaile DJ. Percutaneous absorption of pesticides. In: Roberts MS, Walters KA, editors. Dermal absorption and toxicity assessment. 2nd. ed. New York: Informa Healthcare; 2007.pp. 575–91.
18. Heylings JR, Clowes HM, Hughes L. Comparison of tissue sources for the skin integrity function test (SIFT). Toxicol In Vitro. 2001;15:597–600.
19. Heylings JR, Diot S, Esdaile DJ, Fasano WJ, Manning LA, Owen HM. A prevalidation study on the in vitro skin irritation function test (SIFT) for prediction of acute skin irritation in vivo: results and evaluation of ECVAM phase III. Toxicol In Vitro 2003;17:123–38.
20. Howes D, Guy R, Hadgraft J, Heylings JR, Hoeck U, Kemper F, Maibach H, Marty J-P, Merk H, Parra J, Rekkas D, Rondelli I, Schaefer H, Tauber U, Verbiese N. Methods for assessing percutaneous absorption, report and recommendations of ECVAM workshop report 13. Altern Lab Animals. 1996;24:81–106.
21. IPCS: Dermal Absorption. World Health Organization, International Programme on Chemical Safety. Environmental Health Criteria No. 235. 2006.
22. Judd AM, Scurr DJ, Heylings JR, Wan K-W, Moss GP. Distribution and visualization of chlorhexidine within the skin using ToF-SIMS: a potential platform for the design of more efficacious skin antiseptic formulations. Pharm Res. 2013;30:1896–905.
23. Malkinson FD. Studies on the percutaneous absorption of 14C labelled steroids by use of the gas flow cell. J Invest Dermatol. 1958;31:19–28.
24. OECD: Guideline 427 for the testing of chemicals. Skin absorption: in vivo method. Paris: Organization for economic cooperation and development; 13 April 2004.
25. OECD: Guideline 428 for the testing of chemicals. Skin absorption: in vitro method. Paris: Organization for economic cooperation and development; 13 April 2004.
26. OECD: Guidance document number 28. The conduct of skin absorption studies. Paris: Organization for economic cooperation and development; 5 March 2005.

27. OECD: Guidance notes on dermal absorption. Paris: Organization for Economic Cooperation and Development; 18 August 2011.
28. Ramsey JD, Woollen BH, Auton TR, Batten PL, Leeser JE. Pharmacokinetics of fluazifop-butyl in human volunteers II: dermal dosing. Hum Exp Toxicol. 1992;11:247–54.
29. Ramsey JD, Woollen BH, Auton T R, Scott, RC. The predictive accuracy of in vitro measurements for the dermal absorption of a lipophilic penetrant (Fluazifop-Butyl) through rat and human skin. Fundam Appl Toxicol. 1994;23:230–6.
30. SCCS: Scientific committee on consumer safety. Basic criteria for the in vitro assessment of dermal absorption of cosmetic ingredients. SCCS/1358/10. 2010.
31. Scheuplein RJ. Mechanisms of percutaneous absorption. I. routes of penetration and the influence of solubility. J Invest Dermatol. 1965;45:334–46.
32. Scott RC, Dugard PH. The properties of skin as a diffusion barrier and route for absorption. In: Greaves MW, Schuster S, editors. Handbook of experimental pharmacology, Vol 87/II. Pharmacology of the skin II. Berlin: Springer; 1989.
33. Scott RC, Clowes HM. In vitro percutaneous absorption experiments: a guide to the technique for use in toxicology assessments. Toxicol Methods. 1992;2:113–23.
34. Scott RC, Batten PL, Clowes HM, Jones BK, Ramsey JD. Further validation of an in vitro method to reduce the need for in vivo studies for measuring the absorption of chemicals through rat skin. Fundam Appl Toxicol. 1992;19:484–92.
35. Scott RC, Carmichael NG, Huckle KR, Needham D, Savage T. Methods for measuring dermal penetration of pesticides. Food Chem Toxicol. 1993;31:523–9.
36. SUPAC-SS: Guidance for Industry, Non-Sterile Semi-solid Dosage Forms, Scale-Up and Post-approval Changes: chemistry, Manufacturing and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation, USDHHS, FDA, CDER, May 1997. In Vitro Release Test; 1997. pp. 19–24.
37. Trebilcock KL, Heylings JR, Wilks MF. In vitro tape stripping as a model for in vivo skin stripping. Toxicol In Vitro. 1994;8:665–7.
38. Trottet L, Owen H, Holme P, Heylings JR, Collin IP, Breen AP, Siyad MN, Nandra RS, Davis AF. Are all acyclovir cream formulations bioequivalent? Int J Pharm. 2005;304:63–71.
39. U.S. EPA: Health Effects Test Guidelines. U.S. Environmental Protection Agency, 1998, OPPTS 870.7600. Dermal Penetration 1–12. 1998.
40. U.S. EPA: Proposed test rule for in vitro dermal absorption rate testing of certain chemicals of interest to occupational safety and health administration; proposed rule. Fed Regis. 1999;64(110):31073–90.
41. Ussing HH. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. Acta Physiol Scand. 1951;23:110–27.

Chapter 6
In Vitro Product Quality Tests and Product Performance Tests for Topical and Transdermal Drug Products

Avraham Yacobi, Clarence T. Ueda and Vinod P. Shah

6.1 Introduction

Following regulatory approval of a drug product by the regulatory authority, e.g., US Food and Drug Administration (FDA), the product performance test becomes the sole means of directly monitoring the ongoing performance of the dosage form. The United States Pharmacopeia (USP) formed an advisory panel charged with developing appropriate product quality tests and product performance tests for topical dosage forms [1]. Deliberations of the panel included discussions about regulatory and compendia responsibilities in setting specifications for the performance of topical and transdermal drug products. The discussions included scientific principles underlying the product quality and product performance test methods.

Topically applied drug products fall into two general categories: (i) those applied to the skin to achieve local action or effects, e.g., creams, gels, ointments, solutions, suspensions, lotions, foams, and (ii) those applied to the skin to achieve systemic effects after absorption through the skin, e.g., transdermal drug delivery systems (TDSs, also known as patches).

Two categories of tests are performed with drug products: (i) product quality tests and (ii) product performance tests. Product quality tests are intended to assess attributes of the dosage form such as identification, assay (strength), impurities,

A. Yacobi (✉)
DOLE Pharma, LLC, 13 Oak Trail Rd., Englewood, NJ, 07631, USA
e-mail: avraham.yacobi@gmail.com

C. T. Ueda
Department of Pharmaceutical Sciences, University of Nebraska Medical Center College of Pharmacy, 986025 Nebraska Medical Center, Omaha, NE 68198-6025, USA
e-mail: cueda@unmc.edu

V. P. Shah
11309 Dunleith Place, North Potomac, MD 20878, USA
e-mail: Dr.vpshah@comcast.net

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_6

physicochemical properties, uniformity of dosage units, pH, apparent viscosity, microbial limits, antimicrobial preservative content, antioxidant content, sterility, if applicable, and other tests that may be product-specific that are part of a compendial monograph. Product performance tests are designed to assess the performance of the dosage form that in many cases is related to drug release from the finished drug product.

6.2 Tests for Topical Dermatological Drug Products

6.2.1 Product Quality Tests

Product quality tests for topical dermatological drug product are divided into:

- Universal tests that include description, identification, assay, impurities
- Specific tests that include uniformity of dosage units, water content, microbial limits, sterility, pH, and particle size
- Particle size of active pharmaceutical ingredient (API) for semisolid drug products and suspensions
- Viscosity for semisolid dosage forms

6.2.1.1 Particle Size

The particle size of the active drug substance(s) in topically applied drug products is usually determined and controlled at the formulation development stage. However, topically applied drug products should be examined for evidence of particle size, shape, and form alteration, including aggregation, of the active drug substance that may occur during the course of product processing and storage. Such examinations should be conducted at the time of batch release and at designated stability test time points for batch-to-batch monitoring because changes that are visually (macro- and microscopically) observable would likely compromise the integrity and/or the performance of the drug product. These types of testing are generally formulation-dependent. Therefore, such tests are not included in compendial monographs but are part of the manufacturer's specification(s) for the drug product.

6.2.1.2 Viscosity

Viscosity, a critical quality attribute that influences the physical appearance, stability, and performance of topical products, is a measure of a formulation's resistance to flow and is an assessment of the rheological properties of the dosage form (e.g., semisolids). Because only Newtonian fluids possess a measurable viscosity, which is independent of shear rate, semisolid pharmaceutical dosage forms that are non-Newtonian products exhibit an apparent viscosity. The apparent viscosity

of semisolid drug products should be tested at the time of batch release and initially at designated stability test time points to set specifications for batch-to-batch and shelf-life monitoring. For semisolids that show thixotropic properties and/or irreversible changes in viscosity after shearing, specific attention should be given to sample preparation procedures to minimize variability in the measurement of apparent viscosity caused by variable shear histories (e.g., mixing speed and temperature, filling operation, and sample handling). Furthermore, for all products, it is important to have apparent viscosity specifications at more than one set of conditions (e.g., bulk in-process stage, final packaged product, high and low shear rates, and different temperatures).

Apparent viscosity specifications based on data obtained during product development and shelf life testing should be established for batch release and throughout their proposed shelf life. The apparent viscosity test is formulation and/or process-dependent and is part of the manufacturer's specification for the drug product. Furthermore, the specifications for apparent viscosity of semisolid dosage forms at batch release and during stability testing may be different. The apparent viscosity of the finished drug product at the time of batch release must conform to the product development specifications. For stability testing, the apparent viscosity specifications for the drug product should be based on in-process-derived data and statistical assessment of the product over its shelf life.

6.2.1.3 Container Uniformity

Topically applied semisolid drug products may show physical separation during manufacturing processes and during their shelf life. To ensure the integrity of the drug product, it is essential to evaluate the uniformity of the finished product at the time of batch release and throughout its assigned shelf life. Often, semisolid dosage forms undergo phase separation, change in physical appearance and texture. Based on the packaging container, content uniformity test can be carried out using an appropriate procedure. For details on process and procedures, and ways to estimate container uniformity, see USP General Chapter 3 [2].

Briefly, to determine product uniformity when packaged in a tube, the container is cut horizontally just above the crimp edge and below the shoulder/neck of the tube using a utility knife, and vertically along the middle of the tube body as shown in Fig. 6.1a. The content of the tube is readily exposed by pulling aside the walls. Initial inspection of the tube content will show the integrity or lack of integrity of the formulation. For creams, in case of separation, distinct oily and aqueous phases are observed as shown in Fig. 6.1b and c. In addition, the product may not be smooth and often lumps are observed indicating lack of integrity of the original product. To quantitate drug in various parts of the tube, the tube contents should be divided into individual sections representing the top, middle, and bottom (T, M, B) zones as shown in Fig. 6.1. Samples from each zone should be weighted and analyzed as specified in the test method. Depending on the sensitivity of the assay method, sample sizes may be 0.1–2.0 g. Quantitative analysis of the API will provide a comparative measure of the uniformity and stability of the product.

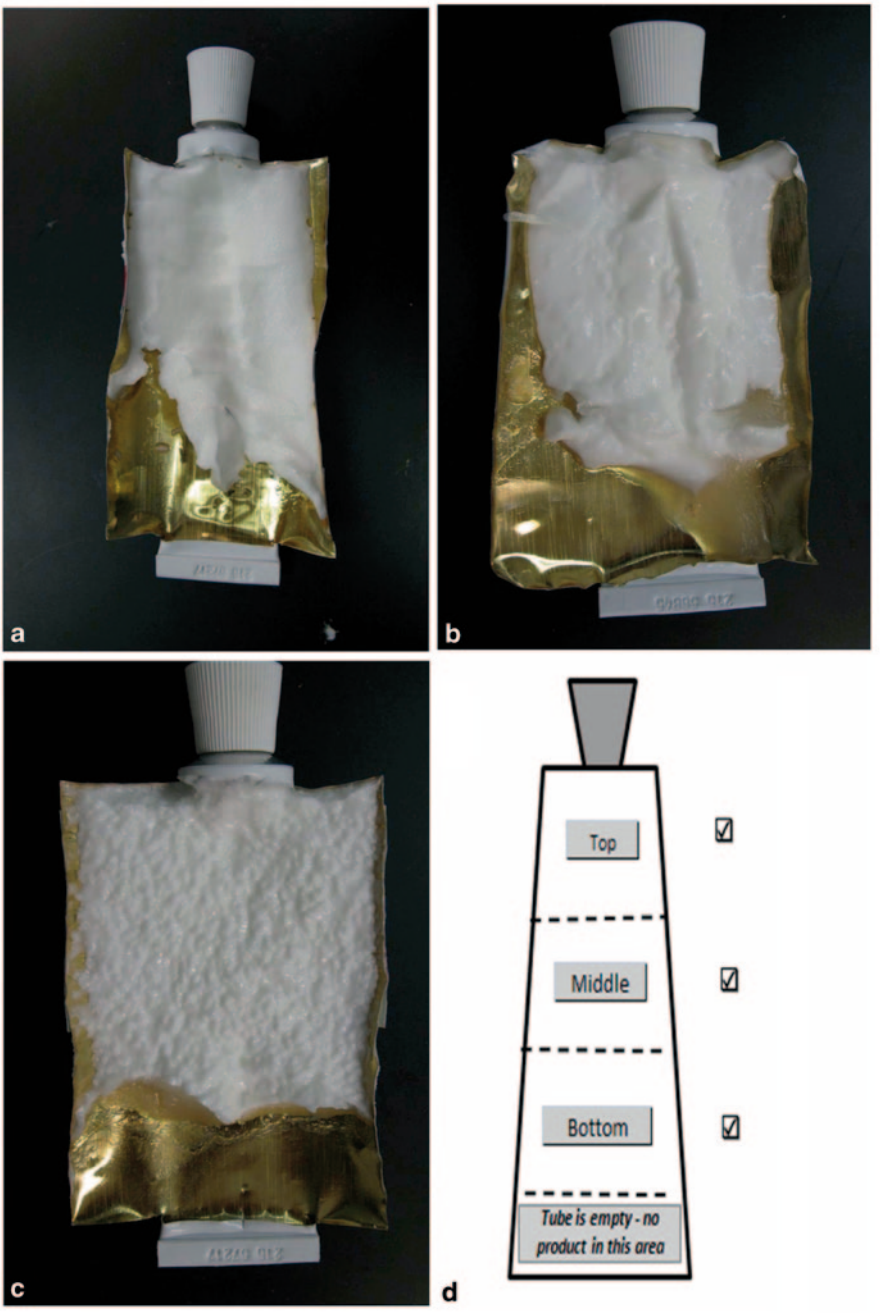


Fig. 6.1 Product uniformity sampling for a tube container. The area of the tube cut away (**a**) that may show oily (**b**) and aqueous (**c**) phase separation. Tubes should be sampled in zones as shown in panel D

For the determination of uniformity of semisolid products packaged in a container other than a tube, such as a jar, other sampling methods may be used. For example, after selecting a disposable plastic syringe of suitable length, remove the plunger and cut off the bottom of the syringe barrel (Fig. 6.2). With the plunger removed, slowly and carefully insert the syringe barrel vertically into the container until it reaches the bottom. Then, gently twist the syringe barrel containing the sample core, and carefully remove the syringe barrel. After removing the core sample, insert the plunger into the syringe barrel and carefully extrude samples representing the top, middle, and bottom zones of the container. Samples from each zone should be weighted and analyzed as specified in the test method. Depending on the sensitivity of the assay method, sample sizes may be 0.1–2.0 g.

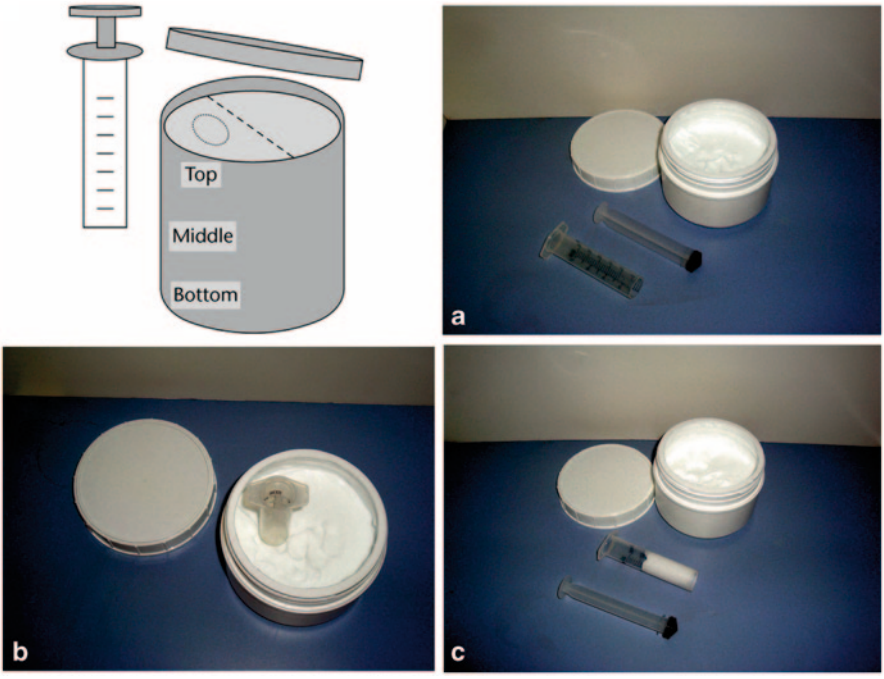


Fig. 6.2 Product uniformity sampling for a jar container. Diagram shows sampling from different depths and panels illustrate the use of a syringe barrel (**a**), inserted to the bottom of the container (**b**), to obtain a core sample (**c**)

6.2.2 Product Performance Test

USP General Chapter 1724 provides procedures for determining drug release from semisolid dosage forms. Semisolid dosage forms include creams, ointments, gels, and lotions. Semisolid dosage forms may be considered extended-release preparations, and their drug release depends largely on the formulation and manufacturing

process. Therefore, the drug release rate from products prepared by different manufacturers is likely to be different due to formulation and manufacturing process differences. This is similar to specifications for dissolution of extended release dosage forms and subsequent drug release from the formulation.

Product performance tests for semisolid drug products are conducted to assess drug release from the dosage forms. *In vitro* performance tests for semisolid products may not, however, directly predict the *in vivo* performance of drugs, as the primary factors that impact bioavailability and clinical performance are the barrier properties of the epithelia to which the product is applied (epidermal or mucosal tissues). Although product performance tests do not directly measure bioavailability and relative bioavailability (bioequivalence), they can detect product changes that may correspond to altered *in vivo* performance of the dosage form. These changes may arise from changes in physicochemical characteristics of the drug substance and/or excipients, or to the formulation itself, changes in the manufacturing process, shipping and storage effects, aging effects, and other formulation and/or process factors.

The vertical diffusion cell (VDC) is a reliable and reproducible means of measuring drug release from semisolid dosage forms. The VDC has a donor and a receptor chamber separated by a synthetic inert, porous support membrane which is in contact with the receptor medium in the reservoir. The support membrane is intended to keep the drug product sample and receptor medium separate and distinct. Typically, about 200–400 mg of the semisolid product is placed on the membrane. The release rate experiment is carried out at 32 ± 1 °C, except in the case of vaginal drug products for which the temperature should be 37 ± 1 °C. Usually a set of six cell assemblies are operated together at one time (i.e., single run). Sampling, generally, is performed over a 4–6 h time period, and the volume withdrawn is replaced with stock receptor medium. To achieve sink condition, the receptor medium must have a high capacity to dissolve the drug, and the drug concentration in the receptor medium at the end of the test ideally should be as low as possible. For each cell, the amount of drug released ($\mu g/cm^2$ at each sampling time (t_1, t_2) is determined, and the cumulative amount released plotted versus $\sqrt{t}$. The slope of the resulting line is a measure of the rate of drug release. The test is often conducted with a group of 6 or 12 cells per test run. The average of six slopes for each test and reference product is a measure of the drug release rate from the dosage form. The VDC system is the most common *in vitro* drug release system. In addition to the VDC system, an immersion cell and modified flow-through cell system can also be used to assess drug release from semisolid dosage forms.

6.3 Application of *In Vitro* Drug Release

The product performance test is used to assess product sameness after scale-up and postapproval changes. Because common testing artifacts, such as air bubbles and membrane defects, yield measurements that are not normally distributed, a

nonparametric statistical technique is used to evaluate the test results. The Mann–Whitney U test is used to calculate the 90% confidence interval for the ratio of the slopes between the test and the reference batches.

Another important application of *in vitro* release testing is the biowaiver for generic drug products. FDA/Office of generic drugs has released a guidance for acyclovir ointment 5% that allows biowaiver of a generic product [3]. Similarly, an *in vitro* release test is available for bioequivalence testing of cyclosporine ophthalmic emulsion [4]. These developments may result in the use of *in vitro* release tests as a routine product performance test for semisolid dosage forms as the dissolution test is for oral dosage forms, and may also be used for biowaiver under certain conditions.

6.4 Tests for TDSs

6.4.1 Product Quality Tests

Product quality tests for transdermal drug delivery systems (TDSs) are divided into:

- Universal tests that include description, identification, assay, and impurities
- Specific tests that include uniformity of dosage units, water content, microbial limits, sterility, pH, and particle size
- Adhesion tests
- Leak test

6.4.1.1 Adhesion Tests

TDS or patches are formulated with an adhesive layer to ensure intimate contact with the skin to allow the delivery of the desired dose of drug. Adhesives in TDS must permit easy removal of the release liner before use, adhere properly to human skin upon application, maintain adhesion to the skin throughout the prescribed period of use, and permit easy removal of the TDS at the conclusion of use without leaving a residue or causing damage to the skin or other undesirable effect(s). Additionally, adhesives must be able to maintain the performance of the TDS throughout its shelf life. Three types of TDS adhesion tests are generally used: peel adhesion test (from a standard substrate), release liner peel test, and tack test. Acceptance criteria are product specific and defined to assure that adhesion of each batch of TDS is within the range defined by the product design and is consistent between batches based on the product development specifications or statistical assessment of multiple product batches over the product's shelf life.

6.4.1.2 Peel Adhesion Test

This test measures the force required to remove (peel away) a TDS attached to a standard substrate surface (e.g., polished stainless steel). The TDS is applied to the substrate using specified techniques for application and is conditioned at a specified temperature and time. Then, the TDS is peeled away from the substrate with an instrument that allows control of peel angle (e.g., 90 or 180°) and peel rate (e.g., 300 mm/min), and the peel force is recorded. This procedure is repeated using a minimum of five independent samples. The product fails the test if the mean peel force is outside the acceptable range determined during product development and/or based on statistical assessment of multiple product batches over the product's shelf life.

6.4.1.3 Release Liner Peel Test

This test measures the force required to separate the release liner from the adhesive layer of the TDS. The test is performed with a finished product sample. The test sample is conditioned using specific procedures (temperature and time). Then, the release liner is pulled away from the TDS with an instrument that allows for control of peel angle (e.g., 90 or 180°) and peel rate, and the peel force is recorded. This procedure is repeated using a minimum of five independent samples. The product fails the test if the mean peel force is outside the acceptable range determined during product development and/or based on statistical assessment of multiple product batches over the product's shelf life.

6.4.1.4 Tack Test

Several tack test methods have been developed. Examples include the probe tack method and the rolling ball method. It is the responsibility of the TDS manufacturer to determine which method is appropriate for their drug product (For details, see USP General Chapter 3).

6.4.1.5 Leak Test

This test is applicable only to form-fill-seal (reservoir or pouched)-type TDS. Form-fill-seal TDS must be manufactured with zero tolerance for leaks because of their potential for dose dumping if leaking occurs (e.g., Fentanyl patch).

6.4.2 Product Performance Test

USP General Chapter 724 provides three procedures using Apparatuses 5, 6, and 7 for testing drug release from transdermal systems. Apparatus 5 is the simplest

system applicable to all types, sizes, and strengths of marketed TDS. Apparatuses 6 and 7 are product specific.

References

1. Pharmacopeial Forum: 32(5) Sept–Oct 2006, 1586–9.
2. USP General Chapters <3>; <1724> and <724.>
3. FDA draft guidance on acyclovir ointment 0.05 %, March 2012.
4. FDA draft guidance on cyclosporine emulsions/ophthalmic, June 2013.

Chapter 7
Safety and Efficacy Testing of Topical Products; Practical Considerations

Rashmikant Mohile

7.1 Introduction

Skin has always been looked upon as our external natural gear which protects the body from environmental factors like sun, pollution, extreme temperatures, etc. Like our attire, the skin too is supposed to describe one's looks and make a firm statement about one's personality. This external organ of our body which we see more often that the hidden complexities of other vital organs has, thus, been nearer and dearer to all of us. No wonder then that the safety of skin has been of paramount importance to all.

In spite of this, the scientific community started considering systematic safety evaluation of skin products relatively late in the day. It was probably in the year 1966 that Prof. Kligman first proposed the standardized method for detecting the contact allergens in a scientific way [1]. Our knowledge of skin safety testing has evolved several folds since then with the use of standardized protocols, techniques, and availability of newer noninvasive biomedical instruments. However, when it comes to skin safety testing, there is no single method that will be apt for a particular study. As Prof. Maibach puts it after his years of deep experience in the field, "It all depends" on a variety of factors.

The practical aspect related to safety and efficacy will always hold the key in defining the successful evaluation of any topical product. In this chapter, I have tried to capture few such points related to the evaluation of cosmetic products and topical drug formulations. Not all of them are technical.

R. Mohile (✉)
C.L.A.I.M.S Private Limited, Dalia Industrial Estate, Off link Road,
Andheri-W, Mumbai 400-058, India
e-mail: rbmohile@claimscro.com

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_7

7.2 Consumer Habits and Its Importance in Designing Safety and Efficacy Studies of Cosmetic Products

The importance of "Consumer habits" cannot be overlooked in designing safety and efficacy studies of cosmetic products. In fact, the variations in patch testing methodologies such as occluded, open, repeat application open, or occluded are all acceptable precisely for the same reason. While selecting any of these approaches, the product knowledge and its intended use by the consumer must be given paramount importance. An overdesigned unrealistic safety protocol may result in rejecting a product/formulation whereas a lenient unthoughtful theoretical approach may put the consumer at undue risk. The role of the principal investigator and his team, who monitor the trial, is to select an appropriate study design, may be slightly stringent and exaggerated than the actual use conditions, so as to provide an adequate safety margin to protect the end-consumer.

The same holds true while designing efficacy studies as well. For example, imagine a study protocol for the evaluation of an antidandruff shampoo among Asian consumers. The Asian consumers on an average shampoo their hair two to three times a week as against their counterparts in western countries who have a habit of shampooing their hair daily. The Asian consumer also applies hair oil before shampooing which has been a traditional habit in this part of the world. A study design wherein a daily hair wash with antidandruff shampoo and restricting the participants with the use of hair oil will result in larger dropouts due to noncompliance. Also, the outcome of this study, even if positive, will have little relevance in the real-life situation in that market.

It is precisely for this reason that the revalidation of safety and efficacy data is necessary when launching a cosmetic product in new markets. It can be done through short but well-designed safety and efficacy trials which are controlled and supervised by experts followed by in-use consumer trials. It will be irrational to assume that the product will do well in new markets since it has done well in the past in other markets.

7.3 Mindset Issues: Testing for Claims vis-à-vis Claiming "What Tested"

The central theme in testing a topical product for safety and efficacy has to be "the patient" who is finally going to use the product or "the end-consumer" in case of cosmetic formulations. The channel partners such as the medical fraternity, domain experts, and others come thereafter. Today, the whole purpose of testing the product has become that of compliance with the regulatory requirements, often country specific, and outsmarting competition to succeed in the market place. While these are crucial considering business imperatives, they by no means can be the main reasons for evaluating the products. This mindset has taken away the inquisitiveness which

is needed in product testing and makes product evaluation a mere ritual. While the ethical guidelines in conducting clinical evaluation do address participant's safety, it does not and cannot address passion needed by the sponsor, principal investigator and other associated members involved in the trial to make it more holistic and prevent it from being a mere ritual.

In the cosmetic industry, very often the need for product evaluation is triggered by marketing needs for product's preconceived claim. The claim thus drives cosmetic product safety and efficacy evaluation rather than the product's safety and efficacy evaluation driving claims. This is not to take away the credit for the efforts being put in by many cosmetic companies doing basic research and by specialty ingredient manufacturers to find new actives. The point being made here is that these efforts often get diluted so as to pass on the real benefit to the end-consumer.

Also, what we see today are the claims from cosmetic companies which are aimed at outsmarting competitor's claims rather than the claims addressing consumer needs. How many such claims help to build credibility of these products in consumer's mind is anybody's guess.

7.4 Efficacy Results and Interpretations—Instrumental, Clinical, and Consumer Perceptions

With advancements in the area of biomedical instrumentation, objective evaluation has become an integral part of efficacy evaluation of cosmetic products. It has found applications in topical drug product evaluation as well. For example, the vasoconstriction assay for bioequivalence of topical corticosteroids as recommended by the United States Food and Drug Administration (USFDA) guidance mentions use of tristimulus colorimeter such as chromameter for quantification of skin blanching effect [2]. The wide range of skin imaging and bioengineering instruments has provided additional tool for the quantification of various skin parameters.

The use of instrumentation does not undermine the clinical evaluation by experts such as dermatologist/cosmetologist. In fact, a comprehensive protocol for evaluation of efficacy of a topical product be it cosmetic or topical drug formulation, should include clinical evaluation, instrumentation, and self-evaluation by participants to capture the holistic product performance. Very often it has been observed that the instrumentation may capture improvement in certain attributes at an early stage during trial period, followed by clinical improvement as recorded by dermatologist, followed by improvement perceived by the participants. This is understandable. Thus, comprehensive evaluation may take longer time. The practical consideration often limits conducting product trial for such a long duration. An instrumental change observed after 1 month may take several months of product application before the change is seen by the user/participants. The product claims, more in the case of cosmetic products, are made based on earliest significant change as detected by instruments or experts. Thus, we often come across claims like "Within 2 weeks" or "longer lasting for 24 h," etc. In practice, it has been observed at our end that

unless the change as captured by instrumentation for critical parameters exceed 25% compared to initial stage, the effect of product performance will not be perceived at the participants/consumers. This is applicable to cosmetic formulations. This is one way of identifying the critical performance parameters which can be taken forward to make product claim and that will be appreciated by the end-user.

The mere use of instruments does not guaranty correctness of the outcome. As Prof. Albert M. Kligman said, "A Fool with a Tool is still a fool." This is so true. Unless one has taken care and pain to understand, standardize, and calibrate these instruments for regular use, the outcome with such instruments has limited utility. The same is the case while using clinical scales to capture clinical improvement in the skin condition. The periodic training for those who grade clinical improvement and aligning these evaluators in case there are more than one evaluator (often being the case with multicentric trails) becomes of paramount importance.

Finally, the self-evaluation questionnaire to be administered to the participants in the trial can reflect interesting practical concerns/benefits, provided this questionnaire is structured with great care to meet the end objective of the trial.

Obviously then, the collective wisdom of experts and participants supported by objective data from the correct use of instrumentation can give immense insights for practical success of the product in the market place.

7.5 Product Knowledge and Testing Methodology

Mindless testing to comply with internal stake holders in the organization or external stake holders (outside the organization) often creates confusing study requirements. How can someone design a study protocol to prove "Non-irritant" claim for an AHA-based product which is supposed to be a skin peel? How to evaluate primary skin irritation of a topical anti-itch or rubefacient product with patch testing methodology since that is an accepted testing protocol for primary skin irritation?

While many such requests seem ridiculous at the first go, they may provide a trigger for doing something new and innovative either with respect to study design, new techniques or building new skills. But then, a sound and holistic knowledge not only of clinical practices but that of product formulation, new ingredients, newer instrumentation, and emerging new claims is needed.

For example, in one study for a modified topical corticosteroid formulation, the sponsor wanted a "Proof-of-Concept" clinical study by adapting vasoconstriction or blanching study protocol. The existing formulation of the same steroid was modified using skin penetration enhancer. The rationale behind this study is that with penetration enhancer the blanching effect will be more pronounced which presumably relates to the amount of drug entering the skin and hence more bioavailability [3].

The preliminary studies, however, indicated lower blanching effect compared to the formulation without penetration enhancer. The possible explanations could

be systemic absorption of the drug due to enhanced penetration thru the skin. This effect, however, is not desirable.

The conceptualization of a product idea with possible effects and likely enhanced side effects therefore is of paramount importance in designing comprehensive safety and efficacy protocols.

7.6 How to Create a Value Proposition for the Sponsor and Consumer Through Safety and Efficacy Testing?

In a commercial setup, no organization would like to undertake any business activity unless it adds significant value either to its top line (read profitability) or bottom line (read cost structure improvement). Very often, while it is easy to identify value adding activities for the immediate future, it is often difficult to visualize value adding propositions for the strategic long-term period, say over 5–10 years. It is all the more difficult to identify such initiatives in the research and development function of an organization since the function itself by the nature has many "ifs" and "buts" to answer. In spite of this, many organizations have successfully accomplished their strategic objectives by coordinating and meshing activities of various functions within organizations responsible for a new product launch including clinical outcomes and product claims. This success often is the outcome of well planned and thought through set of activities connecting seamlessly across various functions.

The thought process for planning these activities invariably starts from markets to be catered to, users in these markets (end-consumers in case of cosmetic products and patients and dermatologists for dermaceuticals and topical pharmaceutical products). The correct insights into their needs is the most important but equally difficult task, which if done correctly, opens will open the path for successful product launch. This is followed by rating and ranking all the needs and asking the consumer/customers to score their level of satisfaction on a simple scale from 0 to 5 or any other suitable scale. This simple exercise helps in identifying those areas or gaps which can then be filled with the product under consideration. Having identified the gaps, the next step is to verbalize them with an exhaustive and comprehensive product brief which is the first step in the research or developmental activity of a new product. The critical activity thereafter for the R&D team is to convert this product brief into a technical brief wherein each and every customer need is effectively measured by one or more quantifiable technical parameters. This is not simple and needs thorough deliberation within the R&D team. Many of these technical parameters can be measured through systematic safety and efficacy studies. The early identification of safety and efficacy evaluation parameters helps in effective identification of product strengths and weaknesses. The entire product improvement, launch and communication strategy then can be designed around these findings. The process is the adaptation of the quality function deployment (QFD) process which is very successfully followed in various industries such as automobile and many others.

This process helps in connecting a consumer need in the case of cosmetic products and a dermatologist or skin expert's needs for pharmaceutical products to product performance and deliverables.

7.7 Ethical Issues in Study Design

The study design for safety and efficacy evaluation is often conceptualized by the sponsor or the company wanting to conduct these trials. Obviously, their objective is to derive as much information as possible with optimal sample size of participants and at a competitive cost. This is natural from their viewpoint but in this process very often the ethical issues in the conduct of the trial get overlooked. For example, a skin lightening trial for a cosmetic face cream often describes a protocol wherein volunteers/participants would apply the product under evaluation to half the face and the other half will be applied a placebo cream. If one looks at the study hypothesis, it states that the product under evaluation is expected to significantly lighten the skin (statistically significant difference at 95 % confidence interval) in a specific time period compared to placebo cream. The reason for doing half-face trial is to have each individual acting as his own control in the trial thereby eliminating an important variable, i.e., of skin type and individual life style and habits.

If this hypothesis was to come true, the trial would end up with number of participants having one side of their face looking significantly lighter than the other side. While the sponsor will be delighted with these results which prove that their product is efficacious, how is it likely to impact the participants? Is it therefore ethically correct to conduct such a trial?

Here is another example for safety evaluation of topical products. In this case, the regulatory body has recommended a protocol for patch testing wherein 3 % sodium lauryl sulphate (SLS) has been recommended as positive control [4]. The test protocol further suggests that only those volunteers showing combined erythema and edema score of more than 4 (Draize scale) should be included in the patch testing study. This may be because it is difficult to visually grade erythema in participants with darker skin (skin type 4 predominantly) thereby necessitating use of such high levels of SLS. The skin damage which 3 % SLS may evoke serious ethical issues more so when the same volunteers repeatedly participate in these safety studies over a period of time.

So, it is not only the sponsoring organization but sometimes even regulatory authorities who need to be sensitive to ethical issues while drafting guidelines in conduct of a trail.

The Ethics Committee/Institutional Review Board approving such trials has a very important role to play in protecting participant's well being which very often the sponsor and regulators themselves may not be in a position to visualize.

The point being made here is that along with the technical considerations one has to consider social and psychological factors in practice to safe guard the interest of all involved parties.

7.8 Summary and Conclusion

While at a macroscopic level, there are differences between Caucasian, Hispanic, Asian, and African skin, structurally, all skin types have similar qualitative structure. At quantitative levels, they differ from each other. For example, African and Asian skins have greater levels and different dispersion of melanosomes because of different photo-protection needs. Similarly, differences in skin thickness exist for different skin sites. Sex and age may also change biomechanical properties of the skin to a certain extent. These changes may be important for the evaluation of certain types of products while they may not significantly impact others.

The practical consideration and understanding therefore, is of paramount importance in conducting any safety or efficacy trial for topical products. Clear understanding of study objective, product–skin interaction, ethical consideration with respect to participant's safety and benefits, and intrinsic desire to offer value to the patients or end-users is the key to innovate newer products and therapies. This in turn will bring credibility and sustainability to the sponsoring companies to create profitable business propositions.

References

1. Albert M. Kligman. The identification of contact allergens by human assay- III The maximization test: A procedure for screening and rating contact sensitizers. J Invest Dermatol. 1966;47(No 5):393–409.
2. Guidance-Topical dermatological corticosteroids: In vivo bioequivalence—issue date: 2 June 1995. U.S. department of Health and Human Services, Food and drug Administration.
3. Perishing Lk, Silver BS, Krueger GG, Shah VP, Skelley Jp. Feasibility of measuring the bioavailability of topical betamethasone dipropionate in commercial formulations using drug content in skin and skin blanching bioassay. Pharma Res. 1992;9:45–51.
4. Indian Standard—Method of test for safety evaluation of cosmetics (second revision) IS 4011:1997 (reaffirmed 2004).

Part III
Bioequivalence of Topical Drug Products

Chapter 8
Challenges in Evaluating Bioequivalence of Topical Dermatological Drug Products

Vinod P. Shah and Roger L. Williams

8.1 Introduction

Topical dermatological drug products are semisolid preparations such as creams, ointments, and gels. They deliver drugs to the skin to prevent or treat disease and/or to alleviate symptoms. Such preparations include antibacterials, antifungals, antivirals, corticosteroids, and retinoids. The onset, duration, and magnitude of therapeutic response for any topical drug product depend on the relative efficiency of three sequential processes: (1) release of the drug substance from the product, (2) penetration/diffusion of the drug through the stratum corneum (SC), and (3) activation of the desired pharmacological effect at the site of action. Assuring the quality of a topical dermatologic product requires a complex set of studies that maintain continuing equivalence of the product relative to the clinical trial material on which the documentation of safety and efficacy was based. These include bioavailability (BA) and bioequivalence (BE) studies, and many other types of studies as well. Transdermal products are also applied topically but are designed to achieve system effect. They are not further considered in this chapter.

Commonly used methods for documenting the BE of oral dosage forms are generally not applicable to topical dermatological drug products. Currently, comparative clinical studies are used to establish bioequivalence for most topical formulations, with the exception of corticosteroids where an *in vivo* pharmacodynamic approach developed at FDA in the 1990s has become available [1]. Over the last quarter century several national and international workshops have been held to discuss the methodologies for assessing BE of topical dermatological drug products [2–6]. The intensity of these dialogues without clear resolution reflects the general challenge of documenting BE for topical dermatologic formulations. This chapter

V. P. Shah (✉)
11309 Dunleith Place, North Potomac, MD 20878, USA
e-mail: Dr.vpshah@comcast.net

R. L. Williams
United States Pharmacopeia, Rockville, MD, USA

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration*, DOI 10.1007/978-1-4939-1289-6_8

considers the pros and cons of the various methodologies either now used or under development.

8.2 Regulatory History

While a detailed review of US regulatory requirements is beyond the scope of this chapter, key milestones include the passage of the Food and Drug Act in 1906, replaced by the Federal Food, Drug and Cosmetic Act in 1938. The latter required safety data through a notification process in a New Drug Application (NDA). In 1962, the Act was extensively revised to include requirements for an Investigational New Drug (IND) application, manufacturing according to good manufacturing practices (GMPs), and—mostly importantly—a preapproval requirement for documentation of efficacy in addition to safety. Out of these revolutionary changes came additional approaches as well, including the possibility of an Abbreviated New Drug Application (ANDA) for pre-1962 medicines [7], the over-the-counter (OTC) monograph system [8], and the Drug Efficacy Study Implementation (DESI) review of drugs approved between 1938–1962 to establish efficacy. In 1984, The Drug Price Competition and Patent Term Restoration Act (Waxman–Hatch Act) extended the ANDA approach to all new drugs, including those approved after 1962.

With the Congressional decisions embodied in these legislative and regulatory milestones, a clear statutory path exits in the USA to allow approval of generic versions of an innovator product following patent or exclusivity expiration, with submission of data documenting bioequivalence between the generic and innovator formulations in an ANDA (Food, Drug and Cosmetic Act, Sections 505(u)(4)(b) and 505(j)(4)(d)). The general concept relies on establishing BA by a first-entry manufacturer for their product (termed the reference listed drug, RLD) in an NDA filing with a multisource manufacture documenting BE relative to the RLD in an ANDA. A generic product must also be pharmaceutically equivalent (PE) to the RLD [9]. With satisfactory documentation of both requirements, the approved generic product may be deemed therapeutically equivalent [9].

BE (relative BA) is defined as the "absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study" (as stated in 21 Code of Federal Regulations (21 CFR) 320.1(e)). BE is documented by performing different types of studies comparing test (T) and reference (R) products. These studies are categorized in order of FDA preference (21 CFR Section 320) as:

- Pharmacokinetic studies in which the active agent or its metabolites are measured in an accessible biological fluid
- Pharmacodynamic effect studies in which one or more measures of drug effect are compared between the generic and the pioneer product

- Comparative clinical trials
- *In vitro* studies

In addition, the inactive ingredients in a generic topical formulation should generally be qualitatively (Q1) the same and essentially quantitatively (Q2) similar (±5%) compared with the innovator product (as defined in 21 CFR 314.94 (a)(9)(v)). Both innovator and generic firms must manufacturer their products in accordance with current Good Manufacturing Practice (cGMP) (as outlined in the 21 CFR Sections 210 and 211]. In addition, all marketed products, innovator and generic, must meet specifications if provided in monographs in the United States Pharmacopoeia-National Formulary (USP-NF) [10], which are official compendia of the USA.

8.3 Regulatory Requirements for BE

BE is a performance comparison of two products, generally a T (generic, multisource) and an R product. BE focuses on release of the drug from the dosage form and subsequent entry into the systemic circulation (for oral products) or site of action (for topical products). They are not studies of the safety and efficacy of the product, which has already been established via the NDA products. In many instances, BE is considered self evident (e.g., solution formulations, gases). For many other formulations (e.g., nonsolution, orally administered dosage forms), rate and extent of absence can rely on pharmacokinetic studies in an accessible biologic fluid such as plasma, which becomes a surrogate marker that should be sufficiently similar to the exposure pattern of the RLD [9].

8.4 Topical Dermatologic Drug Products: BE Methods

Documentation of BE for topical dermatological drug products has posed an important challenge for manufacturers and regulators. Several methods have been developed for documenting BE of these types of formulations and are currently being used. A number of methods, such as the tape stripping technique for measuring drug concentrations in the SC termed dermatopharmacokinetics (DPK), imaging technique, confocal Raman spectroscopy, the blister fluid technique, microdialysis, are under investigation, while still others have been deemed inadequate (Fig. 8.1).

Because a topical dermatological product will generally be applied to diseased skin, formulation/excipient factors may play a much larger role in how the drug moves to the primary site of action within the epidermis or dermis than in the case of a solid oral dose form administered orally, for which drug-excipient interactions after absorption are generally thought not to occur. Despite these and other differences between drugs administered orally and topically, many of the same general methods employed to establish BE for both types of formulations. The following sections of the chapter provide a general review of acceptable methods and methods under investigation.

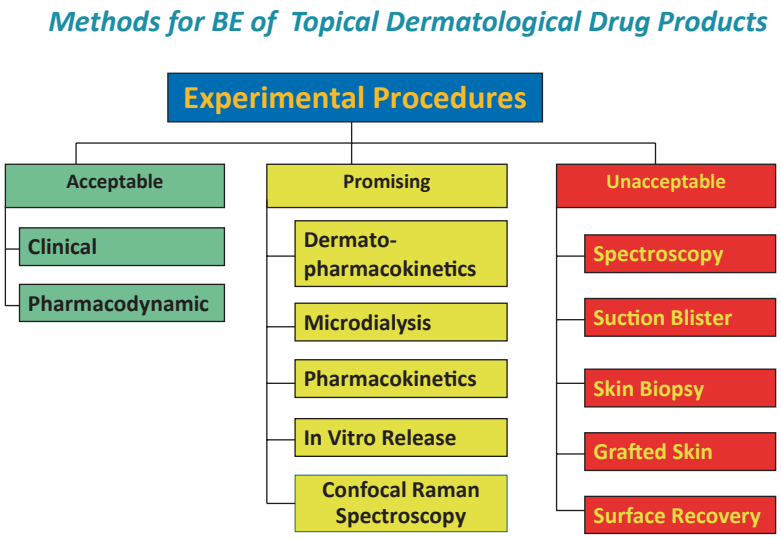


Fig. 8.1 Methods for BE of topical dermatological drug products

8.4.1 BE for Topical Dermtologic Products: Accepted Methods

8.4.1.1 Pharmacodynamic Measurements

When applied topically, corticosteroids produce skin blanching. This blanching response is caused by vasoconstriction, and has been correlated with clinical efficacy of the drug product [11, 12]. Blanching is used to measure bioequivalence of topical formulations in healthy volunteers, but not in patients. It is of interest to note that there have been some publications which suggest possible bioinequivalence between brand name and generic topical corticosteroids [13]. However, no clinical evidence has been provided. As a follow-up, the FDA launched an extensive research program [14, 15] to develop appropriate guidance for corticosteroids [1]. Guidance developed by the FDA suggests the use of a chromometer to quantify the blanching response and to allow measurement of the onset, duration and decline of response, analogous to drug blood/plasma concentrations, as a means of improving the approach in document BE of corticosteroid topical preparations. Some of the issues and limitations surrounding pharmacodynamic dose–response studies, maximum drug effect (Emax) modeling and data fitting, use of naive versus population data, and use of chromameter versus visual blanching reading, are discussed in the guidance [16]. On the basis of population modeling, dose response data from six different potency categories of corticosteroids were defined and described by an Emax model [1]. The dose producing 50% of the maximum drug effect (ED50) decreased with increasing corticosteroid potency. No other classes of topical drug product produce pharmacodynamic response that can be used as a measure of BE.

8.4.1.2 Comparative Clinical Trials

Comparative clinical trials are now required by the FDA for most post-1962 topical dermatological drug products except corticosteroids. These clinical trials require a large patient population, are generally expensive, lack sensitivity, and are resource intensive both for FDA and for industry. However, they are considered "gold standards." Furthermore, they tend to contravene the intent of the 1984 amendments to the FD&C Act, which is to avoid a requirement for repetition of efficacy studies to allow market access to generic manufacturers. For example, for the antiacne retinoic acid derivatives, 275–500 patients are recommended in a three treatment parallel noncrossover study. For antifungal drug products, 275–300 patients are recommended. Given the limitations of clinical trials to establish BE, new approaches are needed.

8.4.2 BE for Topical Dermatologic Products: Promising Approaches

8.4.2.1 Dermatopharmacokinetics

Dermatological drug products after topical drug application do not produce concentration that can be easily measured in an accessible biologic fluid. Given local action, the focus on systemic absorption may relate more to safety (i.e., absence of undesirable systemic response) than to local efficacy. For these reasons, plasma blood-level pharmacokinetic measurement for a topical drug product is used as a surrogate for possible toxicity.

Pharmacokinetics applied to drug concentration measurements in the SC is termed DPK. DPK measures drug levels in the SC as a function of time post application and post removal using tape stripping procedure. The tape stripping is thus analogous to an *in vivo* pharmacokinetic studies, using drug concentrations in the SC to determine drug uptake and elimination from the SC [5, 19]. Whereas a plasma pharmacokinetic study assesses BE after oral administration, DPK measures drug concentration in the vicinity of the site of action following application of a topic product. The approach begins with studies by Rougier et al. have shown a relationship between the amount of drug in the SC (referred to as SC reservoir function) present after a 30-min application from a solution and the amount of drug absorbed, as measured by urinary excretion in 96 h [17, 18]. In these experiments, a radioactive drug was used, and the SC was removed using the skin stripping technique.

DPK Methodology

Unlike blood concentration studies, where multiple samples can be obtained after oral drug administration, only one skin sample can be obtained from a site after topical drug application. To obtain the multiple samples required to define a SC concentration–time curve profile, a DPK study requires application of multiple sites

in each subject. Each site yields a single drug concentration in the SC. Generally, T and R products can be studied at the same time in the same subject [20]. To assess rate of absorption for a BE study, the drug product is applied at multiple sites (four sites in this example) and the SC samples are removed from each site just after removal of the applied drug at sequentially increasing times, for example, at 0.25, 0.5, 1, and 3 h. To assess drug elimination, the drug product is applied to multiple sites, and maintained for a period of time (3 h). At the close of this period, all application sites are cleaned after 3 h and SC samples are removed at 4, 6, 8, and 12 h. SC samples are generally removed by using a tape stripping technique using a clear flexible adhesive tape. Repetition of the procedure with adhesive tape at the same site effectively removes layers of the SC and the drug embedded in it. The tape is applied to the cleaned site of application, pressed uniformly, and removed. The first two tape strips are discarded because they contain drug that has not penetrated through the SC. Amount per surface area is more commonly used because of difficulties in quantification of SC weight. The results are plotted as amount/surface area against time.

The exposure measures in the SC, as determined by area under the concentration–time curve (AUC) to last measured time point and Cmax reflect the rate and extent of diffusion and penetration of the drug reaching site(s) of action. For a generic product to be BE to an RLD, both the AUC and the maximum drug concentration achieved (Cmax) ratios between T and R product should meet average bioequivalence criteria of 80–125 % with 90 % confidence interval [20]. DPK profiles for an example T and R product appear in Fig. 8.2.

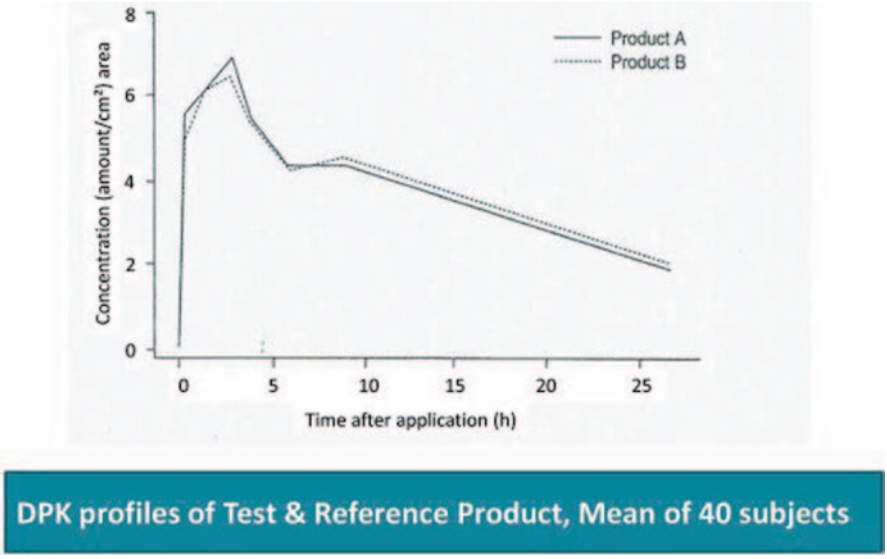


Fig. 8.2 DPK profile of test (T) and reference (R) product; mean of 40 subjects (Source: Presentation at FDA's Pharmaceutical Sciences Advisory Committee meeting)

Challenges to DPK

Preliminary studies indicate that the DPK is proportional to drug concentration at the site of action and is applicable for a variety of topical drug products. According to the primary analogy, two topical products having similar DPK exposure measures are likely to be bioequivalent. Yet, the approach and fundamental assumptions of the DPK approach has challenged on many grounds, as discussed in several public workshops over many years [4–6]. Criticisms center on: (1) measuring drug concentration in "dead" skin; (2) understanding of the relationship of drug concentrations in the SC to topical efficacy; (3) DPK's ability to differentiate between strengths and formulations, reliability; and (4) reproducibility of the DPK method.

Clinical Studies in Support of DPK

Acceptance of any new methodology for BE determination would require information about its relevance to clinical data. An ideal example would involve comparison of DPK results with clinical findings of a RLD; a bioequivalent generic product and a product bioinequivalent to the RLD. Two examples are considered, one for clobetasol and one for tretinoin.

Clobetasol Study A cream and emollient cream of clobetasol products are marketed in the US. Both contain the same amount of active drug (0.05 %). Clobetasol cream was approved first followed by the emollient cream. The two products are not therapeutically equivalent and have different labeling. The cream is considered more potent and should not be applied for more than 2 weeks. In contrast, the emollient cream is comparatively less potent and can be used for 4 weeks. These findings were substantiated by hypothalamic-pituitary-adrenal axis testing.

A DPK study carried out with the clobetasol innovator (reference) cream, clobetasol generic cream and clobetasol emollient cream clearly showed that drug concentrations in the SC after 6 h were not statistically significantly different between the RLD and generic clobetasol cream. They were significantly different between clobetasol cream and clobetasol emollient cream. The concentration of emollient cream in the treated skin was significantly lower (30 %) compared with clobetasol reference cream (Fig. 8.3). These findings suggest a reasonable association between the clinical findings and the DPK results, addressing at least part of the reservations that have been expressed about DPK findings [21].

Tretinoin Study There are three approved tretinoin 0.025 % gel products on the market: the innovator product, a generic product bioequivalent and PE to the innovator product, and a third product which is not equivalent to the innovator product but is approved because it is superior to placebo with fewer adverse effects. This is an unusual situation and provides a basis for assessing the DPK approach.

A DPK study comparing these three tretinoin products was carried out at University of Utah, supported by the FDA. The results of the study concur with earlier clinical findings. The clinically equivalent products showed equivalent DPK

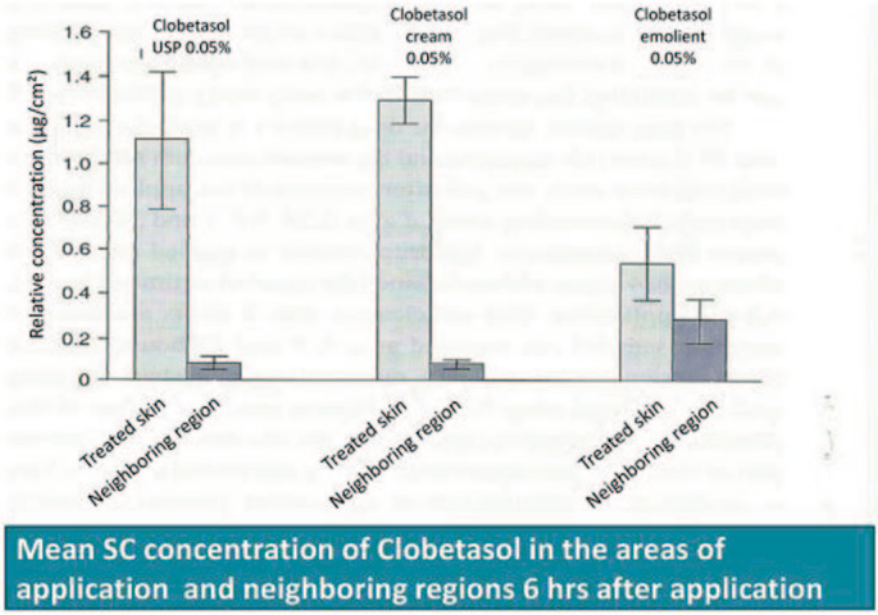


Fig. 8.3 Mean SC concentration of clobetasol in the areas of application and neighboring regions 6 h after application [21]

profile, and the clinically inequivalent product showed significantly lower DPK profile compared to the innovator product (Fig. 8.4). Again, this study supports the clinical observation that reduced toxicity (safety) is associated with lower tretinoin blood levels in support of the general DPK approach [22].

DPK Reproducibility

One of the major questions for using DPK methodology has been the reproducibility of the data/method. DPK data presented on tretinoin study comparing T and R product by two investigators at the FDA's Pharmaceutical Sciences Advisory Committee Meeting in 2001 indicated that the products were bio-inequivalent. Yet contradictory and inconsistent results in the data set [23] resulted in the FDA's withdrawal of a draft topical bioavailability/bioequivalence guidance in May, 2002. Later, the investigation revealed that the protocol and the procedure followed by the two investigators in tretinoin study were different [24]. Based on this experience, the importance of adhering closely to study protocols was emphasized. Furthermore, topical dermatological drug products vary in their rheological properties and consistency. For these reasons some products, when applied, undergo lateral diffusion. It is important to consider this phenomena during development of BE assessment study protocol. Several researchers have further studied DPK methodology to simplify, standardize, and improve the procedure [25, 26]. DPK is a useful

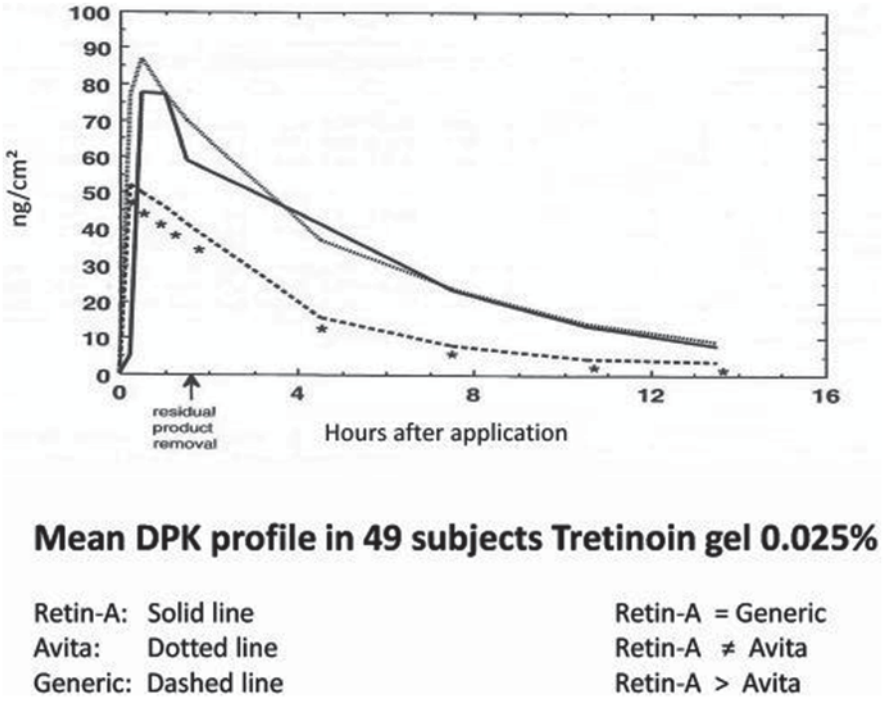


Fig. 8.4 Mean DPK profile in 49 subjects—tretinoin gel 0.025 % [22]

technique for drug products for which the site of action is SC. Overall, a general conclusion is that DPK may not accurately reflect therapeutic effectiveness if the target tissue is not SC.

8.4.2.2 BE of Topical Dermatology Drug Products: *In Vitro* Studies

Drug release from the dosage form is the first and most important step towards the pharmacological and therapeutic activity of the dosage form. When the active and inactive ingredients in a topical dosage form between the T and R product are the same, the only possible difference between the two is the method of manufacture and the micro structure of the formulation. Since for generic products meeting Q1 and Q2, the difference between brand and generic products are essentially similar to Level 2 changes (SUPAC), the *in vitro* release is suggested as a method for assessing BE [27]. This has been utilized in the draft guidance for bioequivalence of acyclovir ointment 0.5 % [28].

8.4.2.3 BE of Topical Dermatology Drug Products: Other Methods

Dermal microdialysis (DMD) and confocal Raman spectroscopy are other promising methods for BE determination of topical dermatological drug products. DMD can be used in healthy as well as damaged skin areas to assess the BE of topical dermatological drug products [29, 30]. DMD is comparatively more invasive than DPK. The method is a challenge for lipophyllic and highly protein bound drugs. Clearly the methods such as suction blister, skin biopsy, and grafted skin model are not acceptable methods because of its invasive nature. In Raman spectroscopy, the drug of interest should have a distinct and a strong absorption to differentiate from the skin spectrum (Fig. 8.1).

8.4.2.4 BE of Topical Dermatology Drug Products: Case-By-Case Solutions

Because of the complexities of demonstrating BE for topical dermatologic drug products, FDA has allowed several approaches on a case-by-case approach [31], summarized as follows:

- Application of pharmacokinetics: Lidocaine topical patch 5%. Lidocaine concentration in plasma can be easily measured. Lidocaine plasma concentration is proportional to its presence in dermal tissue, at the site of action.
- Application of pharmacodynamics: Fluocinolone acetonide 0.01% topical oil. The vasoconstrictor assay can accurately detect the rate and extent of availability in skin. If a generic product is Q1 and Q2, then a biowaiver can be granted.
- Application of clinical approach: 5-flourouracil (5-FU) cream 5%: A clinical endpoint BE study in AK lesions patients is recommended.
- Application of *in vitro* approach: Acyclovir ointment 5%. If the generic product is Q1 and Q2 with the brand name product, approval can be granted based on Q3,that is similar *in vitro* release rate, and similarity in particle size, viscosity, morphic form, PEG molecular weight distribution.
- Application of PK endpoint study and a clinical endpoint study: Diclofenac sodium gel 1%. Two BE studies are required. Topically applied diclofenac is well absorbed, and it acts locally also. A PK study and a BE study with clinical endpoint is recommended.

The FDA considers the drug mechanism and site of action, complexity of the reference formulation, feasibility, and sensitivity of an approach to determine the BE requirements; it can be PK, PD, clinical, or *in vitro,* or a combination of any these techniques.

8.5 Conclusion

Application of drugs to the skin offers both unique opportunities and special challenges to the pharmaceutical scientist and regulator interested in evaluation of bioequivalence of topical dermatologic products. While new approaches are needed,

the question might now be asked—based on many years of research and debate—would it not be sensible for regulatory officials to adopt the most sensitive and specific approach available rather than relying on clinical trials? A small confirmatory clinical trial might used, perhaps in association with a pharmacokinetic blood level study for toxicity. But the primary documentation of BE for these formulations would turn instead either to a DPK approach and/or to an entirely *in vitro* approach such as the diffusion cell discussed in Chaps. 4 and 6. The general argument for this approach would be along the following lines. With a reasonable understanding of the drug substance in terms of its physicochemical characteristics, to include particle size, coupled with requirements for Q1 and Q2 (as noted above), the question becomes: what is reasonable to assure clinicians of BE (and with PE also therapeutic equivalence)? Is it a DPK and/or *in vitro* study or is it a comparative clinical trial. It would seem that the burden of proof should be on those who advocate for this larger, less sensitive, and specific approach, and much more burdensome approach, which might take many years to be generated. In the meantime, patients and practitioners would benefit from timely access to interchangeable multisource products where BE is achieved through simpler means, with resource sparing both for manufacturers and regulatory agencies. Even in the USA, there are attempts to maintain the "gold standard" of comparative clinical trials (which in the case might be more aptly termed "fool's gold"), the rest of the world might well benefit from the much more simple DPK and *in vitro* approaches.

References

1. FDA Guidance: Topical dermatological corticosteroids: in vivo bioequivalence. June 1995.
2. Guy RH, Guy AH, Maibach HI, Shah VP. The bioavailability of dermatological and other topically administered drugs. Pharm Res. 1986;3:253–62.
3. Skelly JP, Shah VP, Maibach HI, Guy RH, Wester RC, flynn G, Yacobi A. FDA and AAPS report of the workshop on principles and practices of in-vitro percutaneous penetration studies: relevance to bioavailability and bioequivalence. Pharm Res. 1987;4:265–7.
4. Shah VP, Flynn GL, Guy RH, Maibach HI, Schaefer H, Skelly JP, Wester RC, Yacobi A. In-vivo percutaneous penetration/absorption. Pharm Res. 1991;8:1071–5.
5. Shah VP, Flynn GL, Yacobi A, et al. Bioequivalence of topical dermatological dosage forms: methods of evaluation of bioequivalence. Pharm Res. 1998;15:167–71.
6. Yacobi A, Shah VP, et al. Workshop Report: current challenges in bioequivalence, quality and novel assessment technologies for topical products. (In preparation).
7. US 21 Code of Federal Regulations: Section 310.6, 2014.
8. US 21 Code of Federal Regulations: Section 330, 2014.
9. DHHS/FDA/ CDER. Orange Book: Approved Drug Products with Therapeutic Equivalence Evaluations, 2014.
10. USP 36/NF 31. The United States Pharmacopeial Convention. 12601 Twinbrook Parkway, Rockville, MD 20852, 2013.
11. McKenzie AW, Stoughton RB. Method for comparing percutaneous absorption of steroids. Arch Dermatol. 1962;86:608–10.
12. Cornell RC, Stoughton RB. Correlation of the vasoconstriction assay and clinical activity in psoriasis. Arch Dermatol. 1985;121:63–7.
13. Stoughton RB. Are generic formulations equivalent to trade name topical glucocorticoids? Arch. Dermatol. 1987;123:1312–4.

14. Caron D, Queille-Rousscl C, Shah VP, Schaefer H. Correlation between the drug penetration and the blanching effect of topically applied hydrocortisone creams in human beings. J Am Arad Dermatol. 1990;23:458–62.
15. Pershing LK, Lambert LD, Shah VP, Lam SY. Variability and correlation of chromameter and tape stripping methods with the visual skin blanching assay in the quantitative assessment of topical 0.05% betamethasone dipropionate bioavailability in humans. lnt J Pharm. 1992;86:201–10.
16. Sing GJ, Adams WP, Lesko LJ, et al. Development of in vivo bioequivalence methodology for dermatological corticosteroids based on pharmacodynamic modeling. Clin Pharmacol Ther. 1999;66:346–57.
17. Rougier A, Dupuis D, Lotte C, Roguet R, Schaefer H. In-vivo correlation between stratum corneum reservoir function and percutaneous absorption. J Invest Dermatol. 1983;81:275–8.
18. Rougier A, Dupuis D, Roguet R, Lotte C. The measurement of stratum corneum reservoir. A predictive method for in-vivo percutaneous absorption studies: influence of the application time. J Invest Dermatol. 1985;84:66–8.
19. Pershing LK, Silver BS, Krueger GG, Shah VP, Skelly JP. Feasibility of measuring the bioavailability of topical betamethasone dipropionate in commercial formulations using drug content in skin and a skin blanching bioassay. Pharm Res. 1992;9:45–51.
20. FDA Guidance for industry: Draft—Topical dermatological drug products NDAs and ANDAs—In vivo bioavailability and bioequivalence. In vitro studies and associated studies. June 1998.
21. Weigman H-J, Ladermann J, Pelchrzim RV, et al. Bioavailabilityof clobetasol propionate quantification of drug concentration in stratum corneum by dermatophrmacokinetic using tape stripping. Skin Pharmacol Appl Skin Physiol. 1999;12:46–53.
22. Pershing LK, Nelson JL, Corlett JL, et al. Assessment of dermatopharmacokinetic approach in bioequivalence determination of topical tretinoin gel products. J Am Acad Dermatol. 2003;48:740–51.
23. FDA: Pharmaceutical Sciences Advisory Committee Meeting, 2001.
24. Shah VP. IV-IVC for topically applied preparations—a critical evaluation. Eur J Pharm Biopharm. 2005;60:309–14.
25. N'Dri-Stempfer B, Navidi EC, Guy RH, Bunge A. Optimizing metrics for the assessment of bioequivalence between topical drug products. Pharm Res. 2008;25:1621–30.
26. N'Dri-Stempfer B, Navidi EC, Guy RH, Bunge A. Improved bioequivalence assessment of topical dermatological drug products using dermatopharmacokinetics. Pharm Res. 2009;26:316–28.
27. FDA Guidance for industry: SUPAC-SS. Nonsterile semisolid dosage forms—scale-up and post-approval changes: chemistry, manufacturing controls; in vitro release testing and in vivo bioequivalence documentation. May 1997.
28. FDA-OGD: Draft Guidance on Acyclovir ointment 0.5%, March 2012.
29. Ortiz PG, Hansen SH, Shah VP, Menne T, Benfeldt E. The effect of irritant dermatitis on cutaneous bioavailability of a metronidazole formulation, investigated by microdialysis and dematopjharmacokinetic method. Contact Dermatitis. 2008;59:23–30.
30. Ortiz PG, Hansen SH, Shah VP, Menne T, Benfeldt E. Impact of adult atopic dermatitis on topical drug penetration: assessment by cutaneous microdialysis and tape stripping. Acta Derm Venereal. 2009;89;33–8.
31. Davit BM. Regulatory approaches for generic drugs: BE of topical drug products. Presentation at PQRI workshop on evaluation of new and generic topical drug products—Current challenges in bioequivalence, quality and novel assessment technologies. Rockville, MD. 11–13 March 2013.

Chapter 9
Methods for the Assessment of Bioequivalence of Topical Dosage Forms: Correlations, Optimization Strategies, and Innovative Approaches

Isadore Kanfer

9.1 Introduction

In the quest to make medicines more affordable and more accessible to the wider population, in 1977, the US Food and Drug Administration (FDA) published its *Bioavailability and Bioequivalence* regulations under 21 Code of Federal Regulations (CFR) [1]. These regulations made provision for the application of surrogate measures to assess the safety and efficacy of drug products. Subsequently, the Drug Price Competition and Patent Term Restoration Act of 1984 (Pub. L. 98–417) (the Hatch-Waxman Amendments) created section 505(j) of the Act which made provision for an Abbreviated New Drug Application (ANDA) approval process in the USA [2]. Unlike the New Drug Application (NDA) requirements to obtain market approval for a new drug product, the ANDA eliminates the requirements that generic drug manufacturers duplicate expensive, time-consuming clinical and nonclinical studies to demonstrate safety and efficacy. Hence, bioavailability (BA) and bioequivalence (BE) studies between a reference drug product (the reference listed drug (RLD), usually the "Brand" or "Innovator" product) and/or the test drug product ("new" or "generic" product) are the current acceptable surrogate approaches to determine the safety and efficacy of generic drug products via the ANDA route.

The current requirements and methods for the assessment of BE between an RLD and generic drug product have largely served the pharmaceutical industry and the public well for many years. When generic drug products are submitted for approval in most countries around the world, they must be both pharmaceutically equivalent and bioequivalent to be considered therapeutically equivalent and as such, approvable and therefore interchangeable (switchable) for a prescribed "Brand" or "Innovator" product.

For topical drug products which are not intended to be absorbed into the systemic circulation, pharmacokinetic measurements in blood/serum/plasma/urine are

I. Kanfer (✉)
Faculty of Pharmacy, Rhodes University, Artillery Road, Grahamstown 6140, South Africa
e-mail: I.Kanfer@ru.ac.za

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_9

generally not acceptable by most regulatory authorities to document BE. Hence, the assessment of BE for such topical products is based on clinical endpoint or pharmacodynamics studies.

9.2 Definitions and Types of Topical Dosage Forms

The term "*TOPICAL*" is defined as "Belonging to a Place or Spot". Hence, when a topical product is applied onto the skin, several implications and consequences need to be considered. Figure 9.1 depicts possible target areas for the active ingredient in the topical dosage form. In the first instance, some drug may well reach the systemic circulation but that consequence is considered to have toxicity implications rather than a therapeutic benefit. Generally, the main objective following application to the skin is that the site of action is "local" as depicted in Fig. 9.1 whereas in certain instances, such as topical products containing a nonsteroidal anti-inflammatory

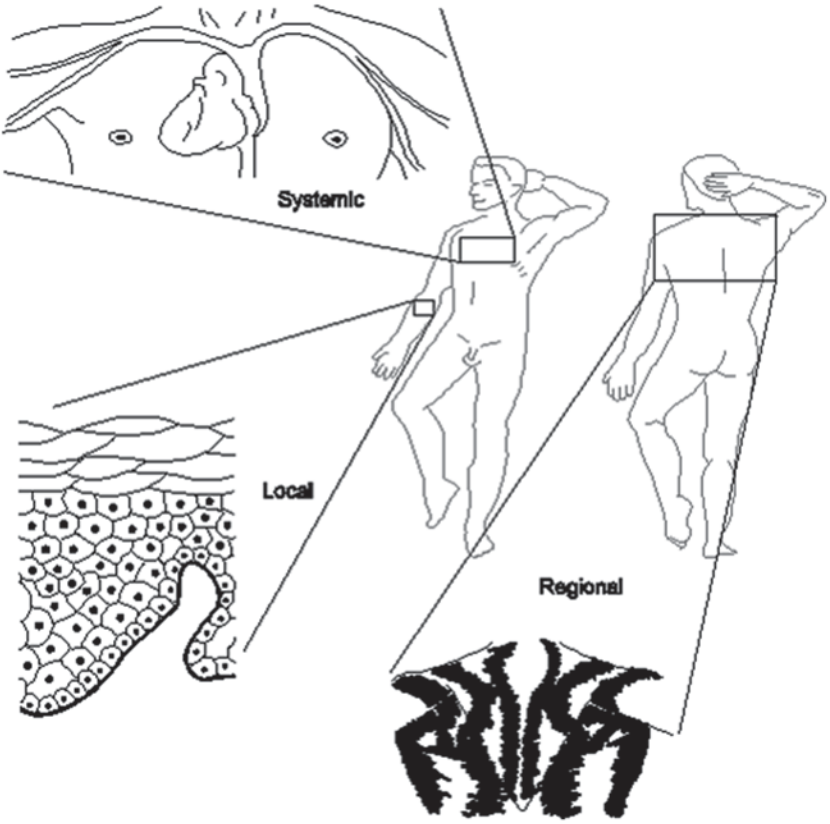


Fig. 9.1 Application sites for topical products

agent (NSAID), the target site may be considered to be in deeper tissue which is indicated as "regional" in the figure [3].

a. Transdermal dosage forms
   These are topical drug products which are intended for the treatment of systemic conditions/diseases. These products are used to achieve systemically active drug concentrations. Hence, percutaneous absorption is a prerequisite for activity, and ideally, no local drug accumulation occurs.
b. Regional drug products are intended for the treatment of disease or symptoms in deeper tissue. The pharmacological action of the active ingredient is affected within musculature, vasculature, joints, and synovial fluid beneath and around the application site. They are more selective with respect to activity compared to systemic delivery, and percutaneous absorption and deposition is intended.
c. Topical drug products are indicated for cutaneous (dermatologic) use and the word topical is synonymous with "local" in this context. The pharmacological or other effects following application of these products are confined to the surface of the skin or within the skin for a local effect and may or may not require percutaneous penetration and deposition.

9.3 Methods

Unlike those drug products which are intended to be absorbed into the systemic circulation, apart from transdermal dosage forms, topical dosage forms are generally not intended to be absorbed, thus the determination of BE of such products presents a formidable challenge.

The application of the principles of BA and BE are, notwithstanding, also applicable to these dosage forms but it is extremely important to make some relevant comparisons.

a. First, in the case of drugs which are systemically absorbed from a dosage form, the rate and extent to which the active ingredient or moiety is absorbed from the drug product and becomes available at the site of action is a primary consideration. However, for drug products where the active ingredient is not intended to be absorbed, the USA CFR [4] describes the type of studies that may be acceptable to demonstrate BE, viz: "For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action."
b. For products where the drug is intended to be absorbed, surrogate measures can be justified by the presumption that the concentration of drug in blood stream is in equilibrium and reflects the concentration at the site of action and by implication, a relationship between effectiveness and systemic blood concentrations of drug exists. However, for topical drug products not intended to be absorbed, such surrogate measures cannot be justified on the same basis as for systemically

absorbed drugs since no apparent relationship between effectiveness and systemic blood concentrations following application to the skin is expected.

The methodology for assessing BE of systemically absorbed drugs and the statistical assessment of data are well-established procedures. In the latter instance, the regulatory BE acceptance criteria are based on the maximum blood (plasma) concentrations (C_{max}) and the area under the plasma drug concentration versus time profiles (AUC) falling within prescribed limits of the confidence interval (CI) of 90 % and the relative means of test to reference product ratios being within 80–125 % on the log transformed basis [5, 6]. However, in the case of topical drug products not intended to be absorbed into the systemic circulation, the methodology for the assessment of BE is still a "work in progress." Currently, apart from the vasoconstrictor assay for the assessment of bioequivalence of topical corticosteroid products [7], the only means whereby a generic company can demonstrate bioequivalence of a topical dosage form intended for local and/or regional activity is through comparative clinical trials with a clinical endpoint using a randomized, double blind, parallel, placebo-controlled study design comparing the generic product versus the RLD in the USA. This has resulted in a dearth of generic topical products reaching the market since conducting clinical endpoint trials are lengthy and expensive. Furthermore, the statistical assessment of data is yet to be defined and apart from dermatological products containing corticosteroids where the FDA Guidance [7] requires that Locke's method [8], which provides an exact confidence interval from untransformed data, is used, no other regulatory guidance permitting the use of surrogate measures has yet been proclaimed.

9.3.1 The Human Skin Blanching Assay (HSBA) also Known as the Vasoconstrictor Assay (VCA) for Topical Corticosteroids

Unlike the determination of the BA/BE of oral dosage forms which involve the comparison of drug concentrations found in biological fluids (blood/plasma/serum/urine) following administration of the dosage form, this approach cannot be used for medicinal products not intended for absorption into the systemic circulation, such as topical dosage forms used for local action. The human skin-blanching assay (HSBA) is a reliable and convenient assay for the comparison of the BA and BE of topical corticosteroid products [9]. The observation of skin blanching following application of a topical corticosteroid to the skin was first observed by Hollander et al. in 1950 [10]. This assay, initially introduced by McKenzie and Stoughton in 1962 [11], relies on the unique ability of topical corticosteroids to produce a blanching response (a skin whitening effect) on the skin following application and is illustrated in Fig. 9.2. This blanching response relates to the amount of corticosteroid that has penetrated into the skin [7]. The HSBA has been found to be very effective for the determination of the effect of formulation on the activity and efficacy of topical corticosteroid products and to examine the comparative bioavailabilities of such topical preparations as the indicators of the efficacy of those products [12]. It is an

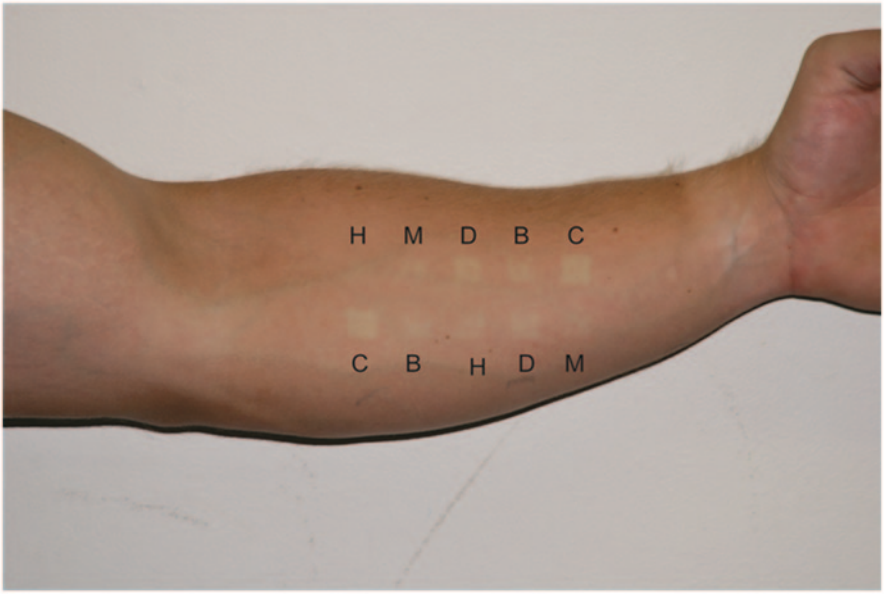


Fig. 9.2 Typical blanching responses following application of topical corticosteroids to the volar aspect of the forearm of a human subject. *H* hydrocortisone 17-butyrate cream (1 mg/gm), *C* clobetasol propionate cream (0.05%), *B* betamethasone valerate cream (5 mg/5 gm), *D* desoximetasone ointment (0.25%), and *M* mometasone furoate cream (0.1%)

indirect measure using a supposed vasoconstriction response following application of topical corticosteroid to human skin. The HSBA was subsequently optimized to facilitate comparisons by evaluating time-response profiles, potencies, and formulations of topical corticosteroids. The optimizations included the application of appropriate statistics, establishment of requisite duration of application of the drug or drug product (dose duration), and intervals of time following application at which the response should be assessed and also a scoring system to facilitate visual assessment [10, 13–16].

9.3.2 Methods for the Evaluation of Skin Blanching

9.3.2.1 Visual Assessment

The initial methodology for the assessment of topical corticosteroid formulations involved the visual assessment of the degree of skin blanching following application to the skin. Visual assessment remained for some period of time as the most commonly used tool when applying the HSBA to compare skin blanching activities between different topical corticosteroids and also formulations [10, 17–19] as well as for the assessment of BA/BE. However, the FDA Guidance document, issued by

the US FDA in 1995 [7], recommended the use of an instrumental method using a chromameter, although the visual assessment method was also retained initially. However, the human eye is sensitive enough for the discrimination of small color changes in skin blanching, different people usually draw upon different references and may express the exact same color in different words. As a consequence, this is perceived as an apparent weakness when using the human eye to evaluate skin whitening owing to the observer's subjectivity. A further criticism on visual assessment relates to the inability to validate the eye as one is able to do when using an instrument [20–22].

However, if observers undergo sufficient training and gain experience in visual evaluation of skin blanching, reproducibility and reliability of visual assessment can be established [23–25].

Visual assessment of skin blanching involves the use of a scoring system to measure skin blanching intensity. A scoring system was developed which involved a graded response based upon the following criteria: absent, faint, faint–moderate, moderate–strong, and strong–intense blanching using the scores of 0, 1, 2, 3, and 4, respectively. This remains the commonly used visual assessment scoring system based on the 0–4 scale introduced by Barry and Woodford [26, 27]. The data are reported and plotted in terms of the percentage of the total possible score versus time (Fig. 9.3) and calculated as follows [7]:

The maximum score per site = 4
The number of independent observers = n

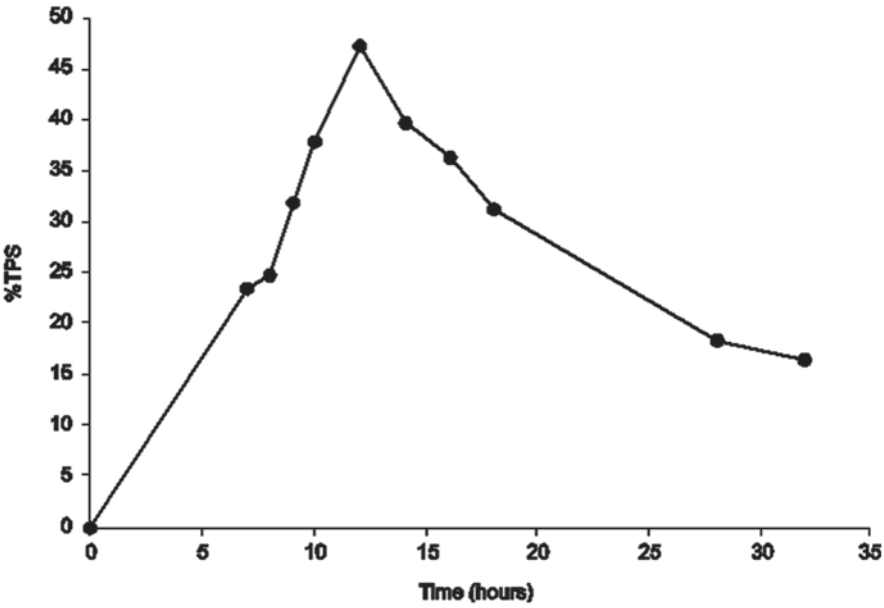


Fig. 9.3 Typical visual blanching profile

The number of sites per preparation per arm = S
The number of volunteers = V
Total possible score (TPS) = $4 \times n \times S \times V$
Percent total possible score (%TPS) = (Actual score/TPS) × 100

9.3.2.2 Chromameter Assessment

An instrumental method involving a tristimulus colorimeter was subsequently introduced as an objective and thus was a "preferred" method. The Minolta® chromameter, which is a portable instrument that uses tristimulus colorimetry involving reflectance spectroscopy, was adapted to measure skin blanching. This approach had subsequently been used for the objective measurement of skin color [28, 29]. The chromameter functions by emitting a white light (using a pulsed xenon arc lamp) onto the chosen area of assessment and measuring the intensity of reflected light through three particular wavelength filters (analyzed at wavelengths of 450, 560, and 600 nm) or using a photodiode array in more recent instruments. The detected signal is converted into three coordinates: L* (luminosity), a* (the amount of green or red), and b* (the amount of yellow or blue). These three coordinates record color in a three-dimensional color system recommended by CIE (Commission International de l'Eclairage)[21, 22, 30]. The skin blanching response is measured relative to the color change in the skin. As the skin blanching response develops, the skin becomes lighter and its redness fades. As the skin becomes paler the L* scale increases, a* scale decreases, and b* scale increases very slightly. It has been shown that the L* and a* coordinates are more discriminative than the b* coordinate in determining skin blanching responses, thus the latter coordinate is omitted from data analysis [31]. However, following the release of the FDA guidance, only the a-scale data has been recommended for use in the statistical analysis [7]. This is possibly due to better correlation with visual skin blanching data found by Pershing et al. [32]. The chromameter can offer reliable and repeatable results provided that certain drawbacks are avoided such as manipulation of the measuring head of the instrument that can affect the quality of the data produced. Skin compression by the measuring head and the angle alignment of the chromameter play a role in obtaining repeatable data [30, 23, 33]. To obtain optimal results, each subject's assessment site as well as ambient temperature should ideally be controlled. It is also important for the operator to hold the chromameter head in such a way that variation in pressure is avoided [23]. The presence of hair and variations in skin glossiness related to the amount of water and lipid on the skin surface, scarring, uneven skin tone, etc., can influence the data obtained [34]. As a result, it is important to avoid these areas of the skin to achieve reliable and reproducible data.

9.3.2.3 Study Designs

Types of Studies—Pilot and Pivotal

The FDA Guidance recommends that two *in vivo* studies, that is, a pilot and pivotal study be conducted in order to determine BE between topical corticosteroid

products. A pilot study provides information on an appropriate dose duration required for the subsequent bioequivalence testing in a pivotal study. The pilot study utilizes a dose duration-response approach, which controls the dose of topical corticosteroid being delivered by comparing different times of exposure of the product on the skin (dose duration is the period of time that the formulation/product is left in contact with the skin). This study is usually conducted only using the reference product. Dose durations required for the pivotal study as recommended by the Topical Corticosteroid FDA guidance [7] are ED_{50}, D_1 (i.e., ½ ED_{50}), and D_2 (i.e., $2ED_{50}$), where ED_{50} is the dose duration at which 50% of the maximum blanching response is achieved. The ED_{50} is chosen since it represents the portion of a dose–response relationship plot where the optimum discrimination of relevant differences can be detected. Using longer dose durations may dampen the assessment of relatively small but significant differences in blanching between a test and a reference product. Furthermore, using shorter dose durations will influence the reliability and repeatability of the assessments. The development and validation of a dose–response curve is therefore essential to determine ED_{50}, D_1, and D_2. These values are determined from an E_{max} model, in accordance to the relevant FDA guidance [7]. The pivotal study is then conducted where a comparison between the responses of a test and reference product is investigated for bioequivalence using the ED_{50}. Furthermore, the Guidance recommends that a subject must be a "detector" in order for inclusion of their data for statistical analyses supporting *in vivo* bioequivalence assessment. Hence, subjects' responses are expected to meet the specified minimum D_2/D_1 ratio of AUEC values in the pivotal study as shown in the equation below.

$$\frac{\text{AUEC at } D_2}{\text{AUEC at } D_1} \geq 1.25$$

9.3.2.4 Comparison Between Visual and Chromameter Assessment

Au et al. [35] conducted a pilot study where Dermovate® cream (containing 0.05% clobetasol propionate) was used as the reference product. The study was performed using the volar aspect of the forearms of 11 healthy human subjects. Visual blanching responses were assessed by three trained observers and a chromameter was also used to assess the degree of blanching at various time intervals over a period of 26 h after removal of the cream. Skin blanching reached a maximum at dose durations longer than 4 h, the maximum blanching occurring at 12 h after removal of the applied formulations at all dose durations for visual and chromameter. The E_{max} model fitting using AUEC data from the visual and chromameter assessments showed that the AUEC of the blanching/vasoconstriction response approaches a maximum at ~1 h dose duration. Based on these values, 0.6 h was utilized as the ED_{50} in the pivotal study. D_1 and D_2 were determined to be 0.3 and 1.2 h, respectively. In the subsequent pivotal study, 34 healthy human subjects were enrolled. The HSBA pivotal study was implemented using Dermovate® cream as both reference and test

Table 9.1 Ninety percentage confidence intervals (CI) using Locke's method for visual and chromameter data [77]

	Visual		Chromameter	
	Mean ratio % (T/R)	90 % CI	Mean ratio % (T/R)	90 % CI
Detectors ($n=23$)	104.6	99.3–111.6	104.6	86.5–129.3
All subjects ($n=34$)	102.9	97.9–109.2	104.3	90.2–120.7

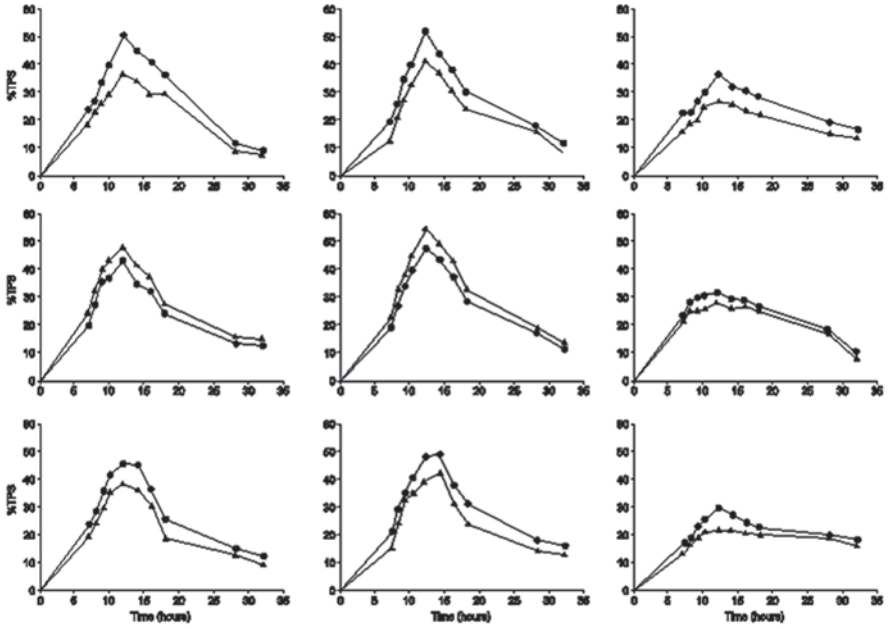
product for the determination of bioequivalence. Skin blanching was evaluated over a period of 30 h after the removal of the applied products. The results revealed that 23 subjects were found to be "detectors" in the pivotal study, but the data for all 34 subjects were included for comparison purposes. The skin blanching profiles were found to be very similar when comparing the data between the two different assessment methods or between "detectors" and "nondetectors" and clearly indicate that the visual and chromameter assessment methods are comparable to each other and both are equally applicable for HSBA. Interestingly, the inclusion of "nondetectors" data did not seem to have a significant effect on the skin blanching profiles or on the outcomes of the comparisons for the assessment of BE.

9.3.2.5 Assessment of Bioequivalence

The acceptance criteria for the declaration of BE generally specify that the 90 % confidence intervals (CIs) for the ratios of the log-transformed C_{max} and AUC for orally administered test and reference products must fall within the range of 80–125 % [36]. However, for the HSBA only the ratios of the AUEC are recommended for use as assessment criteria. As shown in Table 9.1, both assessment methods complied with the BE criteria except for the data for "detectors" using the chromameter where the 90 % CI are 86.5–129.3. This indicated that the power of the study was too low and more subjects should therefore be included in order to increase the power of the study when using the chromameter as the assessment tool. On the other hand, the visual assessment data clearly indicate that the study population of 23 subjects was sufficient for this HSBA BE study.

It is thus seen that although the use of the chromameter is generally recommended as the "preferred" assessment method in favor of visual assessment due to the subjectivity, the above data has shown that visual assessment is a reliable and appropriate assessment technique using the HSBA [35].

Several issues relating to variability of the visual assessment method have also been addressed. Haigh et al. [37] investigated subject- and observer-dependent variation in blanching responses where two different creams containing betamethasone valerate (0.12 %) were compared in three separate trials using three trained



	Celestoderm-V AUBC	Betnovate AUBC	C/B Ratio
Trial 1	733	611	1.28
Trial 2	786	691	1.14
Trial 3	724	593	1.22

Fig. 9.4 Blanching profiles of two different betamethasone valerate cream formulations in three separate trials using three different observers. Celestoderm V® (todermuBetnovate® (▲), *AUBC* Area Under the Blanching Curve

independent observers. Figure 9.4 illustrates the results obtained by each of the observers for each trial where it is seen that the rank order between the two different creams were consistent throughout each trial and for each observer. These data clearly confirm the high degree of reproducibility of the visual method. A further study was undertaken to investigate possible correlations between the visual and chromameter assessments. Figure 9.5 depicts the excellent correlations using a mometasone cream product (1 %) and the data collected by two independent observers.

It is therefore apparent that the visual method of assessment which has largely been discarded by regulatory authorities can provide the same or similar blanching data compared with the chromameter. However, whereas the validation of the visual assessment method remains in contention, attention to detail is essential to ensure accuracy, precision, reproducibility, and subsequent optimized outcomes. These considerations include the use of trained observers, strict adherence to protocols, accuracy of dosing and application to sites, and using appropriate screening methods, among others.

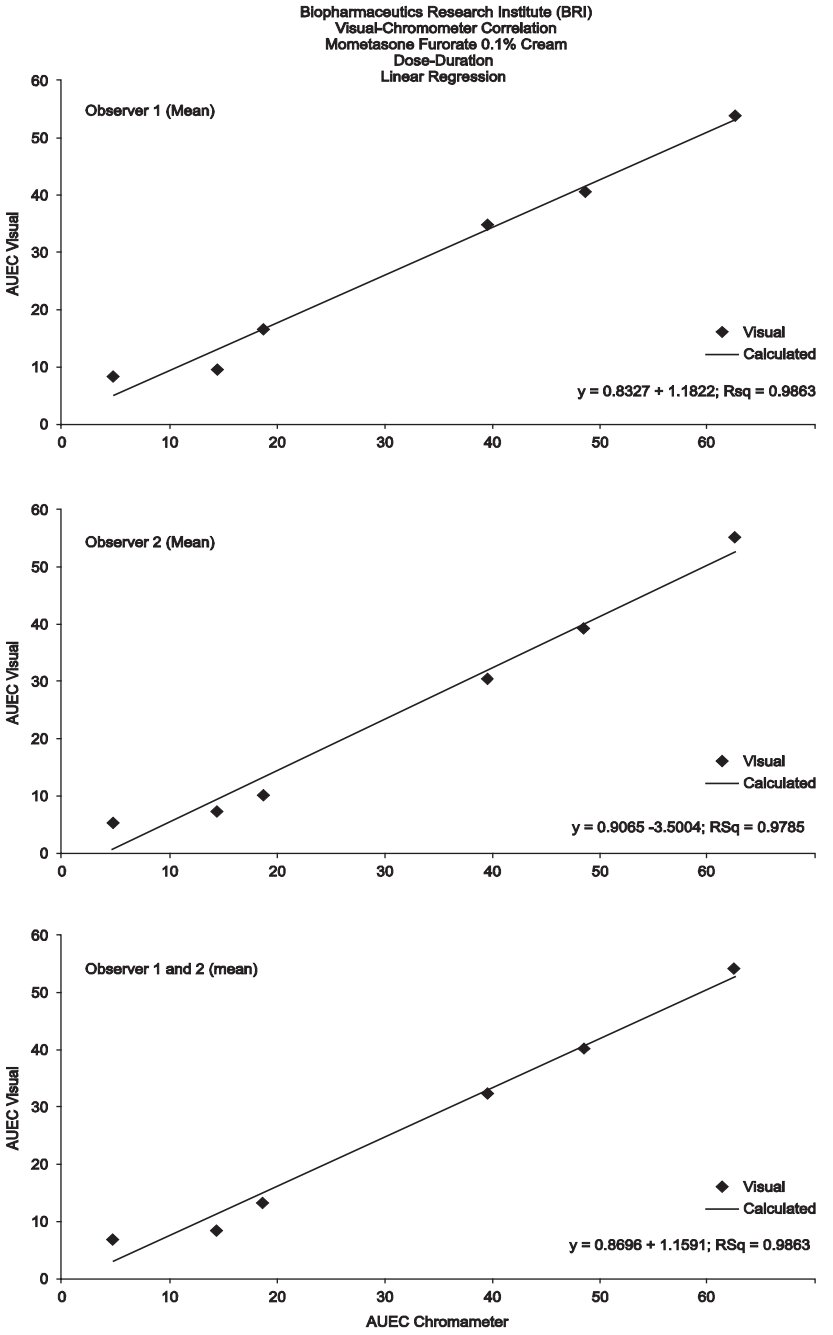


Fig. 9.5 Correlations between visual and chromameter assessments using two observers. *AUEC* Area Under the Effect Curve

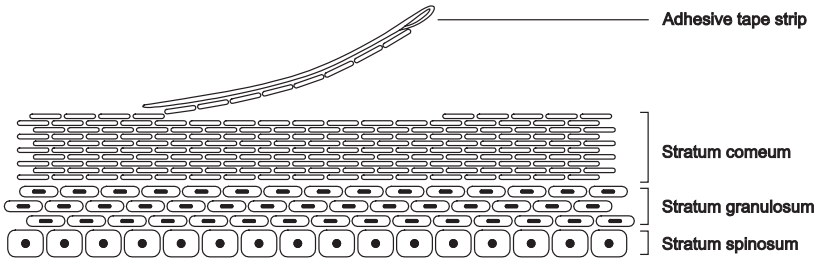


Fig. 9.6 Perceived tape stripping process

9.3.3 Dermatopharmacokinetic (DPK) Methods or Tape Stripping (TS)

This approach to document BE of topical products relies on the measurement of active moiety in the *stratum corneum* (SC) layer of human skin. The methodology involves the determination of the amount of drug which has been permeated into the SC following application of the product to the skin. Adhesive tape strips are used to sequentially remove ~1 μm layers of SC (Fig. 9.6) and are considered relatively noninvasive techniques.

In 1998, the US FDA issued a draft guidance [38]. The initial TS methodology outlining the BA/BE protocol for topical formulations intended for local and/or regional activity was published in that draft guideline. However, in May 2002, the FDA withdrew the guidance as a result of major concerns raised regarding the reproducibility of the DPK method. These concerns were based on contradictory results generated by two reputable independent laboratories regarding the BE assessment of tretinion gel products [39, 40]. The study involved three tretinoin gel products, Retin-A gel, 0.025%, (Ortho-McNeil Janssen Pharmaceuticals, Inc.), Tretinoin gel, 0.025% (Spear Dermatology Products), and Avita tretinoin gel, 0.025% (Bertek Pharmaceuticals). [Pershing LK. BE assessment of three 0.025% tretinoin gel products: dermatopharmacokinetic vs. clinical trial methods; Franz TJ. Study #1, Avita gel 0.025% vs. Retin-a gel 0.025%. http://www.fda.gov/ohrms/dockets/ac/01/slides/3804s2_03_Franz.pdf; Food and Drug Administration Advisory Committee for Pharmaceutical Science, November 29, 2001]. Although subsequent reviews of the data revealed differences in study design and methodologies carried out in the different laboratories, [Conner DP: Differences in DPK Methods. http://www.fda.gov/ohrms/dockets/ac/01/slides/3804s2_05_conner/index.htm, Advisory Committee for Pharmaceutical Sciences Meeting, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), Rockville, Maryland, November 29, (2001), [41] the dermatopharmacokinetic approach using tape stripping was largely discarded as a viable option to pursue for the BE assessment of topical products, mainly due to a number of limitations, in particular the sources of variability and inadequate control. In addition, there were concerns/issues in existing methodology such as trial-and-error approach taken to determine the time points at which TS should take place, the amount of time and effort required to

carry out the procedure, variability in SC characteristics, especially thickness of the SC, between individuals which were not taken into account, the large numbers of subjects deemed necessary to achieve a statistical power greater than 80 %, inherent variability of the method, other sources of variability due to the inconsistency in the amount of SC adhering to each tape strip, variability in the amount of drug present on the discarded tape strips, and variability in the effectiveness of the skin cleaning procedure while removing the product.

Further concerns relate to the appropriateness of using parameters such as AUC and A_{max} parameters derived from the principles of oral pharmacokinetics since there are differences in assessment between topically applied and systemically administered dosage forms. For example, after topical application of a drug, the concentration found at the "site of action" is determined primarily by SC penetration and processes, such as partitioning, diffusion, and keratin binding. In contrast, when using the oral route, the plasma concentration versus time profile obtained is controlled by the processes of absorption, distribution, metabolism, and elimination [42].

When the topical product is applied, the penetration process begins and then at some time after application, the residual formulation is physically removed. Furthermore, when using the oral route, (in the case of an immediate release formulation) absorption occurs until the dosage form is depleted, after which elimination processes start to dominate. Hence, it is clear that oral and topical routes should thus have very different pharmacokinetic profiles (Fig. 9.7).

More recently, the dermatopharmacokinetic approach using the tape stripping method has been revisited by a number of researchers [43]. These authors suggested that the first two tapes which were to be discarded as per the original FDA

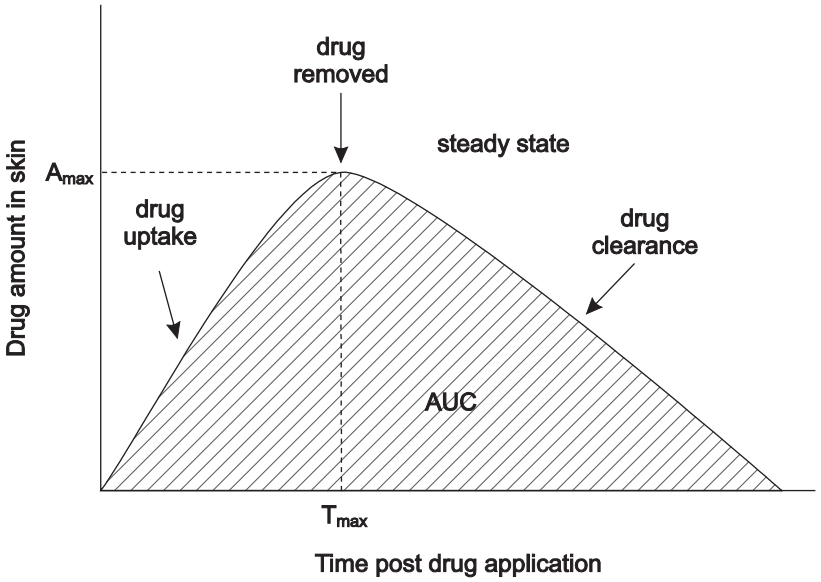


Fig. 9.7 Schematic of profile resulting from a tape stripping method [42]

guidance should be included in the analysis. The initial recommendation in the draft guidance was based on the assumption that residual drug product left on the skin following application might be contained in the first few tapes, thereby resulting in an erroneous overestimation of drug concentration in the SC. They proposed that the product should be rapidly removed from the application site using commercially available alcohol swabs. They also stressed the importance of collecting almost all of the SC to obtain improved reproducibility by controlling several possible sources of variability including type of tape used, subject-to-subject variation, operator, and site of SC collection. According to the FDA draft guidance, a fixed number of tapes should be used but that recommendation could result in incomplete and variable SC collection. Hence, in order to obviate such problems, as much as possible of the SC should be removed using transepidermal water loss (TEWL) data as an indicator to ensure the extent of SC removal. The study design has also been identified by the advisory committee [44] as an important source of variability and implicated as one of the reasons for study failures.

In a paper by Kalia et al. [45], the authors purported that the SC thickness on the forearm varies from 5–20 μm in normal humans. Therefore, the total amount of SC tissue removed from different individuals with the same number of strips may vary. Reevaluation and optimization of the tape stripping technique have been addressed by Herkenne et al. [46] where instead of totaling the amount of drug found in the SC, a profile was obtained which describes the amount of drug present at varying depths of the SC.

In order to reduce intersubject as well as intrasubject variability (between sites within a subject), it is thus important to determine the total SC removed during each tape stripping experiment. In a study conducted by Au et al. [47], refinements to the original tape stripping methodology were made with respect to dosage application, duration of product contact with the application site, removal of excess formulation from the skin, control of temperature and relative humidity of the environment during the study, and normalization of the drug penetration data with skin thickness. The authors emphasized the importance of considering the SC thickness from each individual subject to reduce variability. Normalization of SC thickness (H) was determined from the following equation [45]:

$$\frac{I}{J} = \frac{1}{\mathrm{TEWL}_x} = \frac{H - x}{KD\Delta C}$$

where

J Flux g/m^{2}h
H Total SC thickness
x Partial SC thickness
K Partition coefficient of water in tissue
D Water diffusivity
ΔC Difference in water concentration across the membrane

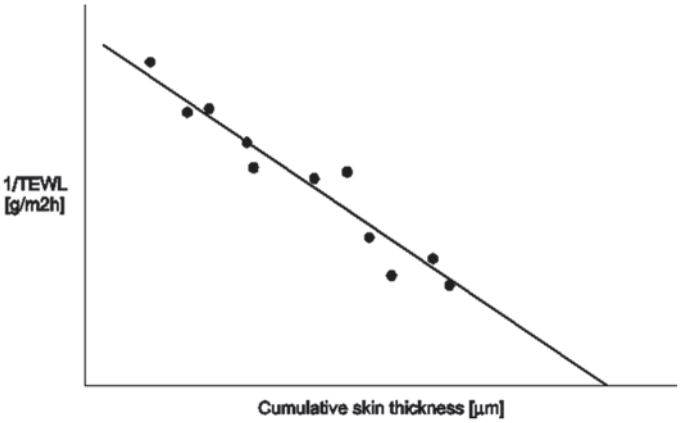


Fig. 9.8 Determination of *stratum corneum* (SC) thickness from TEWL measurements

H can be determined by the x-intercept of the plot 1/TEWL*x* versus x in the following plot shown in Fig. 9.8. The normalized fraction of SC allows for the comparison of data between subjects with varying SC thickness. The corrected area under the curve (AUC_{corr}) of a plot of amount of clobetasol propionate (CP) versus normalized SC fraction was determined and for comparison purposes, the more commonly used data analysis, i.e., the mean amount of drug penetrated into the skin as per uncorrected area (AUC_{uncorr}) was also determined for each formulation in the BE study.

The study compared the tape stripping method with the HSBA to assess BE of topical clobetasol propionate (CP) creams and ointment formulations and determined that the optimum contact time for the drug product with the skin was determined to be 2 h by using a sigmoidal dose-response model [48]. The authors showed that results using tape stripping were comparable to those obtained from the HSBA [47]. Three commercially available products each containing 0.05 % m/m CP were utilized in the studies. The innovator product, Dermovate® cream (Glaxo Wellcome, Midrand, South Africa) was used as both the test and the reference product, and a generic CP cream (Dovate® cream, Aspen Pharmacare Ltd, Port Elizabeth, South Africa) was also assessed. A subsequent TS study was undertaken to compare a CP ointment dosage form containing the same concentration of CP (Dermovate® ointment 0.05 % CP, Sekpharma Pty Ltd, Gauteng, South Africa) versus the CP cream product used as the reference.

BE was demonstrated by both HBSA and TS and similar results were obtained using transformed and Ln-transformed data (Table 9.2). These authors also showed the importance of including SC thickness in data analysis to obtain discriminatory results. The ointment formulation was found to be bioequivalent to the cream (Table 9.3) formulation when the SC thickness determined using TEWL [49] was not taken into account, whereas the two formulations were shown to be nonbioequivalent when the data were corrected for SC thickness (Fig. 9.9).

Table 9.2 Bioequivalence assessment of identical products (Test and Reference: Dermovate® cream). (Adapted from reference [77])

	Mean T/R %		90% confidence interval	
	Untransformed	Ln-transformed	Untransformed	Ln-transformed
HSBA chromameter ($n=34$)	104.3		90.2–120.7	
Tape stripping ($n=7$)	101.8	101.4	88.0–118.3	87.4–117.7

Table 9.3 Bioequivalence assessment of clobetasol propionate creams and ointment products. (Adapted from reference [77])

	Mean T/R %		90% CI	
$n=30$	Untransformed	Ln-transformed	Untransformed	Ln-transformed
AUC_{corr}				
Dovate® cream versus Dermovate® cream	93.8	92.8	84.7–103.6	82.9–103.9
Dermovate® ointment versus Dermovate®cream	66.3	55.2	48.8–82.2	46.1–66.1
AUC_{uncorr}				
Dovate®cream versus Dermovate® cream	93.4	93.6	86.3–101.2	86.2–101.5
Dermovate® ointment versus Dermovate® cream	95.9	96.3	86.8–106.1	86.6–107.1

Whereas the results using tape stripping showed BE between the cream products using either transformed or Ln-transformed data, the difference in bioequivalence between the Dermovate® cream and Dermovate® ointment were masked when using uncorrected AUC data as shown in Table 9.3 and Fig. 9.9 above. Hence, the use of AUC_{corr} data by normalization of the skin thickness appears to provide better discriminatory power and should be considered when using the TS method for BE assessment.

The important question of "How long should the dose be left on the application sites prior to skin stripping?" was also addressed by Au et al. [47] who considered the choice of dose duration which has hitherto generally been unsubstantiated. Sampling when the concentration of drug in the SC is at steady state is likely to mask differences in formulations; thus, it is important to have a validated method of ensuring that the chosen dose duration falls on a sensitive part of a dose–response relationship, such as a plot of the dose duration *versus* drug penetration profile. In order to determine a dose duration which will provide the necessary discriminatory power to identify significant differences or equality between products, the approach

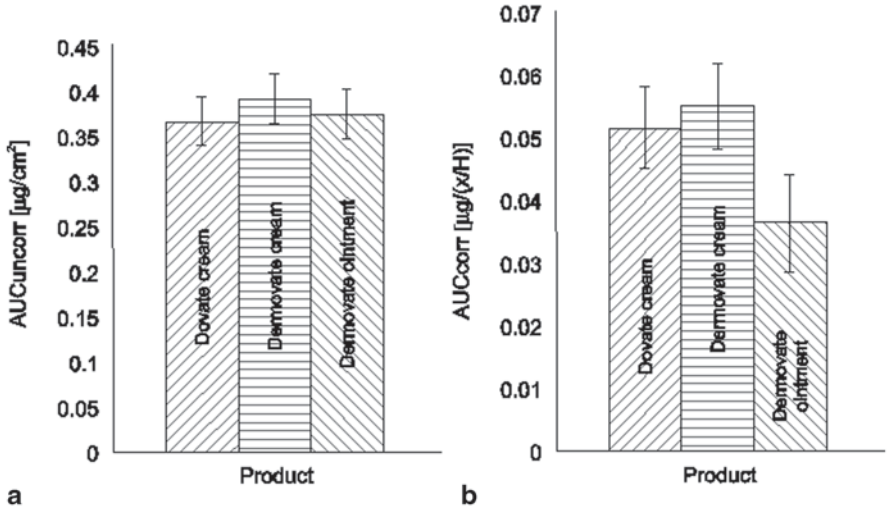


Fig. 9.9 Comparison between the use of **a** AUC_{uncorr} and **b** AUC_{corr} values of the different clobetasol propionate formulations obtained from tape stripping. AUC values with SEM ($n = 30$)

employed in the FDA HSBA guidance was used. This requires that a pilot study be initially conducted with the RLD product alone in order to determine an appropriate "dose duration" to be used later in the pivotal study. The sigmoidal dose-response model [48] was applied to determine the ED_{50} where the maximum sensitivity can be expected, i.e., carried out at the most sensitive part of the dose–response curve. The following relationship is used:

$$E = E_0 + \frac{E_{max} \times D}{ED_{50} + D}$$

where

E	Effect elicited
E_0	Baseline effect in the absence of ligand
E_{max}	Maximum effect elicited
ED_{50}	Dose duration (D) at which effect is half maximal

Figure 9.10 below depicts a dose duration profile following the application of a clotrimazole cream product.

The above data, fitted to the E_{max} model resulted in a $R^2 = 0.9648$, $E_{max} = 89.06$, and $ED_{50} = 0.801$ (10.8 min). Dose duration on the steep part of the curve in accordance with the appropriate sensitivity can then be selected. Although the ED_{50} is considered the time at which maximum sensitivity can be achieved, because it takes approximately 10 min to carry out the TS procedure, dose duration of 15 min can be used instead since a shorter dose duration would be impractical [50].

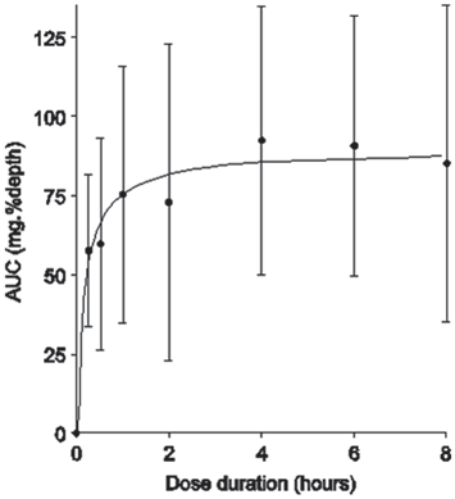


Fig. 9.10 Dose duration profile following the topical application of a clotrimazole cream product

In a small "proof of concept" study investigating the BE of topical clotrimazole formulations using an improved tape stripping method [51], the statistical analyses was performed using both Ln-transformed and untransformed data. The use of Ln-transformed data for the BE assessment of oral products is appropriate since those data have been shown to follow a Ln-normal distribution [52] There is some evidence from *in vitro* work to suggest that dermal absorption measurements also follow this log-normal distribution [53–55]. However, for *in vivo* dermal pharmacodynamic studies (i.e., the HSBA), untransformed data are conventionally used [7] suggesting that untransformed data may be more appropriate for the assessment of topical products. The FDA draft guidance regarding the DPK method [38] endorsed the use of transformed data. However, as that guidance was withdrawn, no guidelines for the analysis of TS data are presently available. Therefore, both Ln-transformed and untransformed data were used for the BE assessments in these studies. The conventional BE limits of 0.8–1.25 for the 90 % CI of the ratio were used to define BE, even though the significance of using these relatively "tight" limits in a BE study involving topical formulations is debatable. For the untransformed data, the point estimate was calculated by dividing the mean AUC_{test} value by the mean AUC_{ref} value and the 90 % CI was determined using Locke's method [7–8]. The CV% associated with the ratio was calculated using the following equation based on untransformed data in order to determine the number of subjects required for 80 % statistical power [56].

$$\mathrm{CV\%} = \frac{\sqrt{\mathrm{MSE}}}{\mathrm{mean}} \times 100$$

Table 9.4 Bioequivalence between clotrimazole cream (1 %) products using conventional BE limits of 80–125. (Reprinted with permission from reference [51])

	Untransformed data	LN-transformed data
n	13	13
AUC_{Test}/AUC_{Ref}	0.94	0.97
90 % CI	0.82–1.08	0.82–1.13
Bioequivalence	Yes	Yes
CV%	23.62	23.40
Statistical power	n/d	47.24 %
n required for 80 % power	19	21

where

MSE Mean square error

For the Ln-transformed data, the Schuirmann two one-sided test (TOST) [57] was used to calculate the 90 % CI and the point estimate. The following equation was used to determine the CV% associated with the ratio using Ln-transformed data:

$$\mathrm{CV}\% = \sqrt{e^{\mathrm{MSE}} - 1} \times 100.$$

The statistical power of the Ln-transformed data was calculated using the MS Excel 2003 spreadsheet accompanying the 5th edition of *Pharmaceutical Statistics: Practical and Clinical Applications* [52]. The following data (Table 9.4) depict the results of the BE study comparing clotrimazole cream products where the same clotrimazole cream was used as both the test and reference products.

The data in Table 9.4 indicate that BE could be declared using either untransformed or transformed data. Although only 13 subjects were used, it is seen that in order to attain a power of 80 % to confirm BE, 19 subjects would be sufficient using untransformed data whereas 21 subjects would be required using Ln-transformed data. Consideration was also given to widening the BE limits and those results are depicted in Table 9.5.

When the BE limits were widened to 75–133 %, 14 subject would be required using untransformed data and 13 using Ln-transformed data. Further widening of the BE limits to 70–143 % would require even less subjects for a power of 80 %, viz: < 13 and 9 using untransformed and Ln-transformed data, respectively.

Table 9.5 Effect of widening the bioequivalence limits and sample sizes required for 80 % power. (Reprinted with permission from reference [51])

90 % CI	Untransformed data	LN-transformed data
0.8–1.25	19	21
0.75–1.33	14	13
0.7–1.43	< 13	9

Table 9.6 Bioequivalence of clotrimazole cream (1%) versus clotrimazole gel (1%). (Adapted from reference [51])

	Ln-transformed data	Untransformed data
n	7	7
AUC_{Test}/AUC_{Ref}	1.67	2.06
90% CI (BE limits 80–125)	0.91–3.23	1.06–3.99
Bioequivalence	No	No
CV%	27.74	24.61

Table 9.6 depicts the results of a subsequent small "Proof-of-Concept" study comparing the BE of a clotrimazole gel (1%) with the clotrimazole cream (1%) reference product. Although only a very small number of subjects were used in this study, these data clearly show that the clotrimazole gel was not found to be bioequivalent to the clotrimazole cream product. The number of subjects required to achieve a power of 80% was calculated to be 29 and 44 using untransformed and Ln-transformed data, respectively. Based on their findings, the authors concluded that although the small sample sizes resulted in the power of the studies being very low and consequently inconclusive BE results, those investigative studies were able to demonstrate that the TS method is capable of detecting both differences and similarities between CLZ formulations, albeit not being able to confirm BE. Furthermore, the vast differences found between the cream and gel formulations suggest the potential of the method to detect formulation differences if such differences truly exist.

The studies clearly show that, if properly powered, it is possible for the TS method to determine BE between topical CLZ products. These results are heartening because they suggest that TS has the potential to be used in a regulatory setting as a tool for the assessment of BE between topical CLZ products. As TS is a more direct method for assessing BE than comparative clinical trials, as well as being less costly and time consuming, it would be extremely advantageous to be able to use such a method. Since the site of action for antifungals is the SC and the TS method takes samples directly from this tissue, this technique is particularly appropriate for the assessment of such formulations. Therefore, by using CLZ as a model compound, these studies have shown that the TS method has the potential to be used as an assessment tool not only for CLZ formulations, but also for other antifungal preparations.

One of the issues surrounding TS is the use of the conventional BE limits of 0.8–1.25. Since the relevance of using these limits in BE studies involving topical products is unsubstantiated, widening the limits may be appropriate. Due to the vast differences with regard to the method of dosing and in particular the usual nondescript size of each dose, the target site, and the pharmacokinetics between oral and locally acting topical products, it is highly unlikely and thus inappropriate that the 0.8–1.25 BE limits will have the same clinical significance for topical products as they have for oral dosage forms.

In summary, it is apparent that the DPK approach using tape stripping has good potential as a method for the assessment of bioequivalence of topical dosage forms not intended for the systemic circulation and more particularly for drugs where the site of action is the SC [58–64].

A particularly attractive aspect using tape stripping is that both the test and reference products are tested in the same individual and as such, each subject serves as an individual control with major advantages such as reduction in intrasubject variability resulting in less subjects being required for the necessary statistical power.

9.3.4 Microdialysis

Microdialysis (MD) is an *in vivo* sampling technique [65–67] following the implantation of a semi-permeable membrane into a specific region of a tissue or fluid-filled space [68]. Details of its application have been extensively reported [66, 69] and although the MD technique was initially developed for use in animals, the first pharmacokinetic study in humans was published in 1997 [70]. Preliminary clinical studies that employed MD demonstrated the potential of this technique for the determination of drug concentrations in the *interstitium* of target tissues. This allowed relative changes of concentration-time profiles of drugs to be described. The development and refinement of this technique over the past two decades has led to its increased acceptance in studies of drug distribution, metabolism, and pharmacodynamics [68, 71]. This technique has been successfully used for the continuous sampling of low molecular weight compounds including glucose, lactose, pyruvate, glycerol, glutamate, and urea as well as pharmacologically active agents in extracellular fluid (ECF) [72] MD has also been used to investigate basic physiology and endogenous substances, as well as the pathophysiology of inflammation and allergic responses, pharmacokinetics and pharmacodynamics of topical and systemic drugs, skin barrier function, and drug penetration into the skin [65, 73–75].

9.3.4.1 Theoretical Principles

The principle of the MD technique is based on passive diffusion of compounds down a concentration gradient across the semi-permeable membrane of a dialysis fiber [76] This technique mimics the functions of a capillary blood vessel that permits the exchange of solutes in and out of the ECF [65, 77, 78–80]. Movement of drug across the membrane is based on Fick's law of diffusion although facilitated diffusion has also been reported with the use of certain types of perfusates [81]

9.3.5 Dermal Microdialysis

Dermal microdialysis (DMD) is a relatively new application of MD, which allows continuous monitoring of endogenous and/or exogenous solutes in the interstitial fluid (ISF) of dermal tissue with minimal tissue trauma [68]. The technique, as illustrated in Fig. 9.11 involves the placement of small perfused membrane systems at given depths within the skin [77].

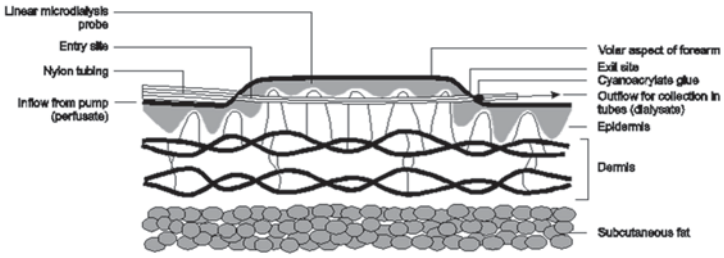


Fig. 9.11 Microdialysis probe with the membrane implanted into the skin of a human subject [98]

Following the implantation of a probe with a microdialysis membrane under the skin and pumping a perfusate through the probe, drug molecules which may have entered the skin from a topically applied product may diffuse from the ISF through the implanted membrane and be collected in the dialysate. This dialysate can be sampled at various intervals of time intervals and the drug concentration in the dialysate can be quantitatively determined [68, 69]. In recent years, DMD has attracted a great deal of interest as a potential technique for the assessment of BA and BE of topical formulations [65, 70, 77, 82].

9.3.5.1 Membrane Systems

Various membrane systems have been used for MD and differ in shape and type of material, depending on the tissue being sampled [67]. Linear systems are normally used and are inserted subcutaneously through a guide cannula and their use requires small skin punctures for entry and exit of the probes where the probes are attached to a microdialysis pump prior to entry and sample collection takes place on exit of the dialysate [77]. Different membrane materials have different chemistries that can affect the recovery and/or delivery of drugs. The molecular weight cut-off (MWCO), a physical property commonly used by manufacturers of these membranes, describes the ability of a membrane to reject 90% of molecules with a specific molecular weight [83]. Although most membrane pore sizes range from 6–3000 kDa, the majority of MD experiments have been conducted using membranes with MWCO of 20 kDa [73] because the pores are small enough to permit diffusion of a large number of drugs, while restricting the entry of large endogenous compounds such as proteins and other macromolecules [84]. The choice of membrane type is an essential element in searching for the optimal probe for a particular application. It is important that the membrane as well as any other component of the MD system does not interact with the drug since this would reduce the drug concentration in the dialysate [85]. The membranes incorporated in linear

probes are usually reinforced with a stainless steel guide wire during manufacture for mechanical strength and also facilitate the determination of the insertion depth by ultrasound image scanning using a frequency of 20 MHz [65, 77]. It has been reported that there is no significant effect on the presence of an intraluminal guide wire in linear MD probes during sampling [86]. When manufacturing MD probes, the length and inner diameter of the outlet tubing should be considered in order to minimize mixing of the dialysate and to prevent hydrostatic pressure build-up across the probe membrane [83].

9.3.5.2 Probe Calibration

MD probes should be checked to ensure reproducible recovery of the study drug to minimize probe-to-probe differences. It is important to note that since MD is a dynamic technique and equilibrium is not established because the perfusate is continuously being pumped through the probe, dialysate concentrations represent only a fraction of the actual concentrations in the ISF [87, 88]. Hence, the extraction efficiency (EE) has to be determined in order to quantitatively relate drug dialysate concentrations in either the tissue or in the surrounding medium. Knowledge of the *in vitro* EE, however, provides information on the reproducibility and patency of the MD probe being used [88]. The general working definition of EE is $(Cp - Cd)/(Cp - Cs)$, where Cd is the drug dialysate concentration, Cs is the known concentration in the external medium, and Cp is the drug concentration in the perfusate. If Cp equals zero, EE is also referred to as the relative recovery (RR). The EE describes the overall mass transport of drugs to and from the MD probe and is commonly used as a means to calibrate the device [83, 89]. Several approaches have been reported to determine EE. The most frequently used calibration methods are the low-flow rate method, the no-net-flux (or zero-net-flux) method, the dynamic (or extended) no-net-flux method, and retrodialysis by drug or by calibrator methods [68]. The simplest approach to calibrate an MD probe is by using a standard solution. For *in vitro* calibration studies, since the *in vitro* drug concentration in the surrounding medium (periprobe) is known and the perfusate contains no drug (i.e., $Cp = 0$), diffusion of the drug occurs from the periprobe into and through the membrane and is collected as dialysate. The EE obtained from a measure of drug concentration recovered in the dialysate (EEr) is described as Cd/Cs. EEr is also referred to as recovery by gain. For *in vivo* and/or *in vitro* calibration studies, EE may be determined by using a standard solution as perfusate with no drug in the tissue or periprobe (i.e., $Cs = 0$). Diffusion occurs from the perfusate into the tissue or periprobe. The EE in this instance, referred to as retrodialysis, recovery by delivery or recovery by loss (EEd), is defined as the ratio of loss of drug from the perfusate relative to the perfusate drug concentration as $(Cp - Cd)/Cp$. It is advisable to perform *in vitro* experiments before human use, to check for *in vitro* adsorption to tubing, time delays in drug movement, and to compare drug gain and loss[65, 68] . The diffusion process is directly proportional to temperature and therefore the MD study should be conducted at a constant, preferably body temperature environment. The

flow rate, thus is an influential factor on EE *and, in general,* low flow rates result in higher recoveries and vice versa, as described according to $EE = (1 - e^{-rA/F}) \times 100$, where r is the mass transport coefficient, A is the surface area of the MD membrane, and F is the flow rate. Low perfusion rates are often limited by the small sample volumes and the quantitation limit of the analytical method. Therefore, it is not advisable to choose minimal flow rates, as this would increase the sample collection interval and consequently result in worse temporal resolution [67, 90] whereas, on the other hand, it is also not advisable to choose high flow rates (> 10 μL/min), as this would significantly result in reduced EE.

9.3.5.3 Assessment of Probe Depth

The depth of the probe insertions, (distance of the dialysis membrane within the skin to the skin surface) can be measured by ultrasound imaging using a frequency of 20 MHz [65, 91, 92]. A probe depth of 0.6–1.0 mm is considered acceptable for DMD studies [65].

9.3.5.4 Composition of Perfusates

The composition, ion strength, osmotic value, and pH of the perfusate, ideally, should be identical to those of the ECF of the dialyzed tissue [85] such that the perfusate chosen should be physiologically compatible with the dermis environment [84]. Perfusates are usually perfused at low flow rates of 1–10 μL/min [88]. A comprehensive list of various perfusates has been reported [85]. Clearly, perfusates should be sterile when used in human experiments and it should be noted that the choice of perfusate used in MD studies may affect drug recovery. Intralipid®, a fat emulsion used for nutritional disorders, and 2-hydroxypropyl-cyclodextrin have been used as perfusates for recovery enhancement of lipophilic, highly protein-bound compounds [81, 93].

9.3.5.5 Exposure and Trauma

Human subjects may experience some trauma caused by the insertion of probes [67] which is a normal result of inflammatory reactions that occur due to the implantation of the probes. However, the inflammation is usually reversible with little bleeding and edema [84]. Probe implantation also causes histamine release into the skin, which returns to baseline after 40 min [94] and histological examinations have shown no cellular infiltration or tissue disruption around dialysis probes in the skin within the first 6–10 h [94, 95]. Apart from the inflammatory response, the probe might also introduce bacteria, therefore causing infection and purulent response. In order to avoid the possibility of infection as a result of probe implantation, the probes should be sterilized prior to implantation. Ethanol solution (70 %) which has been reported not to damage the dialysis membrane may be used [94].

9.3.5.6 Analytical Sensitivity Requirements

Since very low perfusion flow rates (~1–10 μL/min) are generally used, only very small sample volumes are collected (~1–30 μL) during a sampling interval; hence, long sampling collection times may be necessary in order to collect sufficient volumes of dialysate for reliable quantitation [96]. Hence, an analytical method that can either make use of small sample volumes or which has the necessary limit of sensitivity to measure the drug concentration in the dialysate is essential.

9.3.5.7 Advantages and Limitations

DMD offers several advantages for *in vivo* sampling of drugs such as nonremoval of endogenous fluid and thus continuous sampling can be performed with minimal disruption of the physiological system [84]. Although minimally invasive, the procedure is well tolerated by subjects and dermal implantation of the probes may be achieved by placing ice packs directly over the area demarcated for the probes for about 5 min prior to the insertion of the cannulae in order to induce a local anaesthetic effect [82, 97]. The MD technique has a few main limitations. As previously noted, the small sample volumes coupled with associated extremely low drug concentrations necessitate the need for a very sensitive analytical method [81]. Lipophilic compounds may adsorb onto the polymeric materials that are used in manufacturingMD probes as well as the inlet and outlet tubing [83]. Benfeldt and Groth [98] also reported a similar observation when an attempt to measure dermal concentrations of betametasone-17-valerate after topical drug administration failed since no *in vivo* concentrations of the corticosteroid were found.

Many pharmaceutically active compounds demonstrate substantial *in vivo* dermal protein binding. Therefore, the actual recovery of total drug concentration at the site of interest within the skin in an *in vivo* MD study can be well below 1%. This limitation may be overcome by applying high doses or the use of a particularly sensitive analytical technique. Another limitation seems to be the high inter-and intraindividual variability associated with the recovery of exogenous substances. The variability has been reported [99] not to be caused by the MD technique itself, but because of variations in the dermal concentration after the penetration of an exogenous compound. However, some aspects of the MD clinical methodology may contribute toward the variability. One of such aspects is the difficulty in the standardization of a dose application procedure.

9.3.5.8 Bioavailability and Bioequivalence Applications

Apart from the HSBA for topical corticosteroids and the use of clinical endpoint studies, there are currently no other methods acceptable by regulatory agencies which can be used to measure BA or to assess BE of topical drug products intended for local action. Since the use of DMD can facilitate the generation of concentration-time profiles following application of a topical product to the skin, it has the

potential for use to assess BA and BE. Several reports on the application of DMD for investigations of BA and BE have been published [68, 93, 100–102]. Using DMD for the assessment of BE has an important advantage that both the test and reference products can be concurrently applied to sites in each volunteer thereby reducing intrasubject variability which may have significant consequences on the overall coefficient of variation and can result in the reduction of the total number of subjects required to establish BE [102, 103].

Benfeldt et al. [104] investigated the relationship between DMD sampling and the dermatopharmacokinetic method in only eight healthy human subjects to determine the BE of a cream and ointment containing 5% lidocaine. Four microdialysis probes were implanted in one forearm of each subject and dialysate samples were collected for 5 h to measure concentrations of lidocaine. Although it was stated that the study was repeated in the same subjects on two separate occasions, it was unclear which formulations were applied to the various sites and no information was provided regarding the results obtained following each separate occasion. Furthermore, the tables and figures refer to the results obtained from all volunteers ($n=8$) without any mention of whether they were obtained from the first or the subsequent repeated study or average values from both studies? Furthermore, although the two lidocaine formulations were unsurprisingly found to be bioinequivalent, based on an assumption that if the cream and ointment formulations were bioequivalent, the authors calculated the sample size necessary for a bioequivalence study of two formulations using DMD and suggested that 27 subjects are required to establish bioequivalence with 90% CI and 80–125% bioequivalence limits when two probes are used per formulation whereas the number of subjects reduced to 18 when three probes are used per application site. A study was undertaken by McCleverty et al. [103] to determine whether the relatively large degree of variability [82, 99, 104] to be associated with DMD would permit the assessment of bioequivalence of topically applied agents with a reasonable number of subjects. A topically applied vehicle containing methyl salicylate was applied to eight human subjects and samples were collected over a period of 5 h. The results indicated that the demonstration of bioequivalence with 80% power would require approximately 20 subjects. Tettey-Amlalo et al. [82] undertook a study in 18 human subjects to investigate DMD for the assessment of the bioavailability of a ketoprofen topical gel (2.5%) formulation and to evaluate this technique as a tool for the determination of bioequivalence. Four probes were inserted on the left volar aspect of the forearms of each subject. For the assessment of bioequivalence, the same ketoprofen gel product was used as both the test and reference and the gel was applied to four sites on each subject. Sites were perfused for 5 h and dialysate concentrations were determined by UPLC-MS/MS [105]. Although the same ketoprofen product was applied to each site, in order to test the resulting data for bioequivalence, two sites on each arm were designated as test sites (T) and the other two sites as reference sites (R). Designation of the sites was performed according to three randomization sequences: A (TTRR/RRTT), B (TRTR/RTRT), and C (TRRT/RTTR) where the means of probes 1 and 2 versus 3 and 4 were designated as sequence A, the means of probes 1 and 3 versus 2 and 4 were designated as sequence B and the means of probes 1 and 4 versus 2 and

Table 9.7 Bioavailabilities of ketoprofen gel—comparison of application sequences ($n=18$) ([82])

Sequence	PK parameter	Mean ± SD		90% CI	Statistical power	CV%
		Test	Reference			
A	AUC_{0-5} ng.h/ml	155.51 ± 98.89	149.98 ± 107.27	97.39–115.72	92.88	14.88
B	AUC_{0-5} ng.h/ml	152.04 ± 99.23	153.45 ± 103.93	89.86–109.09	95.95	16.72
C	AUC_{0-5} ng.h/ml	139.89 ± 87.28	165.60 ± 116.67	80.37–93.50	53.99	13.04

3 designated as sequence C. The authors reported intra-and intersubject variabilities of 10% and 68%, respectively. Bioequivalence was subsequently confirmed with a power of greater than 90% (Table 9.7), clearly indicating the potential of DMD for the determination of topical formulations intended for local and/or regional activity. It should be noted, however, that AUC values were used to determine bioequivalence. This is justified on the basis of the analogy that for topical corticosteroid products, only the area under the effect curve (AUEC) is used for bioequivalence assessment by VC assay [7]. Furthermore, the C_{max} metric used in bioequivalence assessment of systemically available drugs and may not be a suitable parameter for topical drugs which are not intended for systemic absorption.

DMD was also used by Au et al. [93] for the determination of the BA of CP applied as a 4% ethanolic solution to four sites on one arm of ten human subjects. Linear microdialysis probes with a 2-kDa cutoff were inserted intradermally at the designated sites and a lipid emulsion, Intralipid, was used as the perfusate since the usual perfusates which consist of aqueous saline solutions or similar physiologically acceptable electrolyte/buffer solutions could not be used due to insolubility of CP in such media. The intraindividual variation (CV%) ranged between 10.17–121.65% and the interindividual variation was found to be 50.56%. The interindividual $AUCs_{0-4}$ ranged from 0.74 ± 0.90 to 3.30 ± 1.24 µg/ml.h (Table 9.8). No significant differences were found while comparing the penetration profiles of CP between subjects (p value > 0.05). The flux of CP through the membrane of the probe *in vivo* was found to be 0.15 µg/cm^2.h. Figure 9.12 depicts the comparison of the AUC_{0-4} values between the four sites, A to D, with the sites arranged from the elbow to the wrist. No differences were observed between the sites (p value = 0.8159).

In summary, whereas DMD is associated with several technical difficulties and issues such as low recoveries for highly lipophilic and highly protein-bound drugs, appropriate perfusates, such as Intralipid® for example can help to overcome such problems. A major challenge lies in the necessity of having a sufficiently sensitive analytical method to measure the very low concentrations of drug in dialysate samples, particularly when the topical formulation contains very low concentrations of drug such as 0.05% in the case of topical CP products. A further important consideration is the need to control the high variability of the method. Notwithstanding, based on the various reported applications where DMD has been investigated as a

Table 9.8 Intra-and interindividual variability in AUC_{0-5} values ([93])

Subject No.	AUC (μg/ml.h ± SD)	CV%
1	2.13 ± 1.52	71.34
2	1.41 ± 0.14	10.17
3	2.96 ± 1.64	53.43
4	3.30 ± 1.24	37.60
5	2.78 ± 0.37	13.20
6	1.23 ± 0.29	23.67
7	1.58 ± 1.62	102.90
8	0.74 ± 0.90	121.65
9	1.04 ± 0.94	90.39
10	0.97 ± 1.07	109.46
Mean	1.81 ± 0.92	50.56

possible technique for the determination of BA/BE, this method appears to have great promise and potential for use as a surrogate method and alternative option to replace clinical endpoint studies to assess BE of topical dosage forms intended for local action.

9.3.6 In Vitro *Methods*

Much effort has been directed toward the development and validation of new and alternative approaches to demonstrate BE of topical drug products intended for

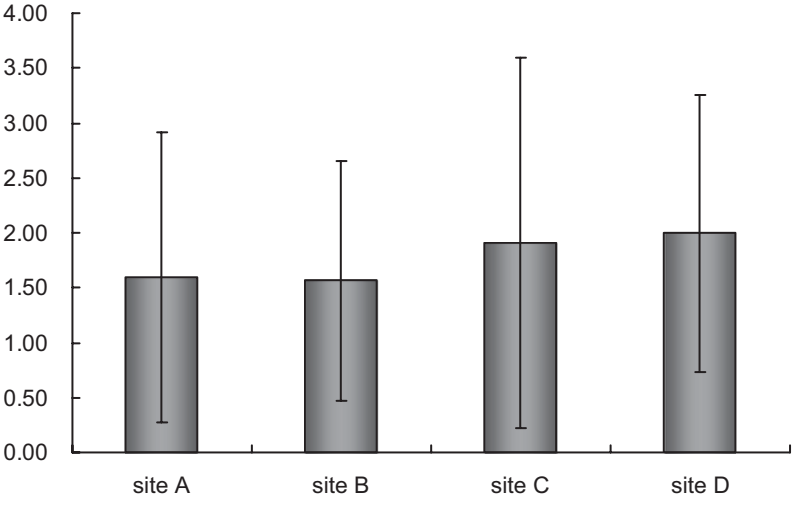


Fig. 9.12 Comparison of clobetasol propionate (AUC_{0-4}) between four sites in human subjects assessed by dermal microdialysis

local action [106]. Although *in vitro* release testing of active ingredients from topical dosage forms can be conducted to characterize performance characteristics of a finished topical dosage form as a quality control procedure and also for justification of scale-up and postapproval changes [107], *in vitro* studies have generally not found acceptance by most regulatory agencies to establish BE until recently when the FDA published a Draft Guidance on Acyclovir [108], which makes provision for an *in vitro* option to establish BA or BE of, specifically, acyclovir topical ointments, only. In terms of that Guidance for acyclovir ointments, all of the following criteria must be met:

1. The test and Reference Listed Drug (RLD) formulations must be qualitatively and quantitatively the same (Q1/Q2).
2. Acceptable comparative physicochemical characterization of the test and RLD formulations must be shown and
3. Acceptable comparative *in vitro* drug release rate tests of acyclovir from the test and RLD formulations.

For many years, vertical diffusion cells (VDCs) have been regarded as the single most powerful *in vitro* model for monitoring the release of active ingredient from semi-solid and transdermal dosage forms and for predicting BA and BE [109]. These cells have been used with various synthetic membranes such as cellulose acetate/nitrate mixed ester, polysulfone, or polytetrafluoroethylene to separate the donor and receiver side for performing *in vitro* drug release testing. Although dermatomed human skin has also been used [110], human skin has largely been used to monitor drug diffusion from transdermal preparations [111]. Whereas artificial membranes do not model the lipid perturbation effects undergone by biological samples, inferences regarding partitioning and diffusion phenomena can be made. Previously reported human skin penetration studies involving ayclovir creams [110] indicated that some generic creams might be bioinequivalent to the innovator and those authors also mentioned that the use of human skin is prone to inconsistent diffusion and that the study protocol needs standardization of skin membranes. Hence, synthetic membranes may be preferred to skin tissue as they are more easily resourced, less expensive, and structurally simpler. This means large-scale studies can be more readily undertaken while mechanisms can be deconvoluted more readily [112]. Furthermore, synthetic membranes exhibit superior permeation data reproducibility since variables such as skin age, race, sex, and anatomical site are eliminated [113]. Nevertheless, the results of artificial membrane studies still tend to yield useful data [114].

Penetration of a drug molecule through skin layers is a complex process, typically rate-limited by the SC. The SC layer of the skin is composed of terminally differentiated corneocytes embedded in a complex lipid matrix comprising primarily ceramides, cholesterol, and free fatty acids [77]. Hence, delivery of drug by passive diffusion and the pharmacological effects elicited are dose-related—the more the permeation of the drug through the skin, the greater the therapeutic effect. Trottet et al. [110] undertook a study of 139 acyclovir cream formulations and concluded that a 40 % propylene glycol concentration in the cream formulation enhanced the availability of acyclovir by tenfold.

A study [115] was undertaken to compare the *in vitro* release of acyclovir from four approved generic cream products using an acyclovir brand/innovator product (Zovirax®) as the reference product. The *in vitro* studies were carried out using vertical diffusion cells with a diffusional surface area of 1.767 cm^2 and various commercially available membranes. Normal saline was used as receptor fluid and the temperature maintained at 32 ± 0.5°C. The *in vitro* release comparisons were based on the recommendations described in the United States Food and Drug Administration Draft Guidance for acyclovir ointment and the SUPAC SS Guidance for nonsterile semisolid dosage forms. The release rates (slope) of the various test products (T) and reference product (R) were monitored and compared. The comparative release rates of acyclovir from the various generic formulations compared with the reference product (Fig. 9.13) were found to be within the limits of 75–133.33 % with a 90 % confidence interval (Table 9.9) for all the generic products tested. These experiments indicate that all the generic acyclovir cream formulations which were tested exhibited release rates that were comparable to the innovator product. These data suggest that such *in vitro* release methods have the potential for use to provide the necessary information for a biowaiver and thereby could be used as a surrogate measure to assess the BE of topical products which are not intended for the systemic circulation.

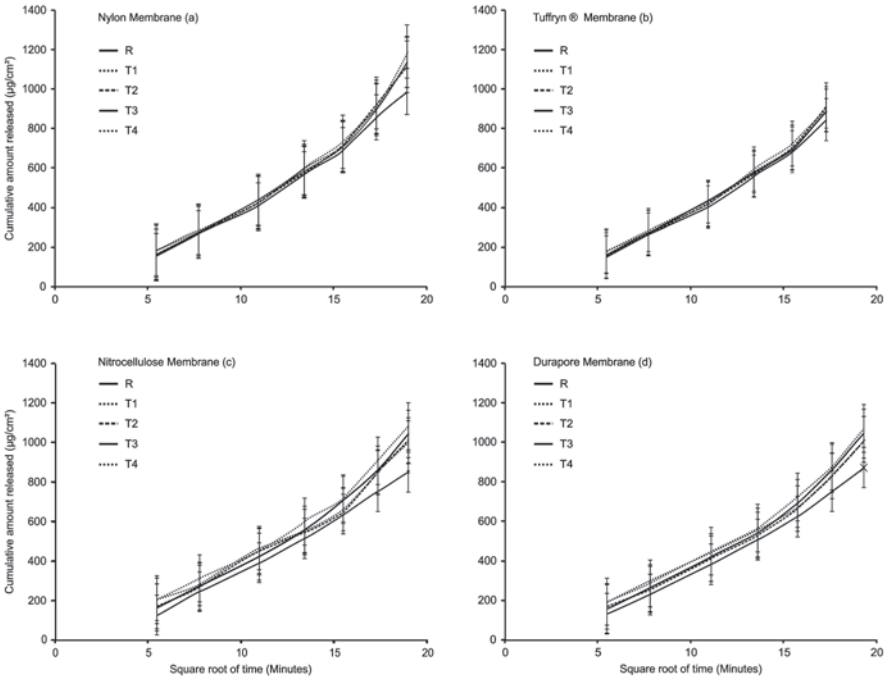


Fig. 9.13 Cumulative amount of acyclovir released from various cream formulations. **a** Nylon membrane. **b** Tuffryn® membrane. **c** Nitrocellulose membrane. **d** Duropore® membrane

Table 9.9 The 90% Confidence interval values (8th and 29th terms) of acyclovir released from various synthetic membranes after 6 h from acyclovir generic cream formulations compared with Zovirax® (innovator)

Membrane	Product T1		Product T2		Product T3		Product F4	
	8th	29th	8th	29th	8th	29th	8th	29th
Nylon	99.43	103.05	96.89	102.81	88.45	94.13	99.85	103.67
Tuffryn®	94.55	99.45	95.15	100.12	87.62	90.03	96.65	99.66
Durapore-HVLP	88.44	97.83	95.73	100.32	86.01	89.20	97.17	100.14
Nitrocellulose-VSWP	87.02	95.33	98.91	102.34	85.49	88.85	98.07	104.43
Strat-M™	Below LOQ							
Fluoropore™ FGLP	Below LOQ							

LOQ limit of quantitation

9.3.7 Open Flow Microperfusion

9.3.7.1 Principles and Application of Open Flow Microperfusion to Study Drug Diffusion Through Skin

DMD is an attractive way to access dermal ISF using MD probes which incorporate a membrane to facilitate exchange between perfusate and dermal ISF and provide filtered ISF which can be readily analyzed for drug content. Furthermore, DMD is excellent for sampling small hydrophilic substances but problems are encountered when attempting to sample high molecular weight or highly lipophilic substances in the ISF. By omitting the membrane in the probe and replacing it with a steel mesh featuring macroscopic openings, a sampling system known as open flow microperfusion (OFM) has been developed and used to sample diluted ISF containing all endogenous and exogenous substances present in the ISF regardless of their molecular weight or lipophilicity [67, 114, 116–118]. The OFM probe is a mesh design catheter with openings of 100 μm rather than a porous membrane (Fig. 9.14).

A wearable light-weight push–pull six-channel micropump is used which enables simultaneous rate-controlled perfusion of six probes in push or three probes in push–pull (Fig. 9.15). The clinical applicability and reliability of this novel wearable dermal open flow microdiffussion (dOFM) device has been evaluated in a clinical setting (Fig. 9.16) where Physicians inserted 141 membrane-free dOFM probes into the dermis of 17 healthy and psoriatic volunteers and sampled dermal ISF for 25 h [120]. A major difference between DMD and OFM is that whereas MD allows measurements of free, unbound drug, OFM only allows measurement of the total drug concentration including the protein-bound fraction. The dOFM technique also differs in probe permeability (semi versus fully permeable), nature

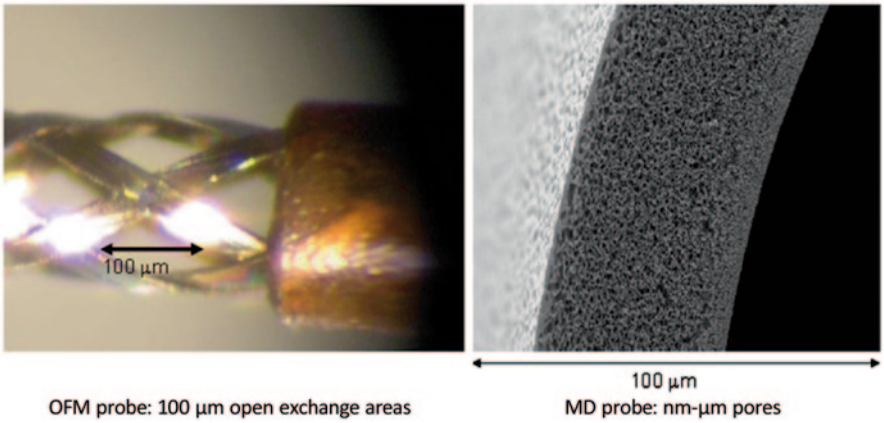


Fig. 9.14 Comparison of OFM and MD probes

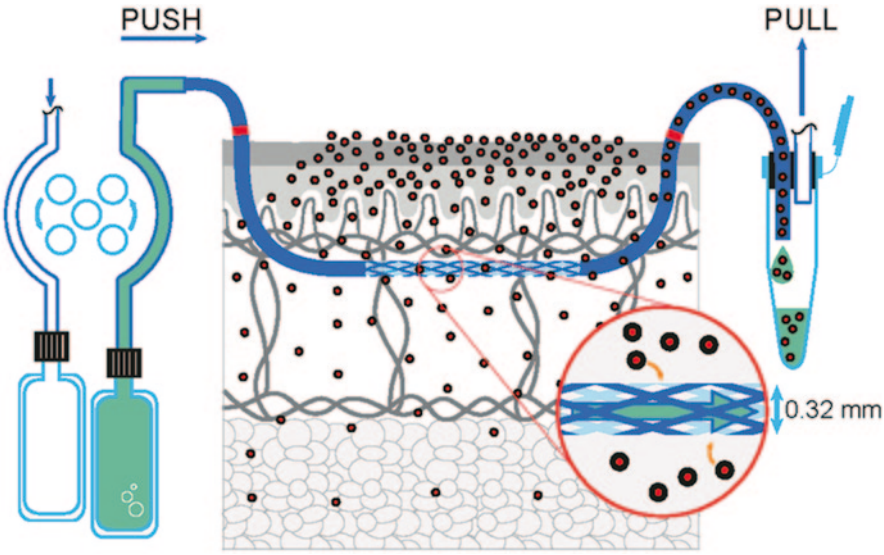


Fig. 9.15 Schematic representation of dermal open flow microperfusion (dOFM) sampling principle

of the probe coating to prevent unspecific adsorption and in the perfusion mode required (push versus push–pull) compared to DMD. Recent reports show that dOFM allows cytokines such as IL-1β, IL-8, IL-6, and TNF-α to be measured in the fluid of subcutaneous adipose tissue [119] and lesional and nonlesional skin of psoriatic patients [120]. Bodenlenz et al. [118] reported a clinical study examining PK and PD of a topical anti-TNF drug (BCT194, 0.5% cream) carried out by dOFM where significant drug concentrations were detected in the skin after 8 days. This

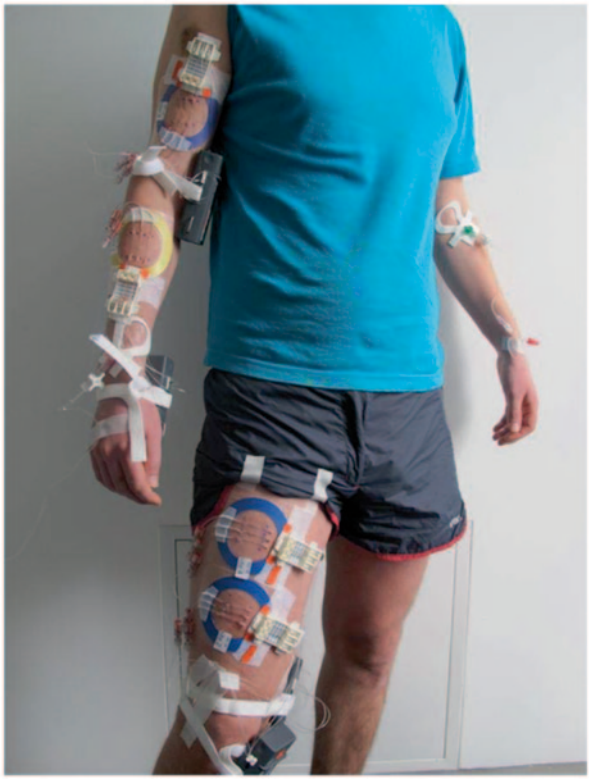


Fig. 9.16 Wearable dOFM device on multiple sites

increased sampling capacity is a significant benefit for the studies of penetration of topical drugs products because such drugs are generally lipophilic. Furthermore, dOFM devices have recently received CE certification for use as medical devices in humans, and there are already standardized procedures in place for its use in clinical trials. When dOFM is used in combination with the latest bioanalytical mass spectrometric techniques, minute quantities of drugs can be measured in the dermis. Because of the open exchange area and the Teflon inner coating of dOFM probes, unspecific adsorption on the OFM materials is inhibited [121]. The OFM probe is a certified (within Europe, CE-labeled), industrially produced (Europe, USA) medical product. In addition, current production of the probes is largely automated, which provides more reproducible probe dimensions than past clinical dOFM and DMD studies that have used in house fabricated probes.

The application of dOFM to study drug penetration into the skin is a new and exciting technique which offers significant advantages over DMD, particularly with respect to the ability to monitor substances present in the ISF regardless of their molecular weight or lipophilicity, and more particularly to permit sampling for prolonged continuous dermal sampling in a multiprobe clinical setting [120].

References

1. Code of Federal Regulations, Title 21, Part 320, Revised as of April1, 2014.
2. Drug Price Competition and Patent Restoration Act of 1984, Public Law 98-417, 98 Stat 1585–1605, Sept. 24, 1984
3. Flynn GL. General introduction and conceptual differentiation of topical and transdermal drug delivery systems. Topical drug bioavailability, bioequivalence, and penetration, pp. 369–91, edited by Vinod P.Shah and Howard I Maibach, Plenum Press, New York, 1993.
4. Title 21 Code of Federal Regulations Part 320. Types of evidence to establish bioavailability or bioequivalence. US Department of Health and Human Service. Revised 1 April 2013
5. FDA Guidance for Industry. Statistical approaches to establishing bioequivalence. January 2001.
6. Schuirmann DJ. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. J Pharmacokinet Biopharm. 1987;15:657–80.
7. FDA Guidance for Industry Topical Dermatological Corticosteroids: In Vivo Bioequivalence. Division of Bioequivalence. Center for Drug Evaluation and Research. Food and Drug Administration, June 1995
8. Locke CS. An exact confidence interval from untransformed data for the ratio of two formulation means. J Pharmacokinet Biopharm. 1984;12:649–55.
9. Barry BW, Woodford R. Activity and bioavailability of topical corticosteroids: in vivo/in vitro correlations for the vasoconstrictor test. J Clin Pharm. 1978;3:43–65.
10. Haigh JM, Kanfer I. Assessment of topical corticosteroid preparations: the human skin blanching assay. Int J Pharm. 1984;19:245–62.
11. McKenzie AW, Stoughton RB. Method for comparing percutaneous absorption of steroids. Arch Dermatol. 1962;86:608–10.
12. Coldman MF, Lockerbie L, Laws EA. The evaluation of several topical corticosteroid preparations in the blanching test. Br J Dermatol. 1971;85:381–7.
13. Heseltine WW, McGilchrist JM, Gartside R. Comparative vasoconstrictor activities of corticosteroids applied topically. Br J Dermatol. 1964;76:71–3.
14. Clanachan I, Devitt HG, Foreman MI, et al. The human vasoconstrictor assay for topical steroids. J Pharmacol Method. 1980;4:209–20.
15. Pepler AF, Woodford R, Morrison JC. The influence of vehicle compostion on the vasoconstrictor activity of betamethasone-17-valerate. Br J Dermatol. 1971;85:171–6.
16. Woodford R, Barry BS. The placebo response to white soft paraffin and propylene glycol in the skin blanching test. Br J Dermatol. 1973;89:53–9.
17. Coleman GL, Kanfer I, Haigh JM. Comparative blanching activities of proprietary diflucortolone valerate topical preparations. Dermatologica. 1978;156:224–30.
18. Coleman GL, Magnus AD, Haigh JM, et al. Comparative blanching activities of locally manufactured proprietary fluocinolone acetonide topical preparations. S Afr Med J. 1979;56:447–9.
19. Magnus AD, Haigh JM, Kanfer I. Assessment of some variables affecting the blanching activity of betamethasone 17-valerate cream. Dermatologica. 1980;160:321–7.
20. Clarys P, Wets L, Barel A, et al. The skin blanching assay with halcinonide: influence of halcinonide concentration and application time. J Eur Acad Dermatol Venereol. 1995;5:250–7.
21. Montenegro L, Ademola JI, Bonina FP, et al. Effect of application time of betamethasone-17 valerate 0.1 % cream on skin blanching and *stratum corneum* drug concentration. Int J Pharm. 1996;140:51–60.
22. Pi'erard GE. EEMCO guidance for the assessment of skin colour. J Env Eng Div Asce. 1998;10:1–11.
23. Schwarb FP, Smith EW, Haigh JM, et al. Analysis of chromameter results obtained from corticosteroid-induced skin blanching assay: comparison of visual chromameter data. Eur J Pharm Biopharm. 1999;47:261–7.
24. Haigh JM, Smith EW. Topical corticosteroid-induced skin blanching measurement: eye or instrument? Letter to the editor. Arch Dermatol. 1991;127:1065.

25. Skelly JP, Shah VP, Peck CC. Topical corticosteroid-induced skin blanching measurement: eye or instrument? Response to the editor. Arch Dermatol. 1991;127:1065.
26. Barry BW, Woodford R. Comparative bio-availability of proprietary topical corticosteroid preparations; vasoconstrictor assays on thirty creams and gels. Br J Dermatol. 1974;91:323–8.
27. Barry BW, Woodford R. Bioavailability and activity of betamethasone 17-benzoate in gel and cream formulations: comparison with proprietary topical corticosteroid preparation in the vasoconstrictor assay. Curr Ther Res Clin Exp. 1974;16:338–45.
28. Kir'aly K, So'os GY. Objective measurement of topically applied corticosteroids. Dermatologica. 1976;152:133–7.
29. Gras PW, Bason ML, Esteves LA. Evaluation of a portable colour meter for assessment of the colour of milled rice. J Stored Prod Res. 1990;26:71–5.
30. Taylor S, Westerhof W, Im S, et al. Noninvasive techniques for the evaluation of skin color. J Am Acad Dermatol. 2006;54:282–90.
31. Chan SY, Po ALW. Quantitative skin blanching assay of corticosteroid creams using tristimulus colour analysis. J Pharm Pharmacol. 1992;44:371–8.
32. Pershing LK, Lambert LD, Shah VP, et al. Variability and correlation of chromameter and tape-stripping methods with the visual skin blanching assay in the quantitative assessment of topical 0.05% betamethasone dipropionate bioavailability in humans. Int J Pharm. 1992;86:201–10.
33. Waring MJ, Monger L, Hollingsbee DA, et al. Assessment of corticosteroid-induced skin blanching: evaluation of the Minolta Chromameter CR200. Int J Pharm. 1993;94:211–22.
34. Fullerton A, Fischer T, Lahti A, et al. Guidelines for measurement of skin colour and erythema: a report from the Standardization Group of the European Society of Contact Dermatitis. Contact Dermatitis. 1996;35:1–10.
35. Au WL, Skinner M, Kanfer I. Bioequivalence assessment of topical clobetasol propionate products using visual and chromametric assessment of skin blanching. J Pharm Pharm Sci. 2008;11:160–6.
36. U. S Food and Drug Administration (FDA). FDA, Guidance of industry: bioavailability and bioequivalence studies for orally administered drug products-General considerations. MD, USA: Centre for Drug Evaluation, Food and Drug Administration, 2000.
37. Haigh JM, Meyer E, Smith EW, Kanfer I. The human skin blanching assay for in vivo topical corticosteroid assessment: II. Subject-and observer-dependent variation in blanching responses. Int J Pharm. 1997;152:185–92.
38. U.S. Food and Drug Administration (FDA). Guidance for Industry, Topical Dermatological Drug Product NDAs and ANDAs—In Vivo Bioavailability, Bioequivalence, In Vitro Release and Associated Studies, 1998.
39. Shah VP. IV-IVC for topically applied preparations-a critical evaluation. Eur J Pharm Biopharm. 2005;60:309-14.
40. Pershing L et al. Assessment of dermatopharmacokinetic approach in the bioequivalence determination of topical tretinoin gel products. J Am Acad Dermatol. 2003;48:740–51.
41. Narkar Y. Bioequivalence for topical products—an update. Pharm Res. 2010;27:2590–601.
42. N'Dri-Stempfer B, Navidi W, Guy RH, Bunge AL. Optimising metrics for the assessment of bioequivalence between topical drug products. Pharm Res. 2008;25(7):1621–30.
43. N'Dri-Stempfer B, Navidi WC, Guy RH, Bunge AL. Improved bioequivalence assessment of topical dermatological drug products using dermatopharmacokinetics. Pharm Res. 2009;26:316–28.
44. Food and Drug Administration, Advisory Committee for Pharmaceutical Science, November 29, 2001.
45. Kalia YN, Alberti I, Sekkat N, Curdy C, Naik A, Guy RH. Normalization of stratum corneum barrier function and transepidermal water loss in vivo. Pharm Res. 2000;17:1148–50.
46. Herkenne C, Naik A, Kalia YN, Hadgraft J, Guy RH. Ibuprofen transport into and through skin from topical formulations: in vitro-in vivo comparison. J Invest Dermatol. 2007;127(1):135–42.

47. Au WL, Skinner M, Kanfer I. Comparison of tape stripping with the human skin blanching assay for the bioequivalence assessment of topical clobetasol propionate formulations. J Pharm Pharm Sci. 2010;13:11–20.
48. Holford NH, Sheiner LB. Understanding the dose-effect relationship: clinical application of pharmacokinetic/pharmacodynamic models. Clin Pharmacokinet. 1981;6:429–53.
49. Russell LM, Wiedersberg S, Delgado-Charro MB. The determination of stratum corneum thickness: an alternative approach. Eur J Pharm Biopharm. 2008;69:861–70.
50. Parfitt NR. Investigations of the assessment of bioequivalence of topical clotrimazole products using a dermatopharmacokinetic approach, MSc Thesis, 2010, Rhodes University.
51. Parfitt NR, Skinner MF, Bon C, and Kanfer I. Bioequivalence of topical clotrimazole formulations: an improved tape stripping method. J Pharm Sci. 2011;14(3):347–57.
52. Bolton S, Bon C. Pharmaceutical statistics: practical and clinical applications. 5th ed. USA: Taylor and Francis; 2009.
53. Williams AC, Cornwell PA, Barry BW. On the non-Gaussian distribution of human skin permeabilities. Int J Pharm. 1992;86(1):69–77.
54. Kasting GB, Filloon TG, Francis WR, Meredith MP. Improving the sensitivity of in vitro skin penetration experiments. Pharm Res. 1994;11(12):1747–54.
55. Cornwell PA, Barry BW. Effects of penetration enhancer treatment on the statistical distribution of human skin permeabilities. Int J Pharm. 1995;117(1):101–12.
56. Chow S, Wang H. On sample size calculation in bioequivalence trials. J Pharmacokinet Pharmacodyn. 2001;28(2):155–69.
57. Schuirmann DJ. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. J Pharmacokinet Biopharm. 1987;15(6):657–80.
58. Pershing LK, Corlett J, Jorgensen C. In vivo pharmacokinetics and pharmacodynamics of topical ketoconazole and miconazole in human stratum corneum. Antimicrob Agents Chemother. 1994;38:90–5.
59. Alberti I, Kalia YN, Naik A, Bonny JD, Guy RH. In vivo assessment of enhanced topical delivery of terbinafine to human stratum corneum. J Control Release. 2001;71:319–27.
60. Alberti I, Kalia YN, Naik A, Bonny J, Guy RH. Effect of ethanol and isopropyl myristate on the availability of topical terbinafine in human stratum corneum, in vivo. Int J Pharm. 2001;219:11–9.
61. Alberti I, Kalia YN, Naik A, Guy RH. Assessment and prediction of the cutaneous bioavailability of topical terbinafine, in vivo, in man. Pharm Res. 2001;18:1472–5.
62. Fernandez C, Nielloud F, Fortune R, Vian L, Marti-Mestres G. Benzophenone-3: rapid prediction and evaluation using noninvasive methods of in vivo human penetration. J Pharm Biomed Anal. 2002;28:57–63.
63. Wissing SA, Muller RH. Solid lipid nanoparticles as carrier for sunscreens: in vitro release and in vivo skin penetration. J Control Release. 2002;81:225–33.
64. Lboutounne H, Chaulet JF, Ploton C, Falson F, Pirot F. Sustained ex vivo skin antiseptic activity of chlorhexidine in poly(epsilon-caprolactone) nanocapsule encapsulated form and as a digluconate. J Control Release. 2002;82:319–34.
65. Groth L, Ortiz PG, Benfeldt E. Microdialysis methodology for sampling in the skin. In: Serup J, Jemec GBE, Grove GL, editors. Handbook of non-invasive methods and the skin. 2nd ed. Florida: CRC Press; 2006. pp. 443–54.
66. Groth L. Cutaneous microdialysis. Methodology and validation validation [Ph.D. Thesis]. Stockholm: Scandinavian University Press; 1996.
67. Plock N, Kloft C. Microdialysis: theoretical background and recent implementation in applied life-sciences. Eur J Pharm Sci. 2005;25:1–24.
68. Chaurasia CS, Müller M, Bashaw ED, et al. AAPS-FDA Workshop White Paper: microdialysis principles, applications and regulatory perspectives report from the joint AAPS-FDA workshop, November 4-5, 2005, Nashville, TN. Pharm Res. 2007;24:1014–25.
69. Müller M. Microdialysis in clinical drug delivery studies. Adv Drug Deliver Rev. 2000;45:255–69.

70. Joukhadar C, Müller M. Microdialysis: current applications in clinical pharmacokinetic studies and its potential role in the future. Clin Pharmacokinet. 2005;44:895–913.
71. Li Y, Peris J, Zhong L, et al. Microdialysis as a tool in local pharmacodynamics. AAPS J. 2006;8:E222–35.
72. Abrahamsson P, Winsö O. An assessment of calibration and performance of the microdialysis system. J Pharm Biomed Anal. 2005;39:730–4.
73. Schnetz E, Fartasch M. Microdialysis for the evaluation of penetration through the human skin barrier-a promising tool for future research? Eur J Pharm Sci. 2001;12:165–74.
74. Leis S, Drenkhahn S, Schick C, et al. Catecholamine release in human skin-a microdialysis study. Exp Neurol. 2004;188:86–93.
75. Schwalbe O, Buerger C, Plock N, et al. Urea as an endogenous surrogate in human microdialysis to determine relative recovery of drugs: analytics and applications. J Pharm Biomed Anal. 2006;41:233–9.
76. Mathy F, Denet AR, Vroman B, et al. In vivo tolerance assessment of skin after insertion of subcutaneous and cutaneous microdialysis probes in the rat. Skin Pharmacol Appl Skin Physiol. 2003;16:18–27.
77. Kanfer I, Tettey-Amlalo RNO, Au WL. Assessment of topical dosage forms intended for local or regional activity. In: Shargel L, Kanfer I, editors. Generic drug product development: specialty dosage forms. Chapter 4. New York: Informa Healthcare; 2010. pp. 54–103.
78. Pena A, Liu P, Derendorf H. Microdialysis in peripheral tissues. Adv Drug Deliver Rev. 2000;45:189–216.
79. Zhao Y, Liang X, Lunte CE. Comparison of recovery and delivery in vitro for calibration of microdialysis probes. Anal Chim Acta. 1995;316:403–10.
80. Brunner M, Derendorf H. Clinical microdialysis: current applications and potential use in drug development. Trends Anal Chem. 2006;25:674–80.
81. Ward KW, Medina SJ, Portelli ST, et al. Enhancement of in vitro and in vivo microdialysis recovery of SB-265123 using Intralipid® and Encapsin® as perfusates. Biopharm Drug Dispos. 2003;24:17–25.
82. Tettey-Amlalo RNO, Kanfer I, Skinner MF, et al. Application of dermal microdialysis for the evaluation of bioequivalence of a ketoprofen topical gel. Eur J Pharm Sci. 2009;36:219–25.
83. Stenken JA, Chen R, Yuan X. Influence of geometry and equilibrium chemistry on relative recovery during enhanced microdialysis. Anal Chim Acta. 2001;436:21–9.
84. Davies MI, Cooper JD, Desmond SS, et al. Analytical considerations for microdialysis sampling. Adv Drug Deliver Rev. 2000;45:169–88.
85. de Lange ECM, de Boer AG, Breimer DD. Methodological issues in microdialysis sampling for pharmacokinetic studies. Adv Drug Deliver Rev. 2000;45:125–48.
86. Klimowicz A, Bielecka-Grzela S, Groth L, et al. Use of an intraluminal guide wire in linear microdialsis probes: effect on recovery? Skin Res Technol. 2004;10:104–8.
87. Brunner M, Langer O. Microdialysis versus other techniques for the clinical assessment of in vitro tissue drug distribution. AAPS J. 2006;8:E263–71.
88. Song Y, Lunte CE. Comparison of calibration by delivery versus no net flux for quantitative in vivo microdialysis sampling. Anal Chim Acta. 1999;379:251–62.
89. Steuerwald AJ, Villeneuve JD, Sun L, et al. In vitro characterization of an in situ microdialysis sampling assay for elastase activity detection. J Pharm Biomed Anal. 2006;40:1041–7.
90. Weiss DJ, Lunte CE, Lunte SM. In vivo microdialysis as a tool for monitoring pharmacokinetics. Trends Anal Chem. 2000;19:606–16.
91. Benfeldt E, Serup J, Menné T. Effect of barrier perturbation on cutaneous salicylic acid penetration in human: in vivo pharmacokinetics using microdialysis and non-invasive quantification of barrier function. Br J Dermatol. 1999;140:739–48.
92. Benfeldt E, Serup J. Effect of barrier perturbation on cutaneous penetration of salicylic acid in hairless rats: in vivo pharmacokinetics using microdialysis and non-invasive quantification of barrier function. Arch Dermatol Res. 1999;291:517–26.
93. Au WL, Skinner MF, Benfeldt E, Verbeeck RK, Kanfer I. Application of dermal microdialysis for the determination of bioavailability of clobetasol propionate applied to the skin of human subjects. Skin Pharmacol Physiol. 2012;25:17–24.

94. Anderson C, Anderson T, Molander M. Ethanol absorption across human skin measured by in vivo microdialysis technique. Acta Derm Venereol. 1991;71:389–93.
95. Ault JM, Riley CM, Meltzer NM, et al. Dermal microdialysis sampling in vivo. Pharm Res. 1994;11:1631–9.
96. Verbeeck R. Blood microdialyis in pharmacokinetic and drug metabolism studies. Adv Drug Deliv Rev. 2000;45:217–28.
97. Tettey-Amlalo RNO. Application of dermal microdialysis and tape stripping methods to determine the bioavailability and/or bioequivalence of topical ketoprofen formulations [PhD Thesis]. Grahamstown, South Africa: Rhodes University, 2008
98. Benfeldt E, Groth L. Feasibility of measuring lipophilic or protein-bound drugs in the dermis by in vivo microdialysis after topical or systemic drug administration. Acta Dem Venereol. 1998;78:274–8.
99. Schnetz E, Fartasch M. Microdialysis for the evaluation of penetration through the human skin barrier-a promising tool for future research? Eur J Pharm Sci. 2001;12:165–74.
100. Holmgaard R, Nielsen JB, Benfeldt E. Microdialysis sampling for investigations of bioavailability and bioequivalence of topically administered drugs: current state and future perspectives. Skin Pharmacol Physiol. 2010;23:225–43.
101. Schmid S, Banks R, Kumar V, Rand KH, Derendorf H. Clinical microdialysis in skin and soft tissues: an update. J Clin Pharmacol. 2008;48:351–64.
102. Benfeldt E, Bashaw ED, Shah VP. In: Walters K, Roberts MS, editors. Assessment of topical bioequivalence using microdialysis and other techniques. New York: Taylor and Francis; 2007. p. 537–48
103. McCleverty D, Lyons R, Henry B. Microdialysis sampling and the clinical determination of topical bioequivalence. Int J Pharm. 2006;308:1–7.
104. Benfeldt E, Hansen SH, Volund A, Menne T, Shah VP. Bioequivalence of topical formulations in humans: evaluation by dermal microdialysis sampling and the dermatopharmacokinetic method. J Invest Dermatol. 2007;127:170–8.
105. Tettey-Amlalo RNO, Kanfer I. Rapid UPLC-MS/MS method for the determination of ketoprofen in human dermal microdialysis samples. J Pharm Biomed Anal. 2009;50:580–6.
106. Lionberger RA. FDA critical path initiatives: opportunities for generic drug development. AAPS J. 2008;10(1):103–9.
107. Chang RK, Raw A, Lionberger R, Yu L. Generic development of topical dermatologic products: formulation development, process development, and testing of topical dermatologic products. AAPS J. 2013;15(1):41–52.
108. Draft Guidance on Acyclovir. 2012. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm296733.pdf. Accessed 22 Jan 2013
109. Raney S, Lehman P, Franz T. 30th anniversary of the Franz cell finite dose model: the crystal ball of topical drug development. Drug Delivery Technol. 2008;8(7):32–7.
110. Trottet L, Owen H, Holme P, Heylings J, Collin IP, Breen A, Siyad MN, Nandra RS, Davis AF. Are all acyclovir cream formulations bioequivalent? Int J Pharm. 2005;304(1/2):63–71.
111. Prausnitz MR, Langer R. Transdermal drug delivery. Nat Biotechnol. 2008;26(11):1261–8.
112. Wester R, Maibach H. Review: percutaneous absorption of drugs. Clin Pharmacokinet. 1992;23:253–66.
113. Michaels A, Chandrasekaran SK, Francis W, Meredith M. Drug permeation through human skin: theory and in vitro experimental measurement. A I Ch E J. 1975;21:985–96.
114. Schaupp L, Ellmerer M, Brunner GA, Wutte A, Sendlhofer G, Trajanoski Z, Skrabal F, Pieber TR, Wach P. Direct access to interstitial fluid in adipose tissue in humans by use of open-flow microperfusion. Am J Physiol. 1999;276:E401–8.
115. Nallagundla S, Patnala S, Kanfer I. Comparison of in vitro release rates of acyclovir from cream formulations using vertical diffusion cells. AAPS PharmSciTech. 2014;15(4):994–99.
116. Ellmerer M, Schaupp L, Brunner GA, Sendlhofer G, Wutte A, Wach P, Pieber TR. Measurement of interstitial albumin in human skeletal muscle and adipose tissue by open-flow microperfusion. Am J Physiol Endocrinol Metab. 2000;278:E352–6.

117. Bodenlenz M, Schaupp L, Druml T, Sommer R, Wutte A, Schaller HC, Sinner F, Wach P, Pieber TR. Measurement of interstitial insulin in human adipose and muscle tissue under moderate hyperinsulinemia by means of direct interstitial access. Am J Physiol Endocrinol Metab. 2005;289:E296–300.
118. Bodenlenz M, Höfferer C, Magnes C, Schaller-Ammann R, Schaupp L, Feichtner F, Ratzer M, Pickl K, Sinner F, Wutte A, Korsatko S, Köhler G, Legat J, Benfeldt EM, Wright AM, Neddermann D, Jung T, Pieber TR. Dermal PK/PD of a lipophilic topical drug in psoriatic patients by continuous intradermal membrane-free sampling. Eur J Pharm Biopharm. 2012;81(3):635–41.
119. Ikeoka D, Pachler C, Korsatko S, Mader JK, Weinhandl H, Bodenlenz M, Plank J, Smolle KH, Ellmerer M, Pieber TR. Interleukin-6 produced in subcutaneous adipose tissue is linked to blood pressure control in septic patients. Cytokine. 2010;50(3):284–91.
120. Bodenlenz M, Aigner B, Dragatin C, Liebenberger L, Zahiragic S, Höfferer C, Birngruber T, Priedl J, Feichtner F, Schaupp L, Korsatko S, Ratzer M, Magnes C, Pieber TR, Sinner F. Clinical applicability of dOFM devices for dermal sampling. Skin Res Technol. 2013;19(4):474–83.
121. Pieber T, Birngruber T, Bodenlenz M, Höfferer C, Mautner S, Tiffner K, Sinner F. Open flow microperfusion: an alternative method to microdialysis? In: Microdialysis in drug development (M.Müller, ed.), AAPS Advances in the Pharmaceutical Sciences Series 2013. ISBN: 978-1-4614-4815-0

Chapter 10
Application of Microdialysis in Assessing Cutaneous Bioavailability

Eva Benfeldt

10.1 Introduction

In this chapter the focus is on microdialysis (MD) methodology with emphasis on cutaneous, i.e., dermal microdialysis (DMD) sampling. MD is an *in vivo* technique used for sampling endogenous and exogenous substances in the extracellular space, routinely used in the clinic as well as in research settings [13].

MD sampling methodology in the skin and subcutaneous tissue introduces a unique opportunity for *in vivo, ex vivo,* and *in vitro* studies of topical drug penetration, and the DMD method has gained ground in the last decade (for an in-depth review, see [20].

MD as a technique provides *in vivo* chronological, real-time information about the pharmacokinetics of drugs, obtained from the extracellular fluid phase at the site of action, i.e., the target tissue.

Today, there are over 14,000 publications describing the applications of MD in numerous tissues and therapeutic areas and there are over 600 publications on the applications of MD in the skin.

This chapter aims to provide a background for understanding the current position of DMD as one of several eligible methodologies for evaluation of bioequivalence of topical products, proposed by regulatory experts [29, 32].

E. Benfeldt (✉)
Department of Dermatology, Faculty of Health Sciences, University of Copenhagen, Roskilde Hospital, Koegevej 7-13, 4000 Roskilde, Denmark
e-mail: benfeldt@post5.tele.dk

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_10

10.2 The Methodology (Brief Description)

MD is a sampling technique that involves insertion of a MD probe in the tissue of interest with the aim of sampling and subsequently measuring local concentrations of the analyte in the sample.

A probe occurs in various sizes and shapes and consists of a cylindrical semipermeable membrane. The membrane is affixed on to nonpermeable tubings. The semipermeable membrane offers an exchange of solutes through the pores of the membrane. The probe is perfused with a physiological solution (called "perfusate"), which is pumped through the probe at a very slow flow-rate—typically between 1 and 5 μL/min. The slow flow rate is selected to prevent excessive periprobe drainage and/or loss of perfusate volume as well as drainage of molecules into or out of the tissue, thus minimizing any disturbance in the tissue microenvironment. The recovery (by dialysis) of a given drug or substance is independent of the concentration of the drug or substance in the tissue. The resulting solution that exits the probe is called "dialysate" and can be directly analyzed using a well validated bioanalytical method. For most probe types this dialysate is free of cells, larger molecules such as enzymes, and protein.

For a detailed review of the methodology, the probe types available, the calibration of probe efficacy, recovery evaluation, and analytical considerations, please consult Holmgaard et al. [20] for a comprehensive technical review.

10.3 Planning an *In Vivo* Bioequivalence Study

There are many choices while preparing for *in vivo* experimentation. The most important one is probably the study design itself, as the between-subjects variability will, for some topical applications, be much larger than the inter-subject variability. Whenever possible, a design using the opportunity for several test and reference areas in the same person should be chosen. Furthermore, the opportunity of pooling samples in collecting vials for larger sample volume as well as optimizing probe length/membrane area should be considered for low-recovery drugs.

10.3.1 Choice of Probe Type and Perfusate

Different types of probes for DMD are available [20] and the majority of these probes are inserted in the skin through a small guide cannula as the probes are made of fragile material. The linear probe has a unidirectional flow and compared to the other probe types has a very small diameter—down to approximately 200 μm for some structures. Linear probes will, however, need to penetrate the skin twice when inserted, since they have both an inlet and an outlet. The typical probe used in clinical studies relating to systemic drugs is the concentric probe which, like the

side-by-side and loop design probes, has an inlet and outlet placed in parallel with each other. The latter probe types require guide cannulas of larger dimensions (e.g., 500 μm) than those necessary for insertion of the linear probe, which leads to a larger insertion trauma (see later)—and this is a drawback.

In cutaneous MD, the perfusate is most often an isotonic saline solution or a Ringer solution. Depending on the lipophilicity of the drug, the perfusate medium may have to be modified to allow more lipophilic substances to enter the probe. Substances such as albumin, Intralipid®, and Encapsin® have been used [22, 46]. The enhancing effect of adding binding agents such as α, β, γ-cyclodextrins to the perfusate has been evaluated by studying the recovery of several eicosanoids *in vitro*. Similarly, the effect of adding small organic molecules such as ethanol, propylene glycol, and dimethylsulfoxide has been studied, and the inclusion of arachidonic acid in the perfusate has been shown to increase *in vitro* relative recovery for hydrophilic analytes [42].

10.3.2 Application Site

In cutaneous MD, the most frequently used area of application is the dermis of the volar forearm [3, 4, 6–8, 16–18, 35, 45].

The reason for this is twofold—this is the "standard area" for investigations into noninvasive measurements of skin blood flow and barrier perturbation. Secondly, the area is easily accessible, usually not hairy and not very convex. If there is a need for shaving, this should be done preferably 3 days before the experiment to prevent an artifact by skin barrier perturbation.

Only one arm is used for cutaneous MD at a time, since that arm must be at rest and tied up during the experiment. It is uncomfortable to have both arms tied up. Blood drawn from the other arm can provide measurements of plasma drug concentrations and thus the systemic drug delivery, if relevant.

10.3.3 Insertion Procedure, Trauma, and Exclusion Criteria

The probes are inserted under clean or sterile procedures after gentle wash of the skin area. To minimize the discomfort during insertion, local anesthesia [18] and application of ice packs onto the skin can be used [1, 24, 45]. The probes are inserted using a 19–23 G cannula as a guide, which is inserted horizontally in the dermis or subcutis. The probe is subsequently inserted in the opposite direction through the open tip of the hollow cannula and tested to secure the functionality. Subsequently, the guide cannula is withdrawn with the probe still in place. The accuracy of placing the probe at the intended depth in the skin depends on the training and experience of the laboratory personnel, and the depth should be measured routinely by ultrasound scanning as part of the experimental protocol, see Sect. 10.6.3.

MD is a minimally invasive technique, but the skin trauma and histamine release evoked by the insertion will cause a reversible increase in the local blood flow, increased skin thickness, and hyperemia in both animals and humans. The tissue trauma needs to subside before sampling can begin and an appropriate equilibration period of minimum 60–90 min in human skin is advisable. The hyperemia reaction lasts between 90 and 135 min, but complete normalization of the skin perfusion may not occur [28]. The use of local anesthesia has been found to reduce the trauma reaction in man [18].

The presence of a MD probe will elicit an inflammatory response after approximately 12 h; infiltration of lymphocytes will begin and after 32 h scar tissue may appear [2]. Following insertion of concentric probes more extensive tissue disruption compared to a linear probe has been reported. The larger reaction could be caused by the larger diameter of the guide cannula used for implantation of concentric probes (e.g., ~500 μm).

The tissue reactions are generally reversible and lasting tissue damage is negligible. However, all the cytokines and cells associated with, e.g., inflammatory disease are involved in the trauma reaction [41]. In my experience the development of skin changes, visible at clinical examination after, e.g., 3 months, is very rare. One exception is if the volunteer or patient is prone to keloid formation, and for this reason we recommend screening for this as an exclusion criterion of the study protocol, thus avoiding DMD in keloid-prone individuals.

10.4 Bioavailability

The bioavailability of a topically applied drug is defined as “the rate and extent to which the active ingredient or active moiety is absorbed and becomes available at the target site. For drugs that are not intended to be absorbed into the bloodstream, bioavailability reflects the rate and extent to which the active ingredient becomes available at the relevant site within the skin” as per FDA “Guidance for Industry” 2002. A recent study demonstrates the use of MD to determine the bioavailability in a study comparing orally administered diclofenac with topically administered diclofenac. Thus, the bioavailability at the target site was measured by placing the MD probe in the subcutaneous tissue as well as the muscular tissue. The relative bioavailability in both muscular and subcutaneous tissue after topical application was significantly higher than after oral administration and the measured plasma levels were much lower when the drug was topically administered compared to oral administration [3, 43, 44]. Similarly, the diffusion of ketoprofen from transdermal patch application to the knee joint fluid has been demonstrated in rats and pigs [38]. A recent study explores, by MD sampling in subcutaneous tissues and skeletal muscle, the effect of alterations in the galenic composition of four novel topical diclofenac formulations under development. An improved drug delivery to the tissue (2.7-fold improved) over the reference product, a commercially available diclofenac gel, was demonstrated by Brunner [11].

10.5 Bioequivalence

FDA describes bioequivalence as a comparative test between two products using specified criteria. Bioequivalence is defined as "the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study." However, the assessment of bioequivalence for locally acting and targeted delivery drugs has presented great challenges to science in the approval of generic drugs.

Kreilgaard et al. published the first human study demonstrating the potential of cutaneous MD for bioequivalence studies of topically applied drugs in 2001 [27]. The aim of that study was to evaluate the cutaneous bioequivalence of a lipophilic drug (lidocaine) applied in a novel topical microemulsion vehicle, compared to a conventional oil-in-water emulsion. Earlier, Kreilgaard had published an experimental study in rats documenting that dermal drug delivery of hydrophilic and lipophilic drugs was improved by microemulsion vehicles [26]. Subsequently, Kreilgaard proved that microemulsion vehicles can increase dermal drug delivery of lipophilic drugs in humans, and that the MD technique combined with an appropriate pharmacokinetic model provides high sensitivity in this bioequivalence study of a topically applied formulation.

When addressing the issue of DMD variability the source of variability in these studies can be extracted from data obtained in hairless rats (which are genetically inbred and of the same sex and age and thus have minimum variability). The increased variability observed in dermal sampling of the topically applied drugs can be seen to arise from interindividual variability in skin penetration kinetics/barrier function and or microcirculation, unrelated to the MD sampling methodology, which is followed by an internal calibrator simultaneously with the ongoing penetration process [40].

The comparability of dermato-pharmacokinetics, so-called tape-stripping methodology, and DMD was demonstrated in a recent study evaluating bioequivalence of lidocaine in ointment and cream [8]. The study showed agreement between the two methods, finding a 3–5 fold higher lidocaine absorption from the cream formulation over the ointment formulation. Statistical calculations from this study indicated that bioequivalence evaluation of topical formulations based on DMD sampling may be conducted using 27 subjects and applying two probes in each application site or 18 subjects using three probes in each application site with 90 % confidence interval and 80–125 % bioequivalence limits, which are the limits applicable to topical bioequivalence evaluations as per FDA protocols [38].

A theoretical-statistical MD paper found, based on data in the literature, that an evaluation of topical bioequivalence, conducted by duplicate sampling of both formulations in the same human volunteer, could be expected to be conclusive within 80–125 % confidence limits when a population of approximately 20 subjects was to be used [30]. This number of participants was similar to what was calculated

in the study on topical lidocaine formulations [8]. In the latter study, 61 % of the experimental variability could be ascribed to inter-subject variability—an important finding for planning of future studies. This result was corroborated by Tettey-Amlalo et al. who demonstrated a very low variability using the exact same probe structure for sampling drug penetration from a topical ketoprofen gel formulation [45]. However, different formulations may demonstrate different variabilities when the cutaneous penetration is studied by DMD; in a study of topical metronidazole creams the variability was higher and the number of participants for a conclusive bioequivalence evaluation would have been 34 [17]. Nevertheless, in comparison with the 6–700 subjects needed for a clinical comparative study, these numbers are small [29].

Another recent study has compared topical penetration of two commercially available tetracycline formulations and also found high variability [23], whereas a study of an aggressive ethanol solution of clobetasol propionate—a drug which it has previously not been feasible to sample by MD in the dermis—showed that using Intralipid ® as the perfusate the drug could now be reproducibly sampled [1].

Dermal MD sampling may also enable bioequivalence studies in diseased skin [6, 16, 36], which can be argued to be closer to the clinical situation than bioequivalence evaluations conducted in healthy subjects. However, variability is likely to be increased and possibly too problematic, see Sect. 10.6.1 below.

10.6 Sources of Variability

10.6.1 Skin Barrier Function

Damage of the skin barrier implies an increase in trans-epidermal water loss (TEWL) which can be quantified by measurements above the skin surface. In a number of *in vivo* DMD studies the impact of experimentally induced skin barrier perturbation on the cutaneous penetration of different substances has been demonstrated [8, 25, 31, 36]. Benfeldt et al. have studied the effect of different barrier disruption methods on cutaneous penetration in hairless rats as well as humans [5, 7]. The studies demonstrated highly increased drug penetration in tape stripped skin (157- and 170-fold respectively, in comparison to the penetration in unmodified skin) and in skin with irritant dermatitis (46- and 80-fold increased penetration). Since the same probe type, perfusate, flow rate, and topical drug solution were used in these studies, a direct comparison between drug penetration in hairless rats and human volunteers could be made. This showed a 46-fold increase in penetration across rat skin when compared with human skin, while increases in penetration, induced by barrier perturbation were of the same order of magnitude [3].

Other more recent studies have compared, e.g., the penetration of acyclovir and salicylic acid on disrupted skin barriers using MD in the dermis and tape stripping [25], and the penetration of a metronidazole cream formulation (1 %) applied to the

forearm skin in areas of both irritant dermatitis and normal skin [36]. Furthermore, DMD sampling showed a significant threefold increased penetration of topically applied metronidazole in skin with atopic dermatitis compared with unaffected skin [16].

10.6.2 Microcirculation

The bioavailability of topically applied products in skin and underlying tissues is not only dependent on the integrity of the skin barrier, but also on the local blood flow. Vasodilatation as well as vasoconstriction can be physiologically or pharmacologically induced and will have a large influence on the local blood flow. The skin concentration of a topically applied drug will increase if the blood flow is diminished, whereas an increased blood flow enhances the uptake and subsequent systemic distribution and elimination of the drug from the skin [2]. Experimental studies which induce vasoconstriction or vasodilatation have demonstrated that the bioavailability of topically as well as systemically applied test substances is highly influenced by changes in the microcirculation of the skin [9, 12, 37]—an influence much stronger than the influence of variations in probe depth.

Topically administered substances have been studied, with added noradrenaline for vasoconstriction and the nitric oxide donor glyceryltrinitrate for vasodilatation, delivered by the MD probe. The changes seen in the dialysate concentration reflected the changes in the microcirculation [10, 14].

10.6.3 Probe Depth in the Skin

An influence of probe depth on the drug levels sampled following topical drug application is likely from a theoretical point of view. The recommendation is to measure (normally by 20 MHz ultrasound scanning) the probe depth in three separate scans along the length of the probe *in situ.* With experience, probes can be inserted with great accuracy and low variability [8, 45], e.g., 0.7 ± 0.15 mm mean ± SD [36]. The preferable insertion depth is 0.6–1.0 mm in the dermis.

The original study regarding transdermal delivery of nicotine showed a correlation between depth and drug concentration, but only when the analysis included different skin layers (both dermal and subcutaneous probe placement) [33]. Following this, several studies have been unable to show a correlation between the depth of the probe and the drug concentration [8, 17, 19, 35, 36]. However, in a very recent MD study, conducted in *ex vivo* human donor skin, an inverse relationship between the depth of the probe in the dermis and the amount of drug sampled following topical penetration is demonstrated [21]. The result is of relevance to the *in vivo* situation, and it can be predicted that the differences in sampling at different probe depths will have a more significant impact in the beginning of a study or in studies of short duration.

Based on this study it can be recommended that studies of topical drug penetration using DMD sampling should include measurements of probe depth and that efforts should be made to minimize probe depth variability (e.g., to have few and similarly trained persons undertaking insertion as well as ultrasound scanning or other imaging technique for feedback of probe depth achieved) [21].

10.7 Advantages and Limitations

The methodological challenges that may influence *in vivo* experiments may typically be identified through well-planned *in vitro* experiments as a part of the planning phase. Founded on pre-experimental troubleshooting, conducted previous to *in vivo* experimentation, reproducible results with acceptable variability and validated analysis can be achieved for most drugs.

10.7.1 Advantages

- MD captures the pharmacological events where they take place in the tissues, providing high-resolution real-time details.
- There is no fluid extraction from the tissue.
- DMD sampling allows testing of both test and reference product at the same time in the same individuals.
- Both the drug of interest and the metabolites.
- The method can provide protein-free samples, which is often an analytical advantage.
- Cessation of enzyme degradation in the samples.
- DMD is a relatively inexpensive method to use once the MD pumps have been acquired.
- The probes allow sampling as well as delivery of substances.
- Multiple application sites.
- Good reproducibility.
- DMD sampling of topical drug formulation in the bioavailability/bioequivalence setting does not depend on drug concentrations in the formulations being the same.
- DMD sampling can be used in the presence of barrier perturbation or skin disease (unlike other methods for skin penetration assessment).

10.7.2 Limitations

- Drugs with a very high lipophilicity are excellent for topical application but less favorable for sampling by MD (the tape-stripping method will often be more suitable).

- An *in vitro* relative recovery of less than 4 % will most often characterize a compound as unsuitable for MD studies due to an expected even lower *in vivo* recovery.
- Some topical drug formulations are of very low drug concentrations—the analysis of the dialysate will unavoidably be very challenging.
- For protein-bound substances it is often necessary to add a protein to the perfusate to increase recovery, which will often result in more complex analytical procedures.
- Low variability in probe insertion and probe manufacturing depends on experienced personnel.
- The dialysate concentration will decrease with increasing flow rate and vice versa since the relative recovery of substances is flow-dependent. However, if very low flow rates are used,the time resolution can be compromised.
- Analytical procedures may require extensive modifications prior to *in vivo* experiments.
- Both drug concentrations in the tissue as well as recovery by MD sampling are known to be influenced by blood flow and this must be considered in the planning stage of an experiment.
- The duration of a DMD experiment is in many instances limited for practical reasons, and this creates a limitation for slowly penetrating substances (this can be overcome by the use of portable pumps, which in turn typically have relatively few syringe spaces).
- As a consequence of the above-mentioned relationships between topical dose, dermal concentration, and the ensuing concentration in the microdialysates, MD sampling in the skin will hardly ever be the right method for toxicological studies of low dose/real life skin exposures—other methods will be more relevant.

10.8 Future Research

A key issue in the development of a standardized protocol is the reproducible insertion of the probe at a consistent depth within the skin. Reproducible probe insertion is a skill which is only improved with practice. It is evident that implantation in either the superficial dermis or the subcutaneous tissue will affect the data collected. A thorough evaluation of this methodological issue, preferably studied under human *in vivo* conditions, using drugs of varying MW and lipophilicity, is needed.

Most MD studies are of limited time duration, typically less than 8 h, and histological studies of the skin response to probe implantation have not showed signs of tissue inflammation [36]. Studies with more extended observation and sampling periods have, however, demonstrated that infiltration of lymphocytes and even development of scar tissue may be observed over [36, 41].

Another concern, particularly relevant to DMD, is the use of MD in studies of inflammatory and immune-mediated diseases. Here the molecules of interest will very often be the same as those generated during the insertion trauma and

subsequent wound repair. Care should be taken to incorporate the proper controls in order to confirm the correct relation between molecule, implantation trauma, and disease process [41] Studies of the exact relation between probe implantation (depth, diameter, time since implantation) and the ensuing tissue damage will be important in the further development of the MD methodology, also from an ethical point of view.

In vitro drug penetration studies using static or flow-through diffusion cells have been extensively used in the past, and there is a pertinent need to establish a correlation between results from *in vitro* penetration studies of topical drugs and *in vivo* data obtained by MD methodology.

Only few MD studies in diseased skin have been performed. An improved knowledge of the impact of the structural changes in diseased or otherwise impaired skin on topical drug penetration *in vivo* will improve the development of topical therapies. Furthermore, the relevance is supported by the fact that a substantial fraction of the general population suffer from different skin conditions that make the skin barrier potentially more permeable—an aspect relevant also to the occupational setting [15].

MD methodology has a place among other methodologies employed in drug research, preclinical test phases, and clinical studies as well as for use in industry for regulatory approval purposes. The potential for studies of bioequivalence of topical formulations and in evaluation of line extensions of topical is huge.

Whether the method is employed for sampling of pharmacokinetic or pharmacodynamic information, MD in the skin as a technique offers a unique opportunity for real-time chronological sampling in the target tissue.

References

1. Au WL, Skinner MF, Benfeldt E, Verbeeck RK, Kanfer I. Application of dermal microdialysis for the determination of bioavailability of clobetasol propionate applied to the skin of human subjects. Skin Pharmacol Physiol. 2012;25(1):17–24. doi:10.1159/000330489.
2. Ault JM, Riley CM, Meltzer NM, Lunte CE. Dermal microdialysis sampling in vivo. Pharm Res. 1994;11(11):1631–9.
3. Benfeldt E. In vivo microdialysis for the investigation of drug levels in the dermis and the effect of barrier perturbation on cutaneous drug penetration. Studies in hairless rats and human subjects. Acta Derm Venereol. 1999;206:1–59. http://www.ncbi.nlm.nih.gov/pubmed/10605601.
4. Benfeldt E, Groth L. Feasibility of measuring lipophilic or protein-bound drugs in the dermis by in vivo microdialysis after topical or systemic drug administration. Acta Derm Venereol. 1998;78(4):274–8. http://www.ncbi.nlm.nih.gov/pubmed/9689295.
5. Benfeldt E, Serup J. Effect of barrier perturbation on cutaneous penetration of salicylic acid in hairless rats: in vivo pharmacokinetics using microdialysis and non-invasive quantification of barrier function. Arch Dermatol Res. 1999;291(9):517–26. http://www.ncbi.nlm.nih.gov/pubmed/10541883.
6. Benfeldt E, Serup J, Menné T. Effect of barrier perturbation on cutaneous salicylic acid penetration in human skin: in vivo pharmacokinetics using microdialysis and non-invasive quantification of barrier function. Br J Dermatol. 1999a;140(4):739–48. http://www.ncbi.nlm.nih.gov/pubmed/10233334.

7. Benfeldt E, Serup J, Menné T. Microdialysis vs. suction blister technique for in vivo sampling of pharmacokinetics in the human dermis. Acta Derm Venereol. 1999b;79(5):338–42. http://www.ncbi.nlm.nih.gov/pubmed/10494706.
8. Benfeldt E, Hansen SH, Vølund A, Menné T, Shah VP. Bioequivalence of topical formulations in humans: evaluation by dermal microdialysis sampling and the dermatopharmacokinetic method. J Invest Dermatol. 2007;127(1):170–8. doi:10.1038/sj.jid.5700495.
9. Borg N, Götharson E, Benfeldt E, Groth L, Ståhle L. Distribution to the skin of penciclovir after oral famciclovir administration in healthy volunteers: comparison of the suction blister technique and cutaneous microdialysis. Acta Derm-Venereol. 1999;79(4):274–7. http://www.ncbi.nlm.nih.gov/pubmed/10429982.
10. Boutsiouki P, Thompson J, Clough G. Effects of local blood flow on the percutaneous absorption of the organophosphorus compound malathion: a microdialysis study in man. Arch Toxicol. 2001;75(6):321–8. doi:10.1007/s002040100245.
11. Brunner M, Davies D, Martin W, Leuratti C, Lackner E, Müller M. A new topical formulation enhances relative diclofenac bioavailability in healthy male subjects. Br J Clin Pharmacol. 2011;71(6):852–9. doi:10.1111/j.1365-2125.2011.03914.x.
12. Chaturvedula A, Joshi DP, Anderson C, Morris R, Sembrowich WL, Banga AK. Dermal, subdermal, and systemic concentrations of granisetron by iontophoretic delivery. Pharm Res. 2005;22(8):1313–9. doi:10.1007/s11095-005-5335-z.
13. Chaurasia CS, Müller M, Bashaw ED, Benfeldt E, Bolinder J, Bullock R, Bungay PM, et al. AAPS-FDA workshop white paper: microdialysis principles, application and regulatory perspectives. Pharm Res. 2007;24(5):1014–25. http://www.ncbi.nlm.nih.gov/pubmed/17458685.
14. Clough GF, Boutsiouki P, Church MK, Michel CC. Effects of blood flow on the in vivo recovery of a small diffusible molecule by microdialysis in human skin. J Pharmacol Exp Ther. 2002;302(2):681–6. doi:10.1124/jpet.102.035634.sampling
15. Fulzele SV, Babu RJ, Ahaghotu E, Singh M. Estimation of proinflammatory biomarkers of skin irritation by dermal microdialysis following exposure with irritant chemicals. Toxicol. 2007;237(1–3):77–88. http://www.ncbi.nlm.nih.gov/pubmed/17574719.
16. Garcia Ortiz P, Hansen SH, Shah VP, Menné T, Benfeldt E. Impact of adult atopic dermatitis on topical drug penetration: assessment by cutaneous microdialysis and tape stripping. Acta Derm Venereol. 2009;89(1):33–8. doi:10.2340/00015555-0562.
17. García Ortiz P, Hansen SH, Shah VP, Sonne J, Benfeldt E. Are marketed topical metronidazole creams bioequivalent? Evaluation by in vivo microdialysis sampling and tape stripping methodology. Skin Pharmacol Physiol. 2011;24(1):44–53. doi:10.1159/000320151.
18. Groth L, Serup J. Cutaneous microdialysis in man: effects of needle insertion trauma and anaesthesia on skin perfusion, erythema and skin thickness. Acta Derm-Venereol. 1998;78(1):5–9. http://www.ncbi.nlm.nih.gov/pubmed/9498017.
19. Hegemann L, Forstinger C, Partsch B. Microdialysis in cutaneous pharmacology: kinetic analysis of transdermally delivered nicotine. J Invest Dermatol. 1995;104(5):838–43. http://www.nature.com/jid/journal/v104/n5/abs/5610897a.html.
20. Holmgaard R, Nielsen JB, Benfeldt E. Microdialysis sampling for investigations of bioavailability and bioequivalence of topically administered drugs: current state and future perspectives. Skin Pharmacol Physiol. 2010;23(5):225–43. doi:10.1159/000314698.
21. Holmgaard R, Benfeldt E, Bangsgaard N, Sorensen JA, Brosen K, Nielsen F, Nielsen JB. Probe depth matters in dermal microdialysis sampling of benzoic acid after topical application: an ex vivo study in human skin. Skin Pharmacol Physiol. 2012;25(1):9–16. doi:10.1159/000330491.
22. Holmgaard R, Benfeldt E, Nielsen JB, Gatschelhofer C, Sorensen JA, Höfferer C, Bodenlenz M, et al. Comparison of open-flow microperfusion and microdialysis methodologies when sampling topically applied fentanyl and benzoic acid in human dermis ex vivo. Pharm Res. 2012;29(7):1808–20. doi:10.1007/s11095-012-0705-9.
23. Incecayir T, Agabeyoglu I, Derici U, Sindel S. Assessment of topical bioequivalence using dermal microdialysis and tape stripping methods. Pharm Res. 2011;28(9):2165–75. doi:10.1007/s11095-011-0444-3.

24. Kellogg DL, Zhao JL, Wu Y. Neuronal nitric oxide synthase control mechanisms in the cutaneous vasculature of humans in vivo. J Physiol. 2008;586(3):847–57. doi:10.1113/jphysiol.2007.144642.
25. Klimowicz A, Farfal S, Bielecka-Grzela S. Evaluation of skin penetration of topically applied drugs in humans by cutaneous microdialysis: acyclovir vs. salicylic acid. J Clin Pharm Ther. 2007;32(2):143–8. doi:10.1111/j.1365-2710.2007.00803.x.
26. Kreilgaard M. Dermal pharmacokinetics of microemulsion formulations determined by in vivo microdialysis. Pharm Res. 2001;18(3):367–73. http://www.springerlink.com/index/j42w1130m307g658.pdf.
27. Kreilgaard M, Kemme MJ, Burggraaf J, Schoemaker RC, Cohen AF. Influence of a microemulsion vehicle on cutaneous bioequivalence of a lipophilic model drug assessed by microdialysis and pharmacodynamics. Pharm Res. 2001;18(5):593–9. http://www.ncbi.nlm.nih.gov/pubmed/11465413.
28. Krogstad A, Lonnroth P, Larson G, Wallin BG. Increased interstitial histamine concentration in the psoriatic plaque. J Invest Dermatol. 1997;109:632–5. http://www.nature.com/jid/journal/v109/n5/abs/5610032a.html.
29. Lionberger RA. FDA critical path initiatives: opportunities for generic drug development. AAPS J. 2008;10(1):103–9. doi:10.1208/s12248-008-9010-2.
30. McCleverty D, Lyons R, Henry B. Microdialysis sampling and the clinical determination of topical dermal bioequivalence. Int J Pharm. 2006;308(1–2):1–7. doi:10.1016/j.ijpharm.2005.09.020.
31. Morgan CJ, Renwick AG, Friedmann PS. The role of stratum corneum and dermal microvascular perfusion in penetration and tissue levels of water-soluble drugs investigated by microdialysis. Br J Dermatol. 2001;148(3):434–43.
32. Mugglestone CJ, Mariz S, Lane ME. The development and registration of topical pharmaceuticals. Int J Pharm. 2012;435(1):22–6. doi:10.1016/j.ijpharm.2012.03.052.
33. Müller M, Schmid R, Wagner O, Osten v. B., Shayganfar H, Eichler GH. In vivo characterization of transdermal drug transport by microdialysis. J Control Release. 1995;37:49–57
34. Müller M, Mascher H, Kikuta C, Schäfer S, Brunner M, Dorner G, Eichler HG. Diclofenac concentrations in defined tissue layers after topical administration. Clin Pharmacol Ther. 1997;62(3):293–9. doi:10.1016/S0009-9236(97)90032-1.
35. Ortiz PG, Hansen SH, Shah VP, Menné T, Benfeldt E. The effect of irritant dermatitis on cutaneous bioavailability of a metronidazole formulation, investigated by microdialysis and dermatopharmacokinetic method. Contact Dermat. 2008;59(1):23–30. doi:10.1111/j.1600-0536.2008.01348.x.
36. Sandberg C, Halldin CB, Ericson MB, Larkö O, Krogstad AL, Wennberg AM. Bioavailability of aminolaevulinic acid and methylaminolaevulinate in basal cell carcinomas: a perfusion study using microdialysis in vivo. Br J Dermatol. 2008;159(5):1170–6. doi:10.1111/j.1365-2133.2008.08795.x.
37. Seki T, Wang A, Yuan D, Saso Y, Hosoya O, Chono S, Morimoto K. Excised porcine skin experimental systems to validate quantitative microdialysis methods for determination of drugs in skin after topical application. J Control Release. 2004;100(2):181–9. doi:10.1016/j.jconrel.2004.08.016.
38. Shah V, Flynn G, Yacobi A. Bioequivalence of topical dermatological dosage forms-methods of evaluation of bioequivalence. Skin Pharmacol Appl Skin Physiol. 1998;11(2):117–24. http://www.karger.com/Article/Fulltext/29817.
39. Shinkai N, Korenaga K, Okumura Y, Mizu H, Yamauchi H. Microdialysis assessment of percutaneous penetration of ketoprofen after transdermal administration to hairless rats and domestic pigs. Eur J Pharm Biopharm. 2011;78(3):415–21. doi:10.1016/j.ejpb.2011.03.005
40. Simonsen L, Jørgensen A, Benfeldt E, Groth L. Differentiated in vivo skin penetration of salicylic compounds in hairless rats measured by cutaneous microdialysis. Eur J Pharm Sci. 2004;21(2–3):379–88. doi:10.1016/j.ejps.2003.11.004.

41. Stenken JA, Church, MK, Gill CA, Clough GF. How minimally invasive is microdialysis sampling? A cautionary note for cytokine collection in human skin and other clinical studies. AAPS J. 2010;12(1):73–8. doi:10.1208/s12248-009-9163-7.
42. Sun L, Stenken JA. Improving microdialysis extraction efficiency of lipophilic eicosanoids. J Pharm Biomed Anal. 2003;33(5):1059–71. doi:10.1016/S0731-7085(03)00363-7.
43. Tegeder I, Muth-Selbach U, Lötsch J, Rüsing G, Oelkers R, Brune K, Meller S, Kelm GR, Sörgel F, Geisslinger G. Application of microdialysis for the determination of muscle and subcutaneous tissue concentrations after oral and topical ibuprofen. Clin Pharmacol Therapeut. 1999;(65):357–68. doi:10.1016/S0009-9236(99)70128-1.
44. Tegeder I, Lötsch J, Kinzig-Schippers M, Sörgel F, Kelm GR, Meller ST, Geisslinger G. Comparison of tissue concentrations after intramuscular and topical administration of ketoprofen. Pharm Res. 2001;18(7):980–6. http://www.ncbi.nlm.nih.gov/pubmed/11496958.
45. Tettey-Amlalo RNO, Kanfer I, Skinner MF, Benfeldt E, Verbeeck RK. Application of dermal microdialysis for the evaluation of bioequivalence of a ketoprofen topical gel. Eur J Pharma Sci. 2009;36(2–3):219–25. doi:10.1016/j.ejps.2008.09.002.
46. Ward KW, Medina SJ, Portelli ST, Mahar Doan KM, Spengler MD, Ben MM, Lundberg D, et al. Enhancement of in vitro and in vivo microdialysis recovery of SB-265123 using Intralipid and Encapsin as perfusates. Biopharm Drug Dispos. 2003;24(1):17–25. doi:10.1002/bdd.332.

Part IV
Bioequivalence: Targeted Drug Delivery

Chapter 11
Follicular Drug Penetration

Jürgen Lademann and Alexa Patzelt

11.1 Introduction

There is continuous effort to increase the penetration of topically applied substances (drugs and cosmetics) into and through the skin, which, however, represents a strong barrier mainly supported by the stratum corneum, the upper layer of the skin. Endeavors to increase the penetration include the application of physical and mechanical manipulations to reduce the barrier function [5] or to facilitate the access via the physiologically available penetration pathways which include the intercellular, the intracellular, and the follicular route.

Whereas the intracellular penetration pathway seems to be of minor interest as a delivery route, the intercellular pathway around the corneocytes within the lipid layers was considered as the only relevant one for a long time.

It is only during the past few years that the follicular penetration pathway has attracted considerable interest, although the influence of hair follicles on the dermal absorption rate had already been detected significantly earlier [4].

In the absence of quantitative model systems, which were definitely free from follicles but still exhibited the structural, biochemical, and barrier properties of normal skin, the investigations into the influence of the hair follicle on the penetration rate presented the greatest challenge. The utilization of scarred and immature skin as performed in the 1990s [7, 26] fulfilled the criteria of follicular absence; however, structural and barrier properties are supposed to be significantly different in these skin models. Also, the utilization of the sandwich model developed by Barry et al. [1], where the top skin layer blocks the shunts in the bottom layer, cannot be considered as an appropriate model as the resulting skin is twice as thick.

J. Lademann (✉) · A. Patzelt
Departments of Dermatology, Venereology and Allergology, and Skin Physiology, Charité—Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany
e-mail: juergen.lademann@charite.de

A. Patzelt
e-mail: alexa.patzelt@charite.de

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_11

Currently, most penetration studies focusing on intercellular or follicular penetration are performed in *ex vivo* or *in vitro* setups in diffusion cells, although there is increasing evidence of lacking *in vitro–in vivo* correlations [20] and inadequateness of diffusion cell experiments for follicular penetration studies [19]. In the meantime, different methods have been introduced, which are suitable for quantitative investigation of the follicular penetration pathway. Next to the selective artificial hair follicle closing technique and the differential stripping technique, which allow the quantitative estimation of the transfollicular and intrafollicular penetration, respectively, novel optical devices are also available that are useful tools for investigating the follicular penetration qualitatively and quantitatively as recently summarized by Meidan et al. [15].

Whereas follicular penetration is a complex process that has not been completely clarified yet, it is understood that this process has to be divided at least into two parts: in the first step, penetration into the hair follicle can be observed. In the second, a transfollicular penetration step can follow, which seems to depend mainly on the size of the substance applied. For the intercellular penetration process, the 500 Da rule has been suggested above which substances are not able to overcome the intact stratum corneum barrier [2]. The threshold below which transfollicular penetration occurs has still to be defined.

This threshold is of significant importance concerning the risk assessment of nanoparticles and other particulate substances that are continuously suspected of inducing harmful interactions with the living cells of an organism. The relevance of the transfollicular penetration pathway for smaller nonparticulate substances has been demonstrated by Otberg et al. [18]. They showed that after blocking the follicular pathway selectively, the absorption of caffeine into the blood circulation took four times longer than in the case of open hair follicles.

In contrast, for particulate substances, a transfollicular penetration into the blood circulation or the surrounding living skin has not been observed yet, as long as the skin barrier is intact. This aspect makes particles interesting as drug delivery systems into the hair follicle which, in turn, hosts similarly interesting target sites such as the stem cells, which are located in the bulge region of the hair follicle. Also, a dense network of capillaries and dendritic cells surrounding the hair follicle closely turns the hair follicle into an attractive target site.

11.2 Influence of Follicular Morphology, Density, and Activity Status on the Follicular Penetration Process

Each human individual displays an estimated number of 5 million hair follicles [9] which can theoretically contribute to the penetration process of topically applied substances.

Especially the infundibulum of the hair follicle, which is its upper part, represents an area of additional absorption and increases the surface area of the skin partly significantly. The follicular influence on the absorption process was shown to

depend mainly on the follicular size, density, and activity status of the hair follicle, which differs across different skin regions. The highest follicular density could be detected in the region of the forehead whereas the calf region exhibits the largest hair follicles. In both of these regions, the hair follicles cover about 10% of the total skin surface with a reservoir capacity that is comparable to the reservoir of the stratum corneum [17].

Also, the activity status of the hair follicle plays an interesting role in determining whether or not the single hair follicle is receptive for topically applied substances. Each hair follicle undergoes continuous cycling and during each cycle, the hair follicle experiences substantial changes in the immune and gene expression status and vascular supply [8]. A recent study showed that hair follicles were only receptive for topically applied substances if sebum flow and/or hair growth were detectable. Hair follicles were unresponsive if neither sebum flow nor hair growth were present. In these cases, a cover consisting of dry sebum, desquamated corneocytes, and other cell detritus were detected in the follicular openings. In the region of the upper forearm, approximately one fourth of the hair follicles were unreceptive for penetration in this study. [16].

11.3 Measuring Methods for Investigating the Penetration of Topically Applied Substances into the Hair Follicles

As mentioned above, several methods exist allowing a reasonable investigation of the follicular penetration process although some methods still provide some disadvantages, which have to be considered when analyzing the results.

The analysis of histological investigations, e.g., is frequently applied to investigate the penetration of topically applied fluorescent or fluorescence-labeled substances into the skin and the hair follicles by laser scanning microscopy and can be effectively utilized to determine the follicular penetration depth of substances as depicted in Fig. 11.1. Quantitative analysis is feasible with limitations measuring the fluorescence intensity. This methodology, however, has the clear disadvantage that it is invasive as the removal of biopsies is indispensable and time consuming. A further limitation is that the model skin utilized for this kind of investigation has to be selected with caution as there is increasing evidence of lack of *in vitro–in vivo* correlations in several skin models, which is discussed in paragraph 4.

Another alternative method is *in vivo* laser scanning microscopy as depicted in Fig. 11.2 which likewise allows the determination of the penetration of dye-labeled or fluorescent substances *in vivo* [13]. Unfortunately, with this method, only qualitative estimations are possible in the upper part of the hair follicles as the penetration depth of the laser radiation into the tissue is limited to approximately 150 µm. In contrast, the differential stripping method represents a quantitative, noninvasive *in vivo* procedure for the determination of the penetration of topically applied substances into the stratum corneum and the hair follicle [24]. The differential stripping

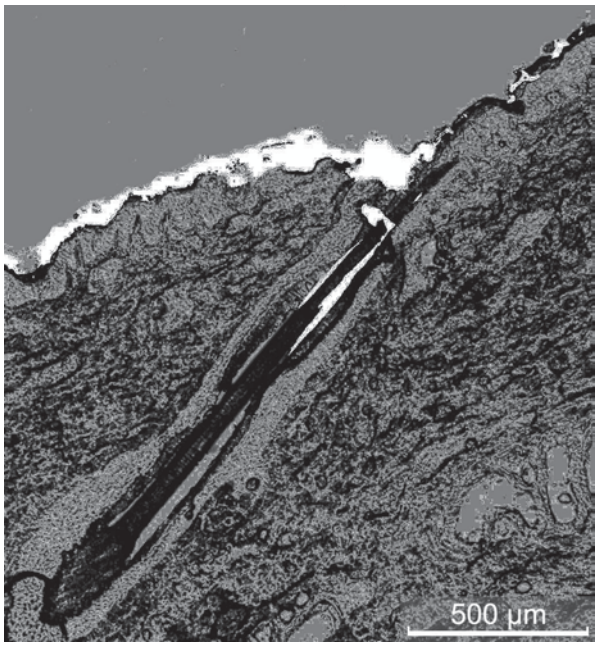


Fig. 11.1 Histological section demonstrating the penetration of a topically applied substance into a hair follicle analyzed by fluorescence measurements using laser scanning microscopy

method combines the tape stripping technique to remove the stratum corneum and the cyanoacrylate skin surface biopsy to extract the follicular content. The tape strips and the cyanoacrylate biopsies allow a selective quantification of the topically applied substance having penetrated into the stratum corneum or the hair follicle by classical analytical methods. A schematic of the method is depicted in Fig. 11.3.

The determination of follicular penetration into the hair follicle has to be strictly separated from the analysis of transfollicular penetration of substances through the hair follicle to the surrounding living tissue, which requires specific analytical methods. One option is the artificial closing of the hair follicles where the follicular pathway is selectively blocked by sealing the follicular orifices [25]. Comparing the penetration rate of topically applied substances through skin with open and closed hair follicles, respectively, allows the calculation of the follicular penetration rate [27].

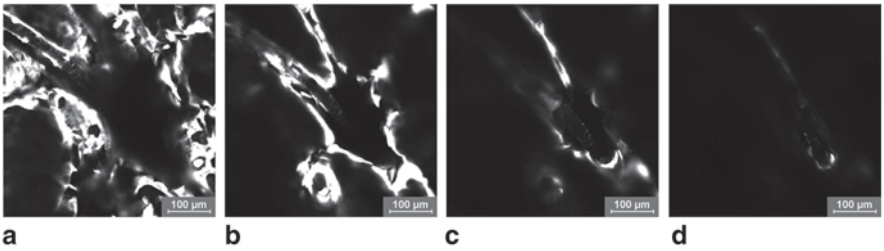


Fig. 11.2 *In vivo* analysis of the penetration of a fluorescent dye into different depths of the hair follicles by *in vivo* laser scanning microscopy (a: 0 µm; b: 50 µm; c: 80 µm; d: 110 µm)

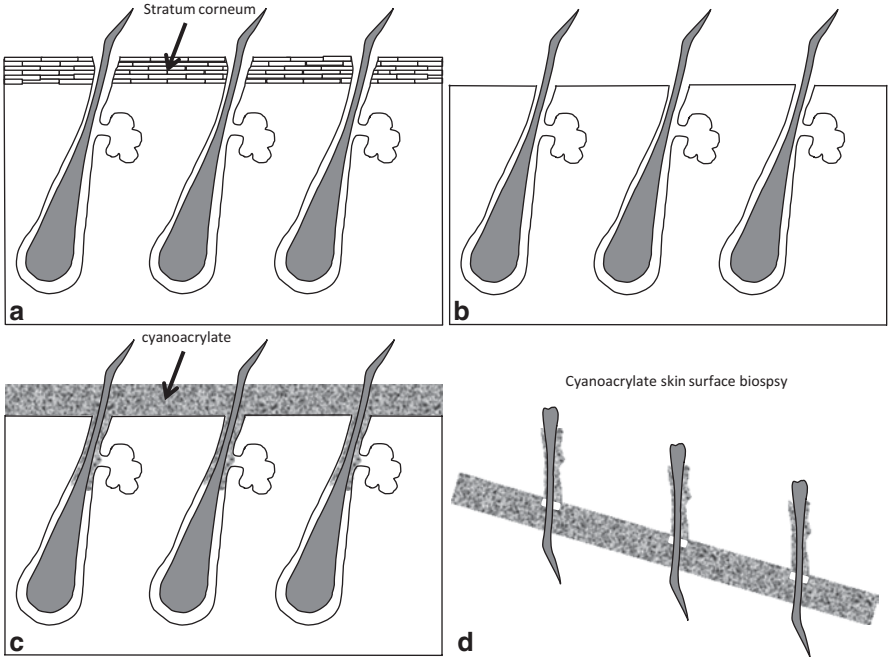


Fig. 11.3 Schematic of the differential stripping procedure: **a** an intact stratum corneum (SC) and hair follicle (HF), the topically applied substance (TAS) has penetrated into the SC and HF, **b** the SC has been removed by tape stripping, the TAS is only located in the HF, **c** cyanoacrylate is applied onto the stripped skin surface, **d** after polymerization of the cyanoacrylate, it is stripped off and contains the follicular content

11.4 Skin Models for the Analysis of Follicular Penetration

The appropriate skin models for each of the above described methods have to be selected with caution as each method has its specific limitations. A previous investigation, e.g., showed that the follicular reservoir in excised skin is only 10 % of the follicular reservoir *in vivo* of the same volunteer and the same skin site suggesting that the elastic fibers surrounding the hair follicles contract irreversibly immediately after excision [20]. Thus, the utilization of excised skin cannot be recommended as appropriate skin model for histological investigations on follicular penetration. On the contrary, the intact porcine ear has been demonstrated to be a reasonable alternative as during the penetration experiments the skin remains fixed on the ear cartilage, which prevents the skin from contracting [13]. Although the porcine skin provides somewhat larger hair follicles than human skin, the porcine ear model has been well established for follicular penetration studies [19].

The follicular contraction effect seems to be a reasonable explanation why diffusion cell experiments are still adequate procedures to investigate intercellular penetration. As mostly split or full epidermis skin is used in these experiments, the subcutaneous fatty tissue is to be removed from the samples. This means that the bottom of the hair follicle, which extends deeply into the subcutaneous fatty tissue, is open and represents a channel into the receptor medium. Similar concerns have also been raised by Senzui et al. [23]. Due to the follicular contraction this effect seems to be decreased.

11.5 Particles, Follicular Penetration, and Safety Aspects

Drug delivery to and through the hair follicles seems to depend on several aspects, including the physicochemical properties of the applied substances and the activity status of the hair follicles as mentioned above.

With regard to the physicochemical properties of the applied substance the characteristics of both the vehicle as well as the active substance obviously play a role [19], whereas the size of the active substance seems to be a predominant parameter.

In the recent years, particulate substances such as liposomes, micro- and nanoparticles were demonstrated to be excellent drug transporters into the hair follicles, exceeding by far the follicular penetration depth of nonparticulate substances.

This effect, however, was only realized when the application protocol of the substance included a massage. Lademann et al. [12] hypothesized that the surface structure of the hair and the hair follicle, which is determined by the thickness of the keratin cells, might act as a ratchet transporting the particles deeply into the hair follicle. The massage application seems to stimulate this ratchet effect *in vitro* whereas *in vivo*, a physiological movement of the hair occurs.

In their study, Lademann et al. [12] also hypothesized that a similar keratin cell thickness and particle size might be advantageous with regard to follicular penetration depth. This assumption was supported by our own investigations demonstrating that particles in the size range from 400 to 700 nm penetrated significantly deeper into the hair follicles than smaller or larger particles as depicted in Fig. 11.4 [21]. In comparison, the thickness of the keratin cells is about 530 nm in human hair [12] and 320 nm in porcine hair, which is in the same range.

These results are of relevance for drug delivery as by the selection of different particle sizes different sites within the hair follicle can be targeted selectively. Sites of special interest can be localized in the infundibulum region, which is surrounded by dense capillary network and dendritic cells, the sebaceous gland and the bulge region where the stem cells are located [21].

Whereas the penetration of particulate substances into the follicular duct has been well investigated, there is still controversy as to whether or not such particulate substances are able to overcome the stratum corneum or to penetrate transfollicularly into the deeper skin layers.

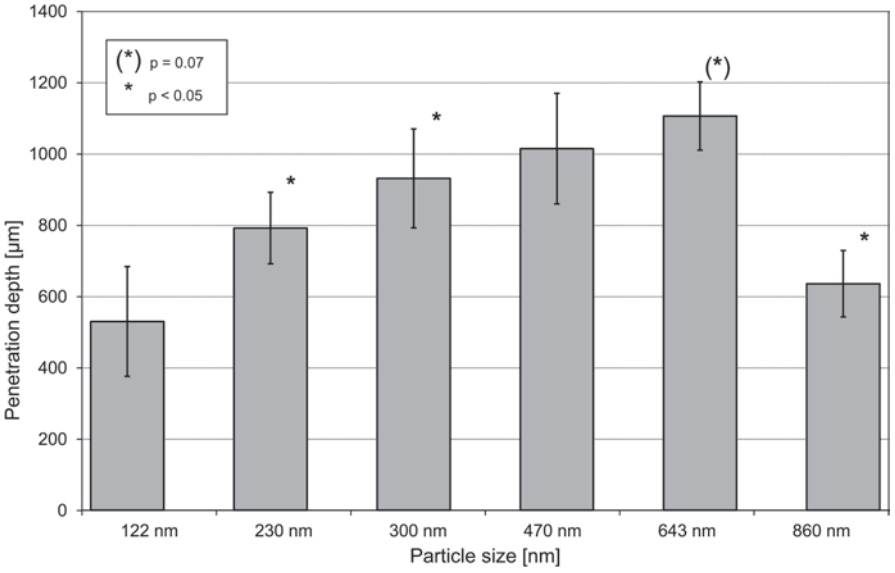


Fig. 11.4 Influence of the particle size on the follicular penetration depth. Data originating from Patzelt et al. [21]

Recently, Labouta et al. [10] reviewed the corresponding literature and found several studies reporting particle penetration or permeation into the living tissue. However, considering the utilized models and methods more precisely it was found that in most of these studies, either penetration enhancers disturbing the skin barrier properties or inadequate model systems such as excised skin were utilized. In the latter case, a violation of the distal hair follicle has to be assumed leading to a direct diffusion into the receptor medium as discussed above. Whereas the contraction of the skin might be able to prevent the diffusion of larger particles only a reduction can be assumed for smaller particles which, then, are erroneously interpreted as having penetrated.

However, the review could not reveal the existence of any study performed *in vivo* on intact human skin that demonstrated transfollicular or intercellular penetration of any particle.

Although this issue is not yet fully explored and clear size thresholds, below which transfollicular or intercellular penetration of particles is feasible, are still missing, there are clear indications that particles larger than 100 nm are not able to overcome the intact skin barrier neither via the stratum corneum nor the hair follicles. These indications are supported by the fact that although particles have been identified as excellent drug transporters, there are still no commercially available products that are based on this principle.

11.6 The Hair Follicle as a Long-Term Reservoir

As previously discussed, transfollicular penetration of especially larger particulate substances is rather unlikely; however, it still remains to be clarified what happens to the particles once they have penetrated deeply into the hair follicle. The hair follicle as a physiological hole in the skin was demonstrated to be a well-protected long-term reservoir hosting the particles over a period of 10 days, whereas the unprotected stratum corneum reservoir was already depleted after 1 day, presumably due to water and textile contact and desquamation [11]. A retrograde depletion of the hair follicle, in contrast, seems to be associated only with slow processes such as hair growth and sebum flow.

11.7 Triggered Release from Particles in the Hair Follicles

The hair follicle can be summarized as an interesting target site for drug delivery due to the above mentioned reasons; however, the problem is that nonparticulate substances are able to penetrate transfollicularly but do not reach significant depths and therefore targets within the hair follicle. In contrast, particles are able to penetrate deeply into the hair follicle close to diverse target sites of interest and have further advantages such as the possibility of sustained release, high surface-to-volume ratio and protection of the active substance from early degradation. However, their big limitation is that they are not able to pass transfollicularly (Fig. 11.5).

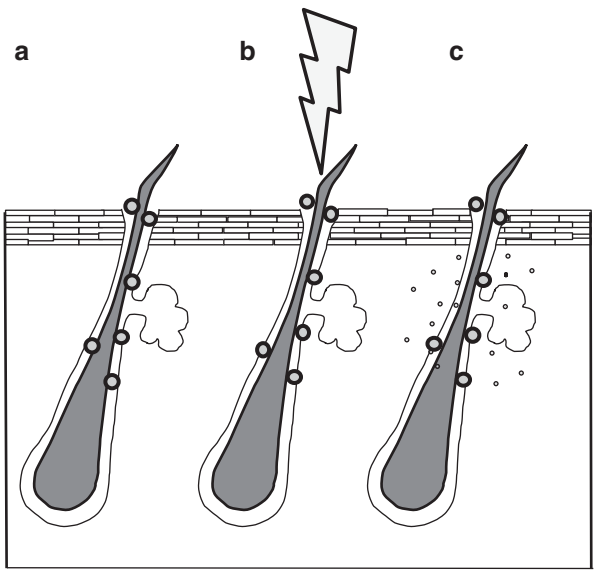


Fig. 11.5 Schematic of the triggered drug release approach. **a** The delivering particles enter the HF and penetrate deeply into the HF. **b** An external or internal trigger induces the release of the active substance from the particles. **c** The active substance translocates independently to the viable tissue

To overcome this problem, the particles have to be equipped with a specific release mechanism. After delivering therapeutic substances deeply into the hair follicles these are released actively and can subsequently translocate independently to their target site, e.g., the viable skin.

Applying this triggered release concept, it is important to quickly release the drug from the particles onto the specific target structure at the appropriate time [14].

Currently, a variety of release triggers are under investigation, including external stimulation factors such as radiofrequency [3], ultrasound [6], light [22], or enzymatic reactions [14], or internal stimulation factors such as pH [28].

11.8 Summary

Human hair follicles are an important target structure for drug delivery that can have a significant influence on the overall penetration and absorption process providing a reservoir comparable to that of the stratum corneum in some body regions. Moreover, compared to the stratum corneum, the hair follicles act as long-term reservoir for topically applied drugs; the hair follicle representing a protected cavity that prevents the content from being depleted easily.

Recent investigations have demonstrated that particulate substances penetrate significantly deeper into the hair follicle than nonparticulate substances; however, a translocation of the particles and thus their loaded drugs into the living tissue is not feasible due to the size of the particles. Currently, several attempts are being made to utilize the positive properties of particles, such as high loading capacity due to the high surface-to-volume ratio and deep follicular penetration, also for drug delivery through the follicular barrier. A promising approach seems to be the triggered release concept where the particles are equipped with a specific release system and only serve as drug transporters. After having reached the desired target region, a trigger signal activates the release system allowing the active drug to be released from the particles and to translocate independently into, e.g., the viable skin.

Reference

1. Barry BW. Drug delivery routes in skin: a novel approach. Adv Drug Deliv Rev. 2002;54(Suppl 1):31–40.
2. Bos JD, Meinardi MM. The 500 Dalton rule for the skin penetration of chemical compounds and drugs. Exp Dermatol. 2000;9(3):165–9.
3. Brazel CS. Magnetothermally responsive nanomaterials: combining magnetic nanostructures and thermally-sensitive polymers for triggered drug release. Pharm Res. 2009;26(3):644–56. doi:10.1007/s11095-008-9773-2.
4. Feldmann RJ, Maibach HI. Regional variation in percutaneous penetration of 14C cortisol in man. J Invest Dermatol. 1967;48(2):181–3.
5. Helmstadter A. Endermatic, epidermatic, enepidermatic-the early history of penetration enhancers. Int J Pharm. 2011;416(1):12–5. doi:10.1016/j.ijpharm.2011.06.005.

6. Huang SL. Liposomes in ultrasonic drug and gene delivery. Adv Drug Deliv Rev. 2008;60(10):1167–76. doi:10.1016/j.addr.2008.03.003.
7. Hueber F, Besnard M, Schaefer H, Wepierre J. Percutaneous absorption of estradiol and progesterone in normal and appendage-free skin of the hairless rat: lack of importance of nutritional blood flow. Skin Pharmacol. 1994;7(5):245–56.
8. Knorr F, Lademann J, Patzelt A, Sterry W, Blume-Peytavi U, Vogt A. Follicular transport route—research progress and future perspectives. Eur J Pharm Biopharm. 2009;71(2):173–80. doi:10.1016/j.ejpb.2008.11.001.
9. Krause K, Foitzik K. Biology of the hair follicle: the basics. Semin Cutan Med Surg. 2006;25(1):2–10. doi:10.1016/j.sder.2006.01.002.
10. Labouta HI, Schneider M. Interaction of inorganic nanoparticles with the skin barrier: current status and critical review. Nanomedicine. 2013;9(1):39–54. doi:10.1016/j.nano.2012.04.004.
11. Lademann J, Richter H, Schaefer UF, Blume-Peytavi U, Teichmann A, Otberg N, Sterry W. Hair follicles—a long-term reservoir for drug delivery. Skin Pharmacol Physiol. 2006;19(4):232–6. doi:10.1159/000093119.
12. Lademann J, Patzelt A, Richter H, Antoniou C, Sterry W, Knorr F. Determination of the cuticula thickness of human and porcine hairs and their potential influence on the penetration of nanoparticles into the hair follicles. J Biomed Opt. 2009;14(2):021014. doi:10.1117/1.3078813.
13. Lademann J, Richter H, Meinke M, Sterry W, Patzelt A. Which skin model is the most appropriate for the investigation of topically applied substances into the hair follicles? Skin Pharmacol Physiol. 2010;23(1):47–52. doi:10.1159/000257263.
14. Mak WC, Richter H, Patzelt A, Sterry W, Lai KK, Renneberg R, Lademann J. Drug delivery into the skin by degradable particles. Eur J Pharm Biopharm. 2011;79(1):23–7. doi:10.1016/j.ejpb.2011.03.021.
15. Meidan VM. Methods for quantifying intrafollicular drug delivery: a critical appraisal. Expert Opin Drug Deliv. 2010;7(9):1095–108. doi:10.1517/17425247.2010.503954.
16. Otberg N, Richter H, Knuttel A, Schaefer H, Sterry W, Lademann J. Laser spectroscopic methods for the characterization of open and closed follicles. Laser Phys Lett. 2004a;1(1):46–9. doi:DOI 10.1002/lapl.200310011.
17. Otberg N, Richter H, Schaefer H, Blume-Peytavi U, Sterry W, Lademann J. Variations of hair follicle size and distribution in different body sites. J Invest Dermatol. 2004b;122(1):14–9. doi:10.1046/j.0022-202X.2003.22110.x.
18. Otberg N, Patzelt A, Rasulev U, Hagemeister T, Linscheid M, Sinkgraven R, Sterry W, Lademann J. The role of hair follicles in the percutaneous absorption of caffeine. Br J Clin Pharmacol. 2008;65(4):488–92. doi:10.1111/j.1365-2125.2007.03065.x.
19. Patzelt A, Lademann J. Drug delivery to hair follicles. Expert Opin Drug Deliv. 2013;10(6):787–97. doi:10.1517/17425247.2013.776038.
20. Patzelt A, Richter H, Buettemeyer R, Huber HJ, Blume-Peytavi U, Sterry W, Lademann J. Differential stripping demonstrates a significant reduction of the hair follicle reservoir in vitro compared to in vivo. Eur J Pharm Biopharm. 2008;70(1):234–8. doi:10.1016/j.ejpb.2008.02.024.
21. Patzelt A, Richter H, Knorr F, Schafer U, Lehr CM, Dahne L, Sterry W, Lademann J. Selective follicular targeting by modification of the particle sizes. J Control Release. 2011;150(1):45–8. doi:10.1016/j.jconrel.2010.11.015.
22. Pissuwan D, Niidome T, Cortie MB. The forthcoming applications of gold nanoparticles in drug and gene delivery systems. J Control Release. 2011;149(1):65–71. doi:10.1016/j.jconrel.2009.12.006.
23. Senzui M, Tamura T, Miura K, Ikarashi Y, Watanabe Y, Fujii M. Study on penetration of titanium dioxide (TiO(2)) nanoparticles into intact and damaged skin in vitro. J Toxicol Sci. 2010;35(1):107–13.
24. Teichmann A, Jacobi U, Ossadnik M, Richter H, Koch S, Sterry W, Lademann J. Differential stripping: determination of the amount of topically applied substances penetrated into the hair follicles. J Invest Dermatol. 2005;125(2):264–9. doi:10.1111/j.0022-202X.2005.23779.x.

25. Teichmann A, Otberg N, Jacobi U, Sterry W, Lademann J. Follicular penetration: development of a method to block the follicles selectively against the penetration of topically applied substances. Skin Pharmacol Physiol. 2006;19(4):216–23. doi:10.1159/000093117.
26. Tenjarla SN, Kasina R, Puranajoti P, Omar MS, Harris WT. Synthesis and evaluation of N-acetylprolinate esters—novel skin penetration enhancers. Int J Pharm. 1999;192(2):147–58.
27. Trauer S, Lademann J, Knorr F, Richter H, Liebsch M, Rozycki C, Balizs G, Buttemeyer R, Linscheid M, Patzelt A. Development of an in vitro modified skin absorption test for the investigation of the follicular penetration pathway of caffeine. Skin Pharmacol Physiol. 2010;23(6):320–7. doi:10.1159/000313514.
28. Zhu Y, Shi J, Shen W, Dong X, Feng J, Ruan M, Li Y. Stimuli-responsive controlled drug release from a hollow mesoporous silica sphere/polyelectrolyte multilayer core-shell structure. Angew Chem Int Ed Engl. 2005;44(32):5083–7. doi:10.1002/anie.200501500.

Chapter 12
Development of Pilosebaceous Unit-Targeted Drug Products

Guang Wei Lu, Kevin S. Warner and Fangjing Wang

12.1 Introduction

12.1.1 Rationale for Pilosebaceous Unit (PSU)-Targeted Drug Delivery

The pilosebaceous unit (PSU) consists of the hair shaft, the hair follicle, sebaceous gland, and the associated erector pili muscle. The flask-like sebaceous glands have ducts that vent into the open space surrounding the hair shaft just below the skin's surface. The space between the hair shaft and the hair follicle enables transfollicular penetration of drug molecules or micro-/nano drug-loaded particles to bypass the stratum corneum (SC) and migrate into the PSU. Sebum produced in sebaceous glands secretes to the surface of skin through follicular ducts. Human sebum is a mixture of multiple components comprising triglycerides, wax esters, squalene, cholesterol esters, and cholesterol [1–3]. Unlike internal body organs and tissues, the sebaceous gland is one of the most accessible sites for targeted drug delivery. Figure12.1 shows the pathways of topically applied medicine into sebaceous glands. Successful targeting of the PSU simply means getting more drug molecules or drug-loaded particles into hair follicles and sebaceous glands than can be delivered with conventional dosage forms. At the same time it implies restricting the amount of drug that reaches therapeutically uninvolved sites, including the systemic circulation. In principle, topically applied agents can reach the lower follicle, hair

G. W. Lu (✉) · K. S. Warner · F. Wang
Pharmaceutical Development, Research & Development, Allergan, Irvine, CA, USA
e-mail: lu_guangwei@allergan.com

K. S. Warner
e-mail: warner_kevin@allergan.com

F. Wang
e-mail: wang_fangjing@allergan.com

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_12

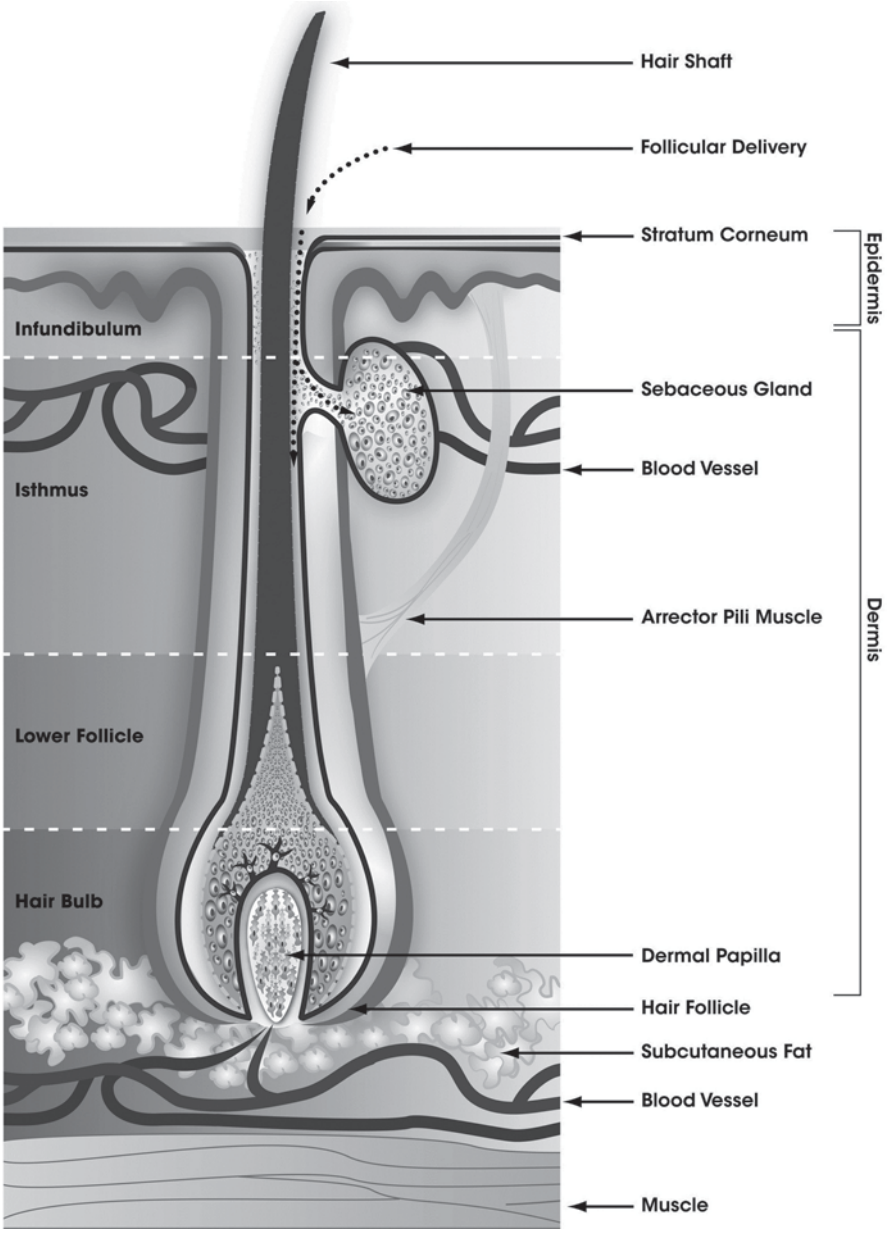


Fig. 12.1 Structure of human skin and drug transport routes via hair follicle and sebaceous gland

bulb, and sebaceous glands either by the transfollicular or the transepidermal route. The former pathway involves drug diffusion or drug-loaded micro-/nanoparticulate migration through the upper reaches of the PSU, while the latter pathway involves secondary local/systemic distribution into hair follicles. In fact, both pathways

occur simultaneously in most cases but the relative contribution of each pathway to the overall delivery should vary substantially depending upon the physicochemical properties of the therapeutic agent, nature of the formulation (e.g., cream or gel), property of particulate system (e.g., polymer or solid lipid), drug release from particulate system, and the elapsed time after topical dosing.

It is generally recognized that targeted drug delivery into and through hair follicles reduces drug exposure to nontargeted tissues (i.e., tissues associated with transepidermal penetration pathway) while enhancing local exposure at the target site (e.g., sebaceous glands) to improve efficacy and/or reduce side effects. This concept has generated considerable interest in developing topically applied PSU-targeted drug products for treating sebaceous gland dysfunctions such as acne.

12.1.2 Properties of the PSU and Sebum

To effectively design and develop a follicle-targeted drug delivery system, it is important to understand the properties of the PSU and sebum. The amount of drug delivered depends on the surface area of the follicles, which is a function of the follicle density and diameter of the follicles. Sebum produced in the sebaceous glands vents through the upper third of the hair follicle in order to reach the skin surface. On average, the secretion rate of sebum from the sebaceous gland to the skin surface has been estimated to be approximately 0.1 mg/cm² (skin)/h [4, 5]. Therefore, the transport rate of topically applied drug molecules into sebum would have to be faster than the outflow of sebum to the skin surface. Transport rate depends on the concentration gradient, and the partition and diffusion properties of the drug molecule, as well as the vehicle effect. In the case of drug-loaded particulate delivery systems, drug release rate from particles into surrounding fluids and/or tissues and topical application with massage have a significant influence on the efficiency of the targeted follicular delivery. Some basic properties of pilosebaceous glands and sebum that are associated with the follicular drug delivery are listed in Table 12.1.

12.1.3 Potential Targets Within the PSU

The PSU has several biological domains that can be targeted, including sebaceous glands, bulge region, and follicular papilla (see Fig. 12.1). In general, drug molecules present at a specified target site in a follicle reach the target site by taking advantage of several transport pathways. Local diffusion of a drug from the surrounding tissues invariably makes its contribution to follicular delivery from the systemic circulation. Over and above the properties of drug molecules and formulations/delivery systems, the contribution of each pathway to the drug concentration at a target site depends upon the distance of the pathway, the mass of the tissue or substance offering the pathway, the physical nature of the local tissue or substance comprising the pathway, and the vasculature surrounding the target site. Due to the

Table 12.1 Basic properties of human pilosebaceous glands and sebum

Investigation	Results			References
Sebum production and secretion	Sebum liberation from sebocytes to the skin surface: 8 days			[1, 3–5]
	Sebum secretion from hair follicle to the skin surface: 14 h			
	Sebum secretion rate: 0.1 mg/cm^2/h			
Sebum composition		*Lumen of gland*	*Skin surface*	[1, 3]
	Squalene	15	15	
	Wax esters	25	25	
	Triglycerides	57	42	
	Fatty acid	0	15	
	Cholesteryl esters	2	2	
	Cholesterol	1	1	
Hair follicles	About 10–100 follicles/cm^2			[6, 7]
	150–900 sebaceous glands/cm^2			
	Follicular diameters: 200–2,000 μm			
Sebaceous glands	50–100 μm below the surface of the skin			[8, 9]
	Excess of 450 sebaceous glands per cm^2 in facial skin			

different nature of pathway tissues/substances, it would be possible for follicular drug delivery to play an appreciable role in targeting sebaceous glands, but a much lesser role in targeting the dermal papilla. Therefore, different delivery strategies and anticipation have to be developed based on the actual target site within a follicle.

12.1.4 Primary Approaches for PSU or Follicular Drug Delivery

Targeted drug delivery (or follicular drug delivery) to the PSU can be managed by two different strategies— first a drug molecule approach and second a formulation design approach [10]. The molecular modification approach involves tailoring the physicochemical properties of a drug molecule, such as its size, polarity (lipophilicity), polar surface area, solubility parameter, and/or charge, any of which has a potential to modulate delivery into the hair follicle [10, 11]. Due to the cost, time, and complexity of new drug molecule development, most studies in this area focus on the second strategy: the formulation approach. A number of studies using the formulation approach have demonstrated that improved localized delivery of drugs to the PSU can be achieved by varying the compositions of applied formulations [12–23]. For example, the gains in localized delivery were achieved by applying a particulate carrier system [21] or by using sebum miscible excipients in the topical preparation [24]. To fully appreciate how the drug mol-

ecule and formulation approaches are utilized one has to become familiar with the structure and physiology of the PSU.

12.2 Recent Development and Commercialization of PSU-Targeted Dermal Products

12.2.1 Particulate Carrier-Based Formulations

Topical formulation development strategies of passive delivery systems have expanded beyond dissolving or suspending a drug (or drugs) into a gel, cream, or ointment formulation to now include particulate/encapsulation technologies [25–29]. Several technology platforms, some yielding commercial formulations, have been developed utilizing an encapsulation technology. These formulations have been investigated for applications such as: cosmetic dermatology, medical dermatology, targeting PSU, and systemic delivery (see Table 12.2). Regardless of the applications, these formulations have a specific dispersed phase (particulate or emulsion), physicochemical properties, and formulation compositions that may leverage the hair follicle as a shunt route or target site.

Table 12.2 lists examples of products that utilize dermal formulations with the potential of follicular delivery. However, there are no prescription dermal products with claims of targeting the hair follicles because it is challenging to clinically differentiate follicular delivery from transepidermal delivery. In the examples listed in Table 12.2, the encapsulation technology is not specifically noted as follicle targeting, but the particle size is consistent with delivery via this route. Rationale for developing particulate or drug-encapsulated carriers may include improved formulation aesthetics, reduced local irritation, improved product stability, and reduced drug exposure to nontargeted tissues (i.e., tissues associated with the transepidermal penetration pathway) while enhancing local exposure at the target site.

12.2.2 Recent Innovations in Follicular Drug Delivery

As summarized in Table 12.3, recent inventions in follicular delivery have concentrated in the areas of delivering large molecular weight compounds into or through the skin, enabling medical diagnostics, and increasing the efficiency of topical preparations and local tolerability. Encapsulation strategies employing polymeric micro/nanoparticles, novel excipients, solid lipid nanoparticles, and liposomes, respectively have been most actively studied [30–37]. In addition, nanoemulsions and biodegradable lipids are becoming more popular as a topical dosage form in part due to their potential in increasing drug solubility, stabilizing active pharmaceutical ingredients (APIs), improving local skin tolerance, and improving aesthetics

Table 12.2 Examples of encapsulation based formulations in development or that are commercially available

Product	Technology description	Indication	Company
Nanosomal amphotericin B gel Nanosomal tacrolimus topical	Proprietary lipid-based (e.g., a phospholipid) nanotechnology formulated in an aqueous medium giving homogenous nanoparticle-sized products	Topical fungal infections Eczema	Jina Pharmaceuticals
Tropazone lotion Tropazone cream	Encapsulation of hydrophilic and lipophilic ingredients utilizing a liposomal technology called Lyphazome	Relief from burning and itching associated with various types of dermatoses	ECR Pharmaceuticals
AcneWorx	Nonphospholipid system comprising a multilamellar bilayer vesicle. Proprietary technology called Novasome®	Acne	Novavax, Inc. Dermworx
SalSphere™ HydroSal® MultiSal™	Microparticles or nanoparticles comprising a biodegradable solid hydrophobic core containing the drug, and a positively charged bioadhesive/mucoadhesive surface	Technology applied to dermal indications such as acne, aging skin, hair loss	Salvona
Phase 3—cold sore product	NanoStat™ technology comprises an oil-in-water emulsion stabilized by surfactants	Herpes labialis	NanoBio
Ameluz	A nanoemulsion in gel formulation. Formulation specific to the active agent 5-aminolevulinic acid	Mild to moderate actinic keratosis on face and scalp	Biofrontera
CeraVe hydrating cleanser CeraVe moisturizing cream CeraVe moisturizing lotion Salex cream Salex lotion	Multivesicular emulsion formed from a quaternary amine salt emulsifier	Moisturizers, facial cleansers and sunscreen applications	Valeant

Table 12.3 Examples of innovations in follicular delivery

Patent #	Technology	Technology application	Assignee
US8168600	Compositions to enhance the delivery of nucleic acids and other nucleosidic moieties via topical routes of administration. Describe two-step process: (1) Use of an aqueous solution to preferentially deliver nucleic acids preferentially to hair follicles. (2) Apply enhancer solution to facilitate delivery from hair follicle to other areas of skin	Inhibiting hair growth	Isis Pharmaceuticals, Inc.
US 7524505	Topically applied formulations comprising hair graying treatment and hair regrowth agents on or through the scalp. Formulations are comprised of liposomes and penetration enhancers to deliver several active ingredients (hair stem cell factor, amino acids, minoxidil, vitamins) into the dermis primarily via follicular pathway	Alopecia and natural hair pigment restoration	Schweitzer Biotech Comp
US 6979440	Nanosphere delivery system with an average sphere diameter from about 0.01 to about 10 μm. Encapsulation technology is comprised of hydrophobic materials, cationic conditioning agent, or cationic conditioning agent in conjunction with a quaternary ammonium compound to assist in adhering the spheres onto hair, skin, and fabric	Application to sundry cosmetic agents to prolong relase rate	Salvona
US 6080127	Technology employs a specific vibrational frequency and amplitude to the skin to enable targeted delivery to the hair follicle. The substances delivered range from small molecules to particles compatible with the mechanical shifts of the hair shaft	Hair growth simulators, hair growth inhibitors, natural hair pigment restoration	AntiCancer, Inc.
US 5753263	Cationic phospholipid based liposomes encapsulating macromolecules, including dyes, proteins, and nucleic acids for gene therapy. These liposomes are claimed to target the hair follicle	Prevent chemotherapeutic agent-induced hair loss	AntiCancer, Inc.
US20090110731	Particles of specific sizes comprising poly[bis(trifluoroethoxy)phosphazene], which include a core having a hydrogel formed from an acrylic-based polymer, and an active ingredient. These particles provide localized delivery of the active into the hair follicle for treatment of alopecia.	Alopecia	Celonova Biosciences, Inc

Table 12.3 (continued)

Patent #	Technology	Technology application	Assignee
WO2013158278	Topical application of light-absorbing submicrometer particles (silica core with gold shell). Particles are delivered into the hair follicle via acoustic vibration or massage of application area followed by energy activation (light) to elicit the therapeutic response	Treating or alleviating diseases of the hair follicle	The General Hospital Corp.
WO2012027728	Utilization of plasmonic nanoparticles of specific size, shape, and composition that will preferentially deposit into hair follicle. These plasmonic nanoparticles are then activated by laser- or light-based systems to treat skin conditions	Cosmetic and therapeutic treatment of dermatological diseases	Sienna Labs
WO2011095970A1	Topical emulsion composition comprising oil-in-polyol dispersion with a mean particle size below 1 μm to enable preferential accumulation in the hair follicle	Treating or alleviating diseases of the hair follicle	JPMed
WO2006109936	Phospholipid-based liposome technology processed to produce small unilamelar vesicles for encapsulating proteins and delivery into follicle	Encapsulation of human growth hormone (hGH) to treat skin diseases	Regeron, Inc

Patent search was conducted on November 4, 2013 and involved searching entire patent utilizing the following keyword searches: "follicular delivery," "sebaceous gland delivery," "follicle drug delivery"

[31, 35]. Other technologies include use of physical enhancement means (i.e., vibrational frequency applied to skin) to enable delivery into follicles [38, 39].

12.3 Research in Developing PSU-Targeted Formulations

Designing a molecule that is appropriate for follicular delivery is no simple task. In addition to the criteria that must be satisfied in the conventional selection of drug candidates (i.e., desirable physiochemical properties, local efficacy, minimal or acceptable dermal and systemic adverse effects, and pharmacokinetics/pharmacodynamics (PK/PD) profiles), follicular accumulation is an additional parameter that has to be added into what is already a complicated equation. There is the chance that changes in molecular structure to improve follicular deliverability could adversely affect other properties of the drug candidate. For example, changes in molecular structure could alter follicle accumulations that would change the pharmacological

or toxicological aspects of the medicine. Therefore, enhancing or targeting follicular drug delivery remains a complicated yet important challenge to researchers in the discovery phases of new drug design.

12.3.1 Development of an Artificial Sebum Model

12.3.1.1 Hypothesis for Application of Artificial Sebum Model

The application of an artificial sebum model in the drug discovery for pilosebaceous unit targeted molecules can be expressed by the following equations. Assuming drug transport through the skin primarily by independent, parallel transepidermal and transfollicular pathways [40, 41] where the primary barrier for the transport is the SC and the sebum respectively, the total steady-state flux is expressed by:

$$J_{total} = J_{sebum} + J_{sc} \tag{1}$$

where J_{total} is the total flux and J_{sebum} and J_{sc} are flux through the sebum and SC, respectively. Since J=AKDC/h, the equation can be expressed as:

$$J_{total} = A\left[f_{sebum}\frac{D_{sebum}K_{sebum}}{h_{sebum}} + f_{sc}\frac{D_{sc}K_{sc}}{h_{sc}}\right]C = \left[A_{sebum}\frac{D_{sebum}K_{sebum}C}{h_{sebum}}\right] + \left[A_{sc}\frac{D_{sc}K_{sc}C}{h_{sc}}\right] \tag{2}$$

where A is the total area of application and C is the concentration of drug in the formulation, f_{sebum} and f_{sc} are the fractional areas of the transfollicular route (primary sebum) and transepidermal route (primary SC), A_{sebum} and A_{sc} are the actual areas of the sebum and SC routes, D_{sebum} and D_{sc} are the functional diffusion coefficients for the drug through sebum and the SC, K_{sebum} and K_{sc} are the partition coefficients for drug in sebum and SC. The terms, h_{sebum} and h_{sc}, are the functional thicknesses of the sebum and SC. In these equations the partition coefficients exhibit the greatest variability between compounds within a family and thus are the parameters that most likely define the transport pathways (transepidermal or transfollicular) [40]. Therefore, when $K_{sebum} >> K_{sc}$, drug molecules will be preferably transported through sebaceous gland and hair follicles. When the reverse is true, that is, when $K_{sebum} << K_{sc}$, the principal pathway for diffusion and accumulation will be that through the SC (the transepidermal pathway). The ratios of K_{sebum}/K_{sc} and $D_{sebum/}D_{sc}$ reflect the potential for follicular drug delivery.

12.3.1.2 Experimental Methods

Preparation of Artificial Sebum Artificial sebum is a mixture of 15% squalene, 10% paraffin wax, 15% spermaceti wax, 10% olive oil, 25% cotton seed oil,

10% coconut oil, 1.4% oleic acid, 5% palmitic acid, 5% palmitoleic acid, 1.25% cholesterol, and 2.4% cholesterol oleate by weight. These ingredients are weighed out in a glass container. The solid mixture in the container is heated to 60 °C with stirring until it becomes a clear liquid. After mixing for approximately 10 min at 60 °C to ensure that all the ingredients are melted or dissolved to form an uniform lipid phase, the mixture is allowed to cool down at room temperature to be solidified for lab use [42].

Determination of the Artificial Sebum and Water Partition Coefficient Artificial sebum (1–20 mg, depending on the partition coefficient of the drug) is weighed in a glass vial containing 1 mL of aqueous drug solution (2–20 μg/mL). The vials are kept at 37 °C in a shaker for approximately 15 h to reach equilibrium. After centrifugation at 8000 rpm for 15 min, the clear aqueous solution in the vials is withdrawn and analyzed using a HPLC method. The amount of drug partitioned into the artificial sebum is estimated by the difference in the drug concentration in the aqueous phase before and after reaching equilibrium. The partition coefficient is expressed as the drug concentration in 1 g of artificial sebum divided by the drug concentration in 1 g of aqueous solution at the equilibrium condition [43].

Determination of Drug Flux Through Artificial Sebum Drug transport through the artificial sebum is carried out in a 24-well format (Transwell®, Corning Incorporated, NY). The supporting membrane (polycarbonate membrane, 0.45 μm thickness) of each insert is coated with 2.1 ± 0.2 mg of the artificial sebum (previously heated at 50–55 °C). A 150 μL aliquot of aqueous suspension of model drug (10 mg/mL in citrate-phosphate buffer (CPB, pH 5.5) is loaded onto the insert and 1 mL of preheated (37 °C) 10% HP-β-cyclodextrin in the CPB is used as the receiver solution. The samples and apparatus are placed in an incubator at 37 °C and rotated at 125 rpm. A sample is taken every 10 min for 2 h. At each sampling time, the entire receiver solution is replaced with the fresh buffer. The withdrawn samples are analyzed for drug content by HPLC. The cumulative quantity of drug collected in the receiver compartment is plotted as a function of time. The flux value for a given experiment is obtained from the slope (steady-state portion) of the cumulative amount of drug permeated vs. time plot. The aqueous solubility of each compound is determined by the centrifugation of the suspension used for the donor phase and analysis of the supernatant with HPLC. The permeability coefficients are calculated from the steady state flux and the drug concentration (solubility) in the vehicle. The diffusion coefficients are calculated using permeability coefficients, thickness of sebum layer, and sebum partition coefficient [44].

12.3.2 In Vitro *Methods for Quantifying Follicular Penetration*

Research in appropriate experimental models that quantify follicular drug penetration into the PSU has met with varied success. Initial development using *in vitro* models provided insight into transfollicular penetration [9, 45, 46]. Subse-

Table 12.4 *In vitro* experimental approaches for quantifying PSU delivery of drugs

Model	Description	References
Tape stripping model	Method comprises tape stripping technique to remove SC layer, followed by application of a cyanoacrylate adhesive to follicle opening, a second tape strip application to remove adhesive and quantify drug amount in adhesive	[48, 53]
Imaging techniques	Utilization of confocal laser light microscopy to show visual localization in PSU	[49–51]
Permeability models	Rodent (mice, hamster) skin, pig and rabbit ear skin, and human cadaver skin have been utilized for assessing permeability, and follicle deposition	[47, 54–56]

quent *in vitro* models were developed aimed at quantifying drug deposition into the PSU [47–52]. Table 12.4 outlines *in vitro* methodologies used to quantify drug deposition into the PSU. Further discussion of these methods as well as the utility of these models has been reviewed previously and the reader is referred to this article for additional discussion [47].

12.3.3 In Vivo Efficacy Models

The methodologies outlined in Table 12.4 provide important insight into the transport behavior of PSU-targeted formulations. However, these models do not provide proof of principle efficacy data that provide insight into whether a particular formulation delivers sufficient quantity of bioavailable drug to the PSU to elicit a pharmcodynamic/efficacy response. Efficacy models for various PSU diseases are outlined in Table 12.5. No single animal model reflects the complete pathology of PSU diseases; however, various animal models are reported to simulate critical elements that enable proof of principle studies to guide formulation development.

A typical example in designing and developing a sebaceous gland targeted molecule for reducing sebum production was published where the authors applied an artificial sebum model to a multi-discipline early development project to screen for new topical androgen receptor antagonists [11]. A hamster ear model was used for the *in vivo* efficacy evaluation based on the correlation between the reduction of total sebum production and the reduction of wax ester in sebum. Drug substance candidates (Compound 1 and Compound 4) were studied for lead selection. For comparison, the artificial sebum/water partition coefficient is 3.92 for Compound 1 and 3.03 for Compound 4 while the skin flux through hamster ear is 0.060 μg/cm²/h for Compound 1 and 0.878 μg/cm²/h for Compound 4, respectively. These data suggested that Compound 1 was relatively favorable for follicular delivery. In the *in vivo* hamster model, Compound 1 had comparable efficacy to Compound 4 even though the skin flux of Compound 1 was much lower than that of Compound

Table 12.5 *In vivo* animal efficacy models

Model	Description	Indications	References
Hamster flank organ model	The Syrian golden hamster flank organ is comprised of clusters of large sebaceous glands. As a result this animal has been used to investigate effects of topically applied hormones, antiandrogens, and retinoids. Measurement of the flank organ sebaceous gland volume is an indication of drug substance efficacy.	Acne	[57, 58]
Stumptailed macaque	As macaques age, they lose most of their hair. The bald scalps of stumptailed macaques are studied to determine the extent of regrowth of terminal hair.	Alopecia	[59]
Hamster ear model	The inner side of the earlobes of Syrian hamsters have a dense concentration of sebaceous glands. These glands are similar to human sebaceous follicles. Measurement of the sebaceous gland volume in the earlobe is an indication of drug substance efficacy.	Acne	[60]
Dundee experimental bald rat and C3H/HeJ mouse models	These two rodent strains develop adult onset alopecia areata. This model is used to study presence, rate and density of hair regrowth.	Alopecia	[61–63]
Wax ester reduction in hamsters	Use wax ester reduction in sebum samples as a biomarker based on the correlation between sebum wax esters and sebaceous gland size.	Excess sebum production and acne	[11]

4, indicating that Compound 1 reached the site of action: sebaceous gland, primarily by follicular route of delivery.

12.3.4 Dosage Forms for Follicular Drug Delivery

Example formulations that can be used for hair follicle delivery, as shown in Table 12.6 and Fig 12.2, include drug suspension formulations, lipid-based formulations, polymer-based formulations, and surfactant-based formulations. Formulations that contain drug crystals with or without a carrier were developed to enhance drug delivery into hair follicles [64, 65]. Liposomes are spherical vesicles composed of amphipathic phospholipids with an aqueous compartment inside. Despite excellent biocompatibility, drug loading, and physical stability are limited due to the small internal volume, drug leakage, and instability in the vascular system [66].

Table 12.6 Examples of topical dosage forms used for pilosebaceuous gland-targeted delivery

Dosage form	Application	Results	References
Drug crystal suspensions in vehicles	Delivery of drug crystals suspended in a gel into hair follicles for the treatment of acne to improve efficacy, safety, and patient compliance.	Microcrystalline suspension of adapalene gel formulation (0.1 % adapalene gel) achieved similar comedolytic effect but much lower irritation than a microcrystalline suspension of tretinoin (0.025 % tretinoin) in a Rhino mouse model. Follicular penetration of microcrystals was observed as early as 5 min after application and massage, and reached a depth of 400 μm after 2 h. Microcrystals may subsequently dissolve into sebum due to its lipophilicity. About 0.01 % of the applied dose was recovered in the receptor fluid, suggesting no appreciable systemic exposure via follicular delivery route.	[64]
Liquid crystalline nanoparticles	Carrier for topical delivery of finasteride to treat androgenetic alopecia.	Nanoparticles based on monoolein material with size range from 154 nm to 170 nm and cubical shape. The drug release rate was < 20 % in the first 24 h, and permeation rate was enhanced with the addition of glycerol or propylene glycol.	[65]
Polymeric biodegradable microspheres	Site-specific delivery of adapalene containing PLGA microspheres to PSU of hairless rat or human skin to improve efficacy of acne treatment.	Particles of 5 μm penetrated into hair follicles, resulting in reduced frequency of drug administration compared to the drug crystal gel formulation.	[20]
	Use of PLGA microparticles and nanoparticles containing insulin peptide to enhance peptide stability and potential for targeting to skin appendages with reduced side effects.	PLGA microparticles with a size above 7 μm remained on the surface of excised human skin, while nanoparticles (< 1 μm) permeated into the viable epidermis and dermis with deposition concentrated around the hair follicles and sebaceous glands.	[74]
	Targeted transfollicular delivery of artocarpin extract by means of alginate/chitosan microparticles to treat androgen-dependent disorders such as male pattern alopecia and acne.	Optimal growth suppression of the hamster flank organs was achieved by topical delivery of microparticles at a size of 2–6 μm with no significant systemic exposure, while same concentration in solution failed to suppress normal growth.	[75]
Non-biodegradable polymeric microparticles	Utilization of polystyrene microspheres to study the penetration profile of microspheres in terminal hair follicles.	Microspheres with a size of 0.75–6.0 μm selectively penetrated into hair follicles (HF). The optimal size was determined to be 1.5 μm, with a 55 % penetration rate into the HF, and with a maximum penetration depth of 2300 μm.	[21]

Table 12.6 (continued)

Dosage form	Application	Results	References
Polymeric nanoparticles	Delivery of poorly water soluble compounds to hair follicles using nanoparticle suspensions for hair growth.	The nanoparticles were well tolerated with no visible skin irritation, and were delivered into hair follicles of rabbit ear with limited systemic exposure. *In vivo* mouse model study demonstrated fast stimulation of hair growth via the use of 100 nm nanoparticles compared to the aqueous vehicle control.	[76]
	Delivery of antigens to hair follicles for transcutaneous immunization.	Ovalbumin was delivered via transfollicular route, and had 2–3 fold higher delivery efficiency than the antigen solution. However, the integrity and bioactivity of antigen within the nanoparticles were slightly impaired.	[77]
Micelles	Development of novel micelle formulations to increase cutaneous bioavailability of azole antifungals.	Deposition of the micelles into skin was 13 fold higher than a marketed liposomal cream formulation. The high drug delivery was mainly via the follicular penetration pathway.	[78]
Solid lipid nanoparticles (SLN) or nanostructured lipid carrier (NLC)	Use of diphencyprone-containing NLC to promote percutaneous absorption and hair follicle targeting for the treatment of alopecia areata.	NLC with soybean phosphatidylcholine had greater follicular deposition and more *in vivo* skin retention, resulting from localization of NLC in follicles and intercellular lipids of stratum corneum (SC).	[79]
	RU58841-myristate prodrug loaded SLN to improve a specific targeting of the hair follicle for topical treatment of acne and androgenetic alopecia.	SLN were found in the deeper layer of porcine hair follicles, while negligible permeation of drug into epidermis was observed.	[80]
Liposomes	Topical delivery of gamma-interferon encapsulated into liposomes for therapeutic applications.	The penetration of drug was highly correlated with the number of follicles/hair in the skin species, suggesting that the transfollicular delivery route is an important pathway for topical therapeutics.	[81]
	Development of liposomes to deliver drugs to hair follicles in mice.	Liposomes delivered molecules into hair follicles and hair shafts of mice in a time-dependent manner. Negligible amounts entered dermis, epidermis or blood stream.	[82]

Table 12.6 (continued)

Dosage form	Application	Results	References
Niosomes	Follicular delivery of finasteride by liposomes and niosomes for the treatment of androgenetic alopecia and some other pilosebaceous unit disorders.	Despite slower *in vitro* skin permeation, the targeted delivery into hair follicles of niosome vesicles *in vivo* was higher than alcohol solution or vesicles in gel state. The deposition was composition dependent.	[83]
Nanoemulsions	Use of hyaluronic acid (HA) based nanoemulsion as transdermal carrier for active lipophilic ingredients.	HA based O/W nanoemulsion formulation penetrated deep into the dermis compared to the control formulation, via follicular and intercellular pathways. Penetration is dependent on concentration gradient, carrier properties, and penetration enhancers.	[84]
	Topical transport of hydrophilic compounds using W/O nanoemulsions.	Drug transport of inulin using nanoemulsions at a size of 25 nm was 5–15 fold higher than micellar dispersion or aqueous solution. Inulin transport was affected by HLB of the surfactant mixture, but independent of drug molecular weight. The transport was predominantly transfollicular in nature.	[85]
Microemulsions	Incorporation of the lipophilic dye curcumin into an O/W microemulsions to study the penetration and distribution in the human skin.	Microemulsions penetrated deeper into the complete follicular infundibula, whereas curcumin amphiphilic cream formulation led to deposition only at the follicular orifices. Micoremulsion also facilitated the transport via SC.	[86]
Foam formulations	Applications of Rogaine 5% foam formulations containing minoxidil on the skin of human volunteers to evaluate follicular and percutaneous penetration pathways.	Drug was detected in blood 5 min after topical application when hair follicles were available for drug delivery, whereas 30 min was needed when hair follicles were closed. This shows the importance and promise of follicular pathway delivery.	[87]

PLGA poly(lactide-co-glycolide), *PSU* pilosebaceous unit

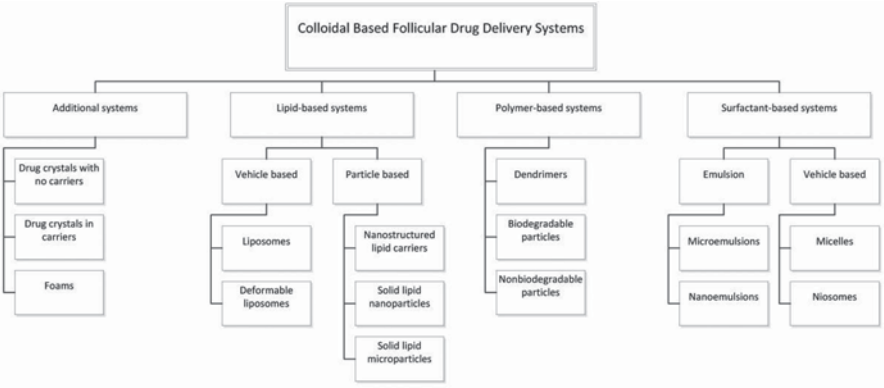


Fig 12.2 Colloidal based topical dosage forms for targeted follicular delivery

Lipid-based formulations such as solid lipid nanoparticles (SLN) or nanostructured lipid carrier (NLC) have gained attention as promising colloidal drug carriers, particularly for drugs with limited solubility. Advantages of lipid-based formulations include the potential of high biocompatibility [67], protection of drugs against chemical and photo degradation, avoiding organic solvents in the manufacturing process, easy modulation of drug release rate, 10–100 fold less cytotoxicity than their polymeric counterparts, wide selection of lipid/surfactant combinations, and large scale production by high pressure homogenization [68, 69]. Different methods have been developed to prepare either SLN or NLC systems [69]. Lower drug loading may be one of the major disadvantages of lipid carrier systems, especially for SLN [69]. NLC systems can address low drug loading exhibited by SLN systems by incorporating liquid lipids into solid lipids which lead to a less ordered lipid structure. The increased disorder in the lipid structure allows for increased drug loading without impairing subsequent physical stability [69]. Polymeric particles have been shown to penetrate into hair follicles when the particles are below 5 μm [20]. Wide selection of polymer types and compositions has enabled high drug loading, especially when lipophilic drugs are encapsulated into microparticles. However, more studies are needed to understand the distribution/elimination and safety of particles once delivered into hair follicles [70–73]. Compared to non-biodegradable polymers, biodegradable polymers are safer to use, but the polymer itself or its degradation products may cause adverse events in the skin. For emulsion based formulations, the use of appropriate surfactants and oil phase may enhance the permeability of the drug formulations into skin [25], in addition to improving deposition into hair follicles. However, limitations occur, including the toxicity of the surfactants, limited prevention of drug cytotoxicity to the skin, and physical instability of the dosage forms due to agglomeration, drug expulsion and breaking of the emulsion.

The selection of appropriate carriers in a formulation to facilitate follicle delivery is dependent on many factors, such as drug properties, required drug concentration at the target site to achieve a therapeutic outcome, commercially viable process, regulatory requirements, and physical and chemical stability of the drug substance

in the formulation. For example, a comparative study of lipid-based versus polymer-based nanocarriers has been conducted [88] that illustrates the impact of carriers on follicle delivery. Compared to drug solutions, lipid nanocapsules (LNC), SLN NLC all increased drug flux into the skin 4–5 fold, while polymeric nanoparticles had a lower permeation enhancing effect. Polymeric nanoparticles were found to have four fold higher accumulation in the skin compared to LNC and twice the accumulation of SLN and NLC. All these nanocarriers were found to accumuale in the hair follicle space. Lipid particles seemed to be efficient transdermal drug delivery vehicles, while polymeric nanoparticles are more suitable for localized dermal delivery. Even though the authors did not discuss the effect of follicular delivery on drug permeation and retention, it might be possible that accumulation in the skin of polymeric nanoparticles was due to follicular delivery.

In addition to chemical enhancement technology, physical enhancement technologies are also applied to improve skin and follicular drug delivery. For example, particulate carriers can be combined with iontophoresis [89, 90], or microneedle systems [91, 92] to improve delivery efficiency. To date there are no prescription products available which target the PSU primarily because of the challenges to clinically differentiate follicular delivery from transepidermal delivery.

12.3.5 Particulate Systems for Follicular Delivery

Polymeric materials have been used as targeted follicular drug delivery systems to effectively protect drug degradation, decrease side effects, and enhance drug cutaneous penetration across the skin barrier by increasing the concentration gradient and directly delivering drugs to the site of action. Selection of appropriate polymers and compositions can effectively enhance drug payload and regulate drug release rate in formulations. Synthetic polymers such as poly(lactide-co-glycolide) (PLGA), poly(ε-caprolactone), polyalkylcyanoacrylates, polystyrene, polyacrylates, and natural polymers such as gelatin, chitosan, albumin, alginate have been used in topical formulations [93].

Hair follicles with its complex vascularization and deep invagination with a thinning SC has enabled hair follicles to be an important penetration pathway for particles (in spite of covering approximately 0.1 % of skin surface area) as well as an important long-term drug reservoir [95]. The physicochemical characteristics of particles such as size, surface charge, and hydrophobicity can influence deposition and/or penetration into hair follicles, as demonsrated in Table 12.7. In particular, particle size plays a crucial role in the delivery of particulate systems into hair follicles. Reports have shown that particles > 10 nm in diameter are unlikely to penetrate through SC into viable human skin but will accumulate in the hair follicle openings, especially after massage [72]. Landemann et al. recommended the optimal size for penetration into hair follicles to be approximately 300–600 nm [70]. It is debateable what is the optimal particle size and whether nanoparticles are better than microparticles in terms of penetration profiles, or more importantly, in

Table 12.7 Examples of follicular delivery studies using polymeric particles

Dosage form	Description	Study results	References
Microparticles	Use of PLGA 50:50 microspheres containing adapalene for the treatment of acne.	See Table 12.6	[20]
	Application of PLGA microspheres and nanoparticles of peptide insulin for deposition in human skin.	See Table 12.6	[74]
	Investigation of the penetration profile of PLGA microspheres at a size of 0.75–6 μm in hair follicles.	See Table 12.6	[21]
	Site-specific methylene blue delivery of highly porous nylon microspheres to PSU structure.	Microspheres at a size of 5 μm were optimally deposited deep within the philosebaceous structure, resulting in the exclusive detection of methylene blue in the hair follicles and sebaceous glands.	[22]
Nanoparticles)	Study of behaviors of polystyrene nanoparticles (diameters of 20 and 200 nm) in skin permeation and distribution.	Accumulation of polystyrene nanoparticles occurred preferentially in the follicular openings, increased with time and was favored by the smaller particle size.	[94]
	Study of penetration and storage behavior of PLGA nanoparticles at a size around 320 nm into hair follicles.	Nanoparticles penetrated deeper into the hair follicles of pig skin and stayed inside the follicles up to 10 days, while the non-particulate dye stayed up to 4 days. Particles in the follicles acted as a reservoir and provided a prolonged drug release into the hair follicle and surrounding sebum. These particles were depleted by hair growth and sebum flow.	[95]
	Use of polylactic acid (PLA) nanoparticles as carriers for transepidermal drug delivery.	Nanoparticles with sizes of 228 nm and 365 nm, respectively, penetrated into the hair follicles as determined by fluorescence microscopy on cryosections of excised human skin. Nanoparticles retained within the sebaceous glands for up to 24 h with the accompanied release of the encapsulant to the viable epidermis.	[96]
	Investigation of the effect of PLGA nanoparticles at sizes of 122, 230, 300, 470, 643 and 860 nm on skin deposition.	Particles with a medium size (643 nm) penetrated deeper into the porcine hair follicles than the smaller or larger particles. By varying the particle size, different sites within the porcine hair follicles can be selectively targeted.	[97]

Table 12.7 (continued)

Dosage form	Description	Study results	References
	Development of self-assembled 40- and 130-nm nanoparticles of poly(ε-caprolactone)-block-poly(ethyleneglycol) for transdermal delivery of minoxidil.	In hairy porcine skin, the permeation of minoxidil incorporated into 40-nm nanoparticles was higher in the epidermal layer and in the receptor solution than that of 130-nm nanoparticles, no size-dependent difference was observed in hairless skin. The nanoparticles containing solutes penetrated mainly via shunt routes like follicles.	[98]
	Use of particles of 40, 750 or 1500 nm to transcutaneously target epidermal CD1a+cells, which resides around hair follicles at a high density.	Only 40 nm nanoparticles deeply penetrated into vellus hair openings and through the follicular epithelium, and entered epidermal Langerhans cells.	[99]
	Use of polystyrene nanoparticles for targeting of vaccine compounds to skin antigen-presenting cells (APCs) by hair follicles.	Nanoparticles of 40 nm, and to a lesser extent 200 nm, penetrated deeply into hair follicles and were internalized by perifollicular APCs and transport to the draining lymph nodes.	[100]
	Research on the uptake of Nile red encapsulated into nanoparticles (<100 nm) composed of different portions of polystyrene (PS) and poly-(2-hydroxyethyl methacrylate) (HEMA).	Nanoparticles could not penetrate through SC, but had affinity for hair follicles. Decreasing hydrophobicity (by increasing HEMA ratio) reduced the encapsulation of the lipophilic dye into nanoparticles subsequent release and the uptake into SC.	[101]
	Evaluation of particles of different size and hydrophobicity for the delivery into the skin.	The skin uptake of lipophilic Nile red encapsulated into nanoparticles, increased with increasing hydrophobicity of polymeric carrier: polystyrene>poly(ε-caprolactone)>cellulose acetate butyrate, due to the resultant high payload of the more hydrophobic polymers.	[102]
	Utilization of chitosan-coated PLGA nanoparticles for transcutaneous immunization.	Follicular uptake of active ingredients from chitosan coated nanoparticles was enhanced by a factor of 2.33±0.52 compared to ovalbumin solution, but slightly lower than non-coated PLGA nanoparticles.	[77]

PSU pilosebaceous unit, *PLGA* poly(lactide-co-glycolide)

terms of overall treatment efficacy. Further investigation is needed to clarify these discrepancies.

Targeted follicular delivery of microparticles or nanoparticles have shown improved efficacy compared to conventional solution formulations [75]. The efficiency of artocarpin (AR), an extract of heartwood of artocarpus incises possessing potent 5a reductase inhibitory effect, at a dose of 0.1 mg in alginate/chitosan microparticles was comparable to a dose of 1 mg AR solution in suppressing the growth of hamster flank organs without causing significant systemic action [75]. Delivery of hair growing ingredients to hair follicles using PLGA 75:25 nanoparticles enhanced scalp-pore permeability (>2–2.5 fold), altered hair growth activity from resting phase to growing phase, and improved the degree of hair growth [103]. Use of noninvasive nanoparticles into hair follicles for transcutaneous immunization improved the delivery efficiency of ovalbumin (OVA) into hair follicles on the excised skin by a factor of 2–3 compared to OVA solution [77]. Particle suspensions in an appropriate vehicle are promising in enhancing the transfollicular delivery of therapeutic drugs into humans.

12.3.6 Characterization of Particulate Drug Delivery Systems

Particle-based formulations have been evaluated for follicular delivery of drugs into skin and enabled enhanced therapeutic effects [20]. The properties of particles need to be evaluated and understood when developing a formulation. Enhanced transfollicular delivery is highly dependent on the particle size and size distribution, which is associated with the penetration depth and distribution in the skin [74]. Also, drug loading of particles and drug release rate affect the overall therapeutic outcome of drug particles in the treatment of diseases [102]. It is essential to quickly release the drug from the particles into the hair follicles to produce the desired efficacy before the particles are removed from the sebum [115]. Zeta potential is an important parameter when the stability of particle formulations is concerned. A higher absolute value of zeta potential (preferably $|\xi| > 30$ mv) is beneficial to the prevention of aggregation of particles during storage [116]. In a formulation composed of a continuous phase and a suspension phase, drug partition between the particles and vehicle is critical in terms of efficacy and side effects. Nanoparticles or microparticles provide a sustained and controlled drug release into the hair follicles, while the existence of free drug crystals outside of the nano/micro particles may cause cytotoxicities to the skin. The formation of free drug crystals, which can be measured by the polarized microscope, may be affected by the crystallinity of carrier and drugs, which can be measured by XRD and DSC [117]. Examination of the morphologies of particles by SEM, TEM or light microscope is important because the shape of particles plays an important role in transport or penetration [118], and drug release rate is associated with the surface morphology of particles. Commonly applied methods

Table 12.8 Characterization of particulate systems

Method	Description	Examples
Differential scanning calorimetry (DSC)	Differential measurement of the amount of heat required to increase the temperature of a sample and reference as a function of temperature. Used for measurement of the melting point, glass transition temperature of the drug or carrier, drug/polymer interaction, or lipid or drug crystallinity.	[74, 104, 105]
X-ray diffraction (XRD)	Characterize a crystalline material by measuring the diffracted X-rays, whose wavelength is related to the diffraction angle and the lattice spacing in a crystalline sample. Used for the measurement of crystallinity and crystal size of the carrier or the drug.	[106–108]
Laser light scattering technique	Measure particle size and distribution by using light diffraction or scattering technique (0.02–2000 μm) or photon correlation spectroscopy (1 nm-5 μm).	[76, 98, 109]
Zeta potential	Determine the surface charge of nanoparticles in solution (colloids) and predict the colloidal stability by examining the magnitude of the zeta potential. Formulations with a high zeta potential tend to have better solution stability due to the particle repulsion, while particles tend to aggregate with a low zeta potential due to van der Waals inter-particle attractions.	[74, 110–112]
Light microscope	Evaluate particle size and morphologies of particles; particularly useful for detection of drug crystals using polarized microscope.	[20, 21, 83]
Scanning electron microscopy (SEM)	Examine the morphologies and shape of each individual particles or distinguish the aggregation state of particles.	[20, 103, 113]
Transmission electron microscope (TEM)	Examine the morphologies and shape of nanoparticles and distinguish the aggregation state of particles.	[76, 78, 98]
In vitro drug release/diffusion	Measure the fraction of drug released at different time using HPLC or UV after drug-encapsulated particles are resuspended in a release vehicle or applied onto or inside a membrane. Used to detect lot to lot variation of particles or screen for optimal formulations.	[20, 75, 96, 114]

for the characterization of the particulate drug delivery formulations or systems are summarized in Table 12.8.

12.3.7 Stability Assessment

The stability assessments for various research formulations and delivery systems are summarized in Table 12.9.

At an early development stage (i.e., pre-phase 1 study), formulation properties need to be measured within defined range for certain periods of time (e.g., > 3 months) before Good Laboratory Practice (GLP) and initial clinical studies are performed. Stability study design is highly dependent on the formulation, e.g., drug used, type of particles, type of carrier, as well as vehicles used to deliver the particles. Some critical parameters for particles that could be considered include: visual appearance, clarity, particle size, particle size distribution, zeta potential, chemical stability of the drug, melting point or transition temperature of the carrier, and drug release rate. Particle agglomeration may increase the particle size and broaden the particle size distribution. A continuous measurement of the particle size and zeta potential can be used to detect stability of the particles as a function of time. For solid lipid particles, DSC and XRD can be used to detect the crystallinity of the lipids during storage. An increase of lipid crystallinity may facilitate the instability of nanoparticles, resulting in drug leakage from the carriers. For gelled formulations containing particles, the viscosity of the gel formulations and phase separation of compositions should be evaluated [124].

Particle size, zeta potential, drug or carrier crystallinity, and viscosity were most frequently analyzed in early stage stability studies. However, most of the reported stability studies target a fit-for-use approach. A more comprehensive stability study is required to support PSU-targeted formulations for clinical and commercial development. Example formulation development approaches are discussed in Section 12.4

12.3.8 Safety and Tolerability

Proper understanding of dermal pharmacokinetics, irritation, and toxicity is important to establish the safety of a topical drug product. Determination of dermal formulation safety is a systematic process involving pre-clinical animal studies to characterize performance and potential adverse events that could occur in a clinical study. Typical non-clinical program to support clinical trials for a topical formulation (supporting new molecular entities (NME) and excipients) could include studies to address potential safety risks associated with the drug product in the following areas: acute and repeat dose toxicology, localized skin irritation, allergic skin sensitization, genotoxicity, carcinogenicity, reproductive toxicity,

Table 12.9 Examples of PSU-targeted formulation stability studies

Dosage form	Methods	Results	References
Nanoparticle suspensions	Particle size measured by dynamic laser scattering, and percentage of aggregation particles measured by drug potency test after filtration using a 0.2 μm syringe filter.	No significant drug loss and aggregation was found for 3 months at room temperature.	[76]
	The integrity and activity of OVA after encapsulation into PLGA nanoparticles determined by SDS PAGE and ELISA, respectively.	No degradation of OVA was observed, but dimers and trimers of OVA were formed during the preparation process. Also the activity of the encapsulated OVA was reduced.	[77]
SLN/NLC	Particle size and zeta potential measured at day 0 and day 30 after storage, DSC and drug entrapment efficiency used to evaluate physical stability.	Suspensions were stable, with zeta potential values in the range of −15 to −17 mV. There was no change in particle size 30 days after preparation. DSC thermograms and UV analysis indicated the stability of nanoparticles with negligible drug leakage.	[119]
	Zeta potential, DSC, particle size measured after particles were stored under different conditions: e.g., exposure to light, variable storage temperatures, packing materials, and shear forces.	Samples stored in the dark, at lower temperature (8 °C) and in siliconized glass vials showed better stability, while exposure to light (daylight, or artificial light), high temperature (20 and 50 °C) and nontreated glass vials reduced zeta potential, increased particle size, and caused recrystallization of lipids.	[120, 121]
	Physiochemical stability of the NLC evaluated by using zeta potential, particle size, XRD, and HPLC.	It was demonstrated that low storage temperature, high drug loading, antioxidants, nitrogen gas flushing, and use of suitable surfactants and solid lipids improved stability.	[122]
	Physical stability of chitosan-coated nanoparticle dispersion at 4 °C storage temperature evaluated by examining mean particle size and zeta potential.	The positive charge of chitosan provided a high zeta potential, and the mean diameter of the particles remained nearly unchanged after 1 year storage at 4 °C, indicating a high physical stability. Cell cytotoxicity data did not show adverse effects (i.e., irritation).	[123]
Water-in-oil nanoemulsions	Isotropic phase stability determined by centrifugation (5000 rpm × 15 min) and dilution method.	A clear isotropic solution with a particle size of 25 nm was obtained indicating that a stable formulation at room temperature was prepared.	[85]

Table 12.9 Examples of PSU-targeted formulation stability studies

Dosage form	Methods	Results	References
Nanogel composed of polymeric PLGA nanoparticles	Appearance visually inspected for clarity, particle size analyzed with time after gels were filled in scintillation glass vials and incubated at 2–8 °C and 40 °C/65 %RH for 3 months.	PLGA/chitosan nanoparticles were embedded into Carbopol and HPMC nanogels. There was no change in the appearance and clarity. The particle size of formulations stored at 4 °C remained unchanged, while that of formulations stored at 40 °C increased with time. Viscosity changed with storage conditions and time.	[124]
Gels containing SLN/NLC	Particle size and zeta potential of the gel formulations measured at day 0 and day 90 at room temperature, drug entrapment efficiency evaluated for particle stability purpose.	No sedimentation was observed after centrifugation of formulations. After 90 days of storage at different temperatures the mean diameters of SLN and NLC remained the same (< 1 μm). Zeta potential of the formulation had no change or slightly decreased.	[125]
	Zeta potential, particle size, viscosity and chemical stability of gel formulations measured from day 1, month 3 and year 1 at 4, 25 and 40 °C, respectively.	Physical stability of dispersion was highly dependent on drug and storage temperature, while hydrogel formulations yielded increased zeta potential and higher stability in particle size. The chemical stability of entrapped drug into the carriers was highly dependent on storage temperature and carrier systems, with the 4 °C and NLC systems providing the best protection.	[126]
Hydrogel-thickened microemulsion system containing stable lipid nanoparticles	The long term stability of the nanoparticles and silica-coated nanoparticles assessed via the observation of clarity and phase separation for up to 6 months, centrifugation used (30 min at 13000 rpm) for physical stability.	No phase separation or aggregation of solid oil droplets was observed up to 6 months. The nanoparticle systems showed no phase separation after centrifugation.	[107]

OVA ovalbumin, *PLGA* poly(lactide-co-glycolide), *SLN* lipid nanoparticles, *NLC* nanostructured lipid carriers, *HPMC* hydroxypropyl methylcellulose

local toxicity, pharmacology, and pharmacokinetics. Non-clinical safety study guidelines required for human clinical trials can be found in ICH M3(R2) [127].

12.4 Discovery and Development Process for Topical Products Targeting Pilosebaceous Unit

Discovery and development of a novel drug product is an expensive and time-consuming process. Due to its complexity, a project management strategy is dependent on development stages. Pharmaceutical companies traditionally divide the whole process into three stages: (1) discovery research, preclinical development and clinical development, or discovery, (2) early development (preclinical and phase 1/2) and (3) late development. Although development programs vary among therapeutic areas, indications, and complexity of drug substance and drug products, Paul and coworkers developed an analytical model to illustrate the timeline, cost, and success rate from discovery to launching a new drug product (see Table 12.10). The average cost for large pharmaceutical companies to launch a new molecular entity (NME) is now estimated up to $1.8 billion, and the average time from discovery to market is about 14 years [128]. One typical example is the discovery and development of topical retinoids for dermal indications. After the first discovery that vitamin A was effective in treating acne in 1943, tretinoin was developed for dermal indications in 1959 as the first generation of retinoids [129]. Since then, several new retinoid drug products have been developed and marketed for treating skin conditions including acne, psoriasis, and photodamaged skin. Figure 12.3 shows the generations of retinoid family which are primarily for dermal indications.

12.4.1 Discovery and Early Stage Development Process

The objective of formulation support at the discovery stage focuses on enabling potential active compounds for early development, rather than developing commercializable products. Considering the nature of the drug discovery stage, which

Table 12.10 Cost and time for discovering and developing a new drug. (Adapted from reference [128])

Parameter	Target-to-hit	Hit-to-lead	Lead optimization	Preclinical	Phase 1	Phase 2	Phase 3	Submission to launch	Total
Cycle time (year)	1.0	1.5	2.0	1.0	1.5	2.5	2.5	1.5	13.5
Cost (million)	$ 94	$ 166	$ 414	$ 150	$ 273	$ 319	$ 314	$ 48	$1778

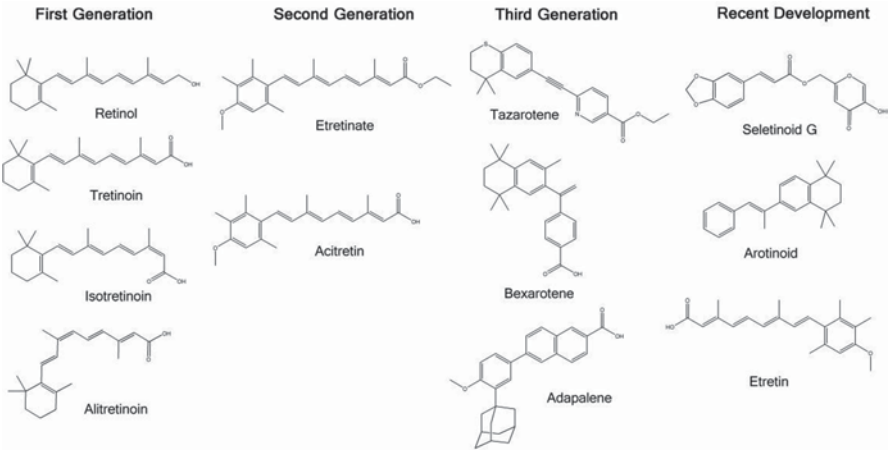


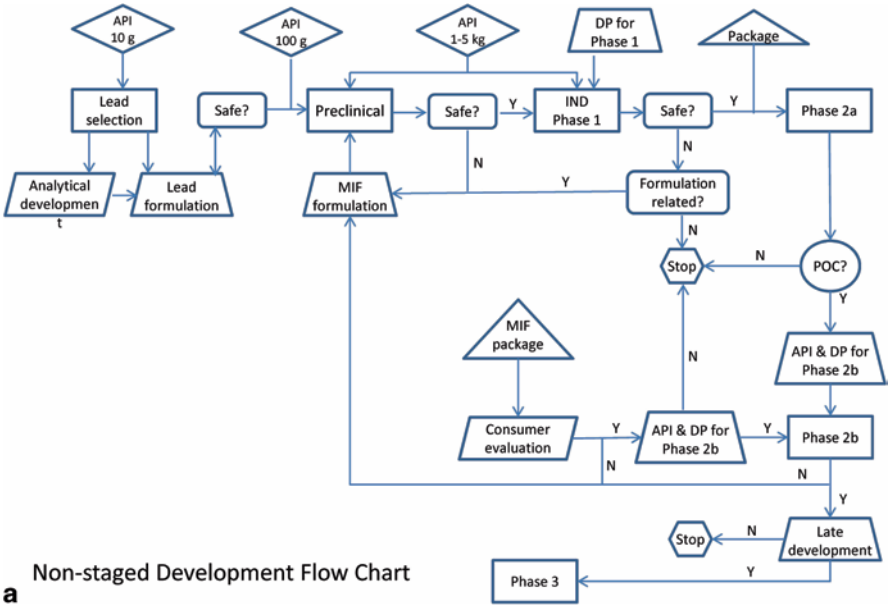
Fig. 12.3 Discovery and development of retinoids

usually involves high throughput screening and high attrition rate, a simple and fit-for-purpose formulation or a simplified delivery system requiring limited formulation development supporting a quick turnaround time is highly preferable. This staged approach is illustrated in Fig. 12.4a. As an alternative approach, a systematic formulation development could start earlier if the estimated probability of success is high. Figure 12.4b outlines the activities and timeline for the non-staged approach.

The formulation activity starts with collecting the relevant information through internal and external reference search to understand: (1) the biological properties including indications, target site, skin integrity at application site, potential dose, duration of treatment, systemic effect (efficacy and side effect), PK, local irritation, skin permeability, etc., (2) physicochemical properties of the NME including chemical structure, molecular weight (MW) log P (D), solubility, pKa, melting point, stability, excipient and packaging compatibility, etc., (3) quantity and quality of drug substance(s), (4) information about marketed products or competitor products, patent status or intellectual property (IP) potential. In terms of project management and process at the discovery stage, a multi-functional team formed by the representatives from chemistry, pharmacology or biology, pharmaceutics, safety, adsorption, distribution, metabolism and excretion (ADME), or PK leads project management and process. Clinical, commercial, legal, and regulatory representatives play an increasing role when the project advances into early development stage. The primary responsibility and activities of each functional line at the discovery and early development stages are summarized in Table 12.11.

12.4.2 Late Stage Development or Reformulation Process

Late stage drug product development strategy should employ a quality by design (QbD) approach to develop a formulation and manufacturing process that will reli-



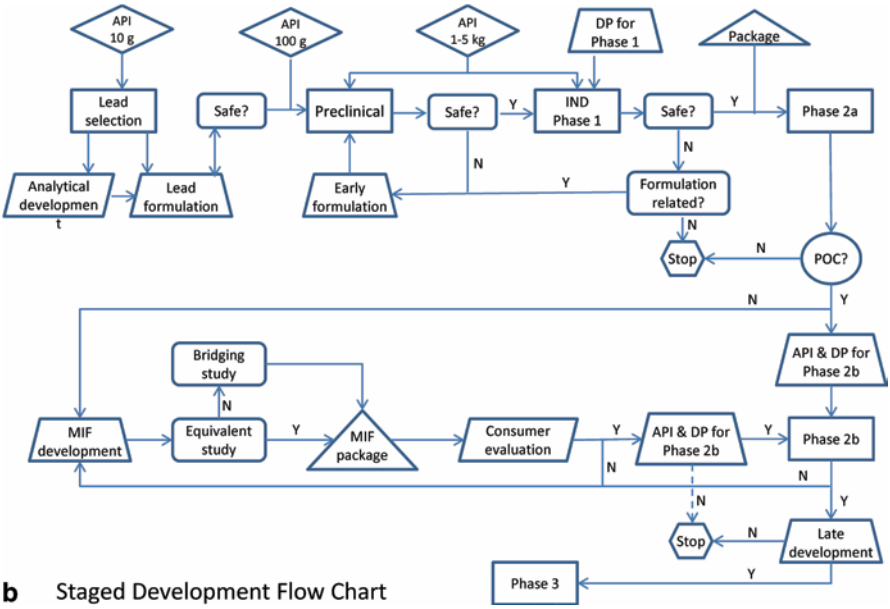


Fig. 12.4 **a** Staged development flow chart. **b** Non-staged development flow chart. *API* active pharmaceutical ingredient, *DP* drug product, IND investigational new drug, POC proof of concept, *MIF* market image formulation

Table 12.11 Primary activities in early-stage development of a new molecular entity (NME) dermal product

Function	Lead selection	Preclinical activities	Phase 1/2 activities
Chemistry	Identify leads and synthetic feasibility	Structural alert Scale up (steps, raw materials, stereocenters, COG, impurities)	GMP scale up Process optimization
Pharmaceutics	Initial solubility, clogP, pKa	Solubility/excipient compatibility/solid form selection UV spectrum for phototoxicity assessment/analytical method Vehicle/formulation development for toxicity/phase 1/2, GLP manufacture API and formulation stability	Develop and validate analytical and antimicrobial methods GMP manufacture Stability
ADME		Systemic exposure/skin permeation and deposition Plasma protein binding/clearance/major clearance mechanisms and metabolites PK/PD assessment/prediction ADME in human/recommendation for clinical dose and dosing regimen	Clinical PK
Pharmacology	*In vitro* functional binding/assay	*In vitro* assay *In vivo* efficacy/biomarker	Clinical pharmacology
Safety		Acute toxicity study Safety pharmacology: effect on neuro, pulmonary, and cardiovascular functions Repeat-dose studies in rats and mini-pigs Genetic toxicity studies Special studies: photoirritation, local lymph node assay, reversibility	Clinical safety Bridging study if formulation is changed
Clinical		IND strategy/early clinical development plan Protocol for phase 1/2	Clinical trials Bridging study if formulation is changed
Commercial/ marketing		IND strategy Strategy for product development and target product profile	
Legal		Freedom to operate and patent opportunity for indication/molecules/API solid form/formulation	File patent application(s)
Regulatory		IND strategy/pre-IND meeting IND filing	IND Amendment

COG cost of goods, GMP good manufacture practice,*GLP* good lab practices, *API* active pharmaceutical ingredients, *ADME* adsorption, distribution, metabolism and excretion, *PK* pharmacokinetics, *PD* pharmacodynamics, *IND* investigational new drug

ably and consistently meet critical quality attributes (CQA) upon release and during shelf-life [130]. The initial step in a development strategy utilizing a QbD approach is to define a quality target product profile (QTPP). QTPP provides guidance on key formulation attributes that must be met during development. The QTPP should address product attributes such as: dosage form and route of administration, container closure, aesthetic attributes of formulation, dosage strength(s), storage condition.

Once the QTPP is defined, the next step in the development strategy is to identify CQA of the drug product. Compiling of drug product CQAs will highlight specific product characteristics that need to be studied to ensure product quality. Drug product CQA of topical formulations usually cover aspects of physical stability, chemical stability, and permeability. For example, studies to define and show control of drug product CQAs for topical products include *in vitro* permeability studies, *in vivo* efficacy studies, and vehicle wear studies to evaluate aesthetics and drug product stability. *In vitro* and/or *in vivo* studies can provide insight into formulation deposition into the hair follicle and subsequent release of drug from encapsulated systems into sebum. For additional detail regarding the conduct of *in vitro* and *in vivo* studies see Tables 12.4 and 12.5. Aesthetic studies to determine cosmetic acceptability can be conducted with a consumer focus group study. Additional CQAs are defined based on QTPP, risk assessment, and formulation team knowledge. As experience and data are obtained from experiments and from early stage formulation development, CQAs should be modified.

Formulation(s) selected for clinical studies (which have been vetted through the above development process) now require an appropriate GMP manufacturing process. Process development activities important to developing an appropriate process include knowing the impact that unit operations in the manufacturing process may have on drug product CQA. As process understanding increases, the significance of raw material and/or drug product controls can be refined and a control strategy can be defined to ensure process performance and product quality.

Stability Protocol Stability protocol for late stage formulation is based on ensuring that the drug product CQA are within defined specifications at release and during shelf-life. Formulations are typically placed on long-term (25 °C/60 % RH), accelerated (40 °C/75 % RH), and if needed, intermediate storage conditions (30 °C/65 % RH) as outlined in ICH Q1 A(R2) [131]. Stability studies may cover a period of at least 2 years for long-term conditions and 6 months for accelerated conditions. Typical time points for long-term storage conditions may include: $t = 0, 1, 3, 6, 9, 12, 18,$ and 24 months. At accelerated storage conditions, at least three time points including the initial and final time points are recommended (e.g., for a 6 month study time points would be initial, 1, 3, and 6 months). At each timepoint formulation testing may include: container closure intregity, assay, impurities of drug substance, total impurities, physical appearance, microbiology testing, and specific testing to evaluate drug release rate from encapsulated systems [132]. Additional testing may be required for micro-/nanoparticulate systems.

12.5 Conclusion

The anatomic structure of pilosebaceous unit provides the possible pathway for targeted drug delivery into hair follicles and sebaceous glands. Although significant progress has been made in the area of PSU-targeted drug delivery over past 20 years, it is still challenging to develop commercial products based on the PSU-targeted mechanisms. With the discovery of new active molecules which can selectively partition in and diffuse through the sebum-filled follicles, it remains an area with great potential. Development of targeted formulations using existing drugs is a more cost-effective and quicker approach to launch a commercial product. Among the emerging technologies, micro-/nanoparticulate systems appear to be the most promising.

References

1. Downing DT, Stewart ME, Strauss JS. Changes in sebum secretion and the sebaceous gland. Dermatol Clin 1986;4(3):419–23.
2. Rosenthal M. Biological role and practical uses of squalene and scalene. In: Libowe I, Wells F, editors. Cosmetics and the skin. New York: Reinhold Publishing Corporation; 1964.
3. Walters KA, Roberts MS. The structure and function of skin. In: Walters KA, editor. Dermatological and transdermal formulations. New York: Marcel Dekker; 2002. pp. 1–39.
4. Downing DT, Strauss JS. On the mechanism of sebaceous secretion. Arch Dermatol Res. 1982;272(3–4):343–9.
5. Greene RS, Downing DT, Pochi PE, et al. Anatomical variation in the amount and composition of human skin surface lipid. J Invest Dermatol. 1970;54(3):240–7.
6. Scheuplein RJ. Mechanism of percutaneous absorption. II. Transient diffusion and the relative importance of various routes of skin penetration. J Invest Dermatol. 1967;48(1):79–88.
7. Woodburne R. Essentials of human anatomy. New York: Oxford University Press; 1965.
8. Whiting D. Histology of normal hair. In: Hordinsky M, Sawaya M, Scher R, editors. Atlas of hair and nails. Philadelphia: Churchill Livingstone; 2000. pp. 918.
9. Barry BW. Drug delivery routes in skin: a novel approach. Adv Drug Deliv Rev. 2002;54(Suppl 1):S31–40.
10. Lauer AC, Lieb LM, Ramachandran C, et al. Transfollicular drug delivery. Pharm Res. 1995;12(2):179–86.
11. Mitchell LH, Johnson TR, Lu GW, et al. Rational design of a topical androgen receptor antagonist for the suppression of sebum production with properties suitable for follicular delivery. J Med Chem. 2010;53(11):4422–7.
12. Touitou E, Godin B, Weiss C. Enhanced delivery of drugs into and across the skin by ethosomal carriers. Drug Dev Res. 2000;50(3/4):406–15.
13. Tata S, Weiner N, Flynn G. Relative influence of ethanol and propylene glycol cosolvents on deposition of minoxidil into the skin. J Pharm Sci. 1994;83(10):1508–10.
14. Sumian CC, Pitre FB, Gauthier BE, et al. A new method to improve penetration depth of dyes into the follicular duct: potential application for laser hair removal. J Am Acad Dermatol. 1999; 41(2 Pt 1):172–5.
15. Schaefer H, Watts F, Brod J, et al. Follicular penetration. In: Scott R, Guy R, Hadgraft J, editors. Prediction of percutaneous penetration: methods, measurements, modelling. Proceedings of the conference held in April, 1989 IBC Technical Services; 1989, pp. 163–73.

16. Motwani MR, Rhein LD, Zatz JL. Deposition of salicyclic acid into hamster sebaceous glands. J Cosmet Sci. 2004;55(6):519–31.
17. Lieb LM, Ramachandran C, Egbaria K, et al. Topical delivery enhancement with multilamellar liposomes into pilosebaceous units: I. In vitro evaluation using fluorescent techniques with the hamster ear model. J Invest Dermatol. 1992;99(1):108–13.
18. Lademann J, Weigmann HJ, Rickmeyer C, et al. Penetration of titanium dioxide microparticles in a sunscreen formulation into the horny layer and the follicular orifice. Skin Pharmacol Appl Skin Physiol. 1999;12(5):247–56.
19. Bamba FL, Wepierre J. Role of the appendageal pathway in the percutaneous absorption of pyridostigmine bromide in various vehicles. Eur J Drug Metab Pharmacokinet. 1993;18(4):339–48.
20. Rolland A, Wagner N, Chatelus A., et al. Site-specific drug delivery to pilosebaceous structures using polymeric microspheres. Pharm Res. 1993;10(12):1738–44.
21. Toll R, Jacobi U, Richter H, et al. Penetration profile of microspheres in follicular targeting of terminal hair follicles. J Invest Dermatol. 2004;123(1):168–76.
22. Mordon S, Sumian C, Devoisselle JM. Site-specific methylene blue delivery to pilosebaceous structures using highly porous nylon microspheres: an experimental evaluation. Lasers Surg Med. 2003;33(2):119–25.
23. Turner NG, Guy RH. Visualization and quantitation of iontophoretic pathways using confocal microscopy. J Investig Dermatol Symp Proc. 1998;3(2):136–42.
24. Pena LE. Topical pharmaceutical compositions of drugs susceptible to oxidative or hydrolytic degradation. WO9407478A1. 1994.
25. Gupta M, Agrawal U, Vyas SP. Nanocarrier-based topical drug delivery for the treatment of skin diseases. Expert Opin Drug Deliv. 2012;9(7):783–804.
26. Musthaba M, Baboota S, Athar TMD, et al. Patented herbal formulations and their therapeutic applications. Recent Pat Drug Deliv Formul. 2010;4(3):231–44.
27. Prabhu P, Patravale V, Joshi M. Nanocarriers for effective topical delivery of anti-infectives. Curr Nanosci. 2012;8(4):491–503.
28. Muller RH, Petersen RD, Hommoss A, et al. Nanostructured lipid carriers (NLC) in cosmetic dermal products. Adv Drug Delivery Rev. 2007;59(6):522–30.
29. Papakostas D, Rancan F, Sterry W, et al. Nanoparticles in dermatology. Arch Dermatol Res. 2011;303(8):533–50.
30. Dokka S, Cooper S, Kelly S, et al. Compositions and methods for topical delivery of oligonucleotides. US Patent 8,168,600. 2012.
31. Friedman D. Hair follicle targeting compositions. WO2011095970A1. 2011.
32. Fritz O, Fritz U, Wojcik R, et al. Loadable polymeric microparticles for therapeutic use in alopecia and methods of preparing and using the same. US20090110731A1. 2009.
33. Harris TJ, Chen AA. Compositions and methods for targeted thermomodulation. WO2012027728A2. 2012.
34. Lishko V, Li L. Method to deliver compositions conferring resistance to alopecia to hair follicles. US5753263A. 1998.
35. Oh DK, Lee KY. Methods for preparing nanoliposome encapsulating proteins and protein-encapsulated nanoliposome. WO2006109936A1. 2006.
36. Lin CC, Kuo TY, Lin YJ. Composition comprising human stem cell growth factor for treatment of hair graying and balding. US7524505. 2007.
37. Shefer A, Shefer SD. Compositions and method for targeted controlled delivery of active ingredients and sensory markers onto hair, skin, and fabric. US6979440. 2005.
38. Paithankar D, Blomgren R, Anderson RR, et al. Compositions and methods comprising energy absorbing materials for follicular delivery. WO2013158278A1. 2013.
39. Li L, Baranov E. Skin vibrational method for topical targeted delivery of beneficial agents into hair follicles. US Patent 6,080,127. 2000.
40. El Maghraby GM, Williams AC, Barry BW. Skin hydration and possible shunt route penetration in controlled estradiol delivery from ultradeformable and standard liposomes. J Pharm Pharmacol. 2001;53(10):1311–22.

41. Flynn G. Cutaneous and transdermal delivery. In: Banker G, Rhodes C, Editors. Modern pharmaceutics. 3rd ed. New York: Marcel Dekker; 1996. pp. 262–9.
42. Lu GW, Valiveti S, Spence J, et al. Comparison of artificial sebum with human and hamster sebum samples. Int J Pharm. 2009;367(1–2):37–43.
43. Valiveti S, Wesley J, Lu GW. Investigation of drug partition property in artificial sebum. Int J Pharm. 2008;346(1–2):10–16.
44. Valiveti S, Lu GW. Diffusion properties of model compounds in artificial sebum. Int J Pharm. 2007;345(1–2):88–94.
45. Trauer S, Lademann J, Knorr F, et al. Development of an in vitro modified skin absorption test for the investigation of the follicular penetration pathway of caffeine. Skin Pharmacol Physiol. 2010;23(6):320–7.
46. Roberts MS, Cross SE, Pellett MA. Skin transport. In: Walters KA, Editor. Dermatological and transdermal formulations. 119th ed. New York: Marcel Dekker; 2002. pp. 89–195.
47. Meidan VM. Methods for quantifying intrafollicular drug delivery: a critical appraisal. Expert Opin Drug Delivery. 2010;7(9):1095–108.
48. Teichmann A, Jacobi U, Ossadnik M, et al. Differential stripping: determination of the amount of topically applied substances penetrated into the hair follicles. J Invest Dermatol. 2005;125(2):264–9.
49. Grams YY, Whitehead L, Cornwell P, et al. Time and depth resolved visualization of the diffusion of a lipophilic dye into the hair follicle of fresh unfixed human scalp skin. J Controlled Release. 2004;98(3):367–78.
50. Bhatia G, Zhou Y, Banga AK. Adapalene microemulsion for transfollicular drug delivery. J Pharm Sci. 2013;102(8):2622–31.
51. Lee WR, Shen SC, Al-Suwayeh SA, et al. Skin permeation of small-molecule drugs, macromolecules, and nanoparticles mediated by a fractional carbon dioxide laser: the role of hair follicles. Pharm Res. 2013;30(3):792–802.
52. Grice JE, Ciotti S, Weiner N, et al. Relative uptake of minoxidil into appendages and stratum corneum and permeation through human skin in Vitro. J Pharm Sci. 2010;99(2):712–8.
53. Patzelt A, Richter H, Buettemeyer R, et al. Differential stripping demonstrates a significant reduction of the hair follicle reservoir in Vitro compared to in vivo. Eur J Pharm Biopharm. 2008;70(1):234–8.
54. Frum Y, Eccleston GM, Meidan VM. In-Vitro permeation of drugs into porcine hair follicles: is it quantitatively equivalent to permeation into human hair follicles? J Pharm Pharmacol. 2008;60(2):145–51.
55. Meidan VM, Bonner MC, Michniak BB. Transfollicular drug delivery—is it a reality? Int J Pharm. 2005;306(1–2):1–14.
56. Bronaugh RL, Hood HL, Kraeling MEK, et al. Determination of percutaneous absorption by in vitro techniques. In: Bronaugh RL, Maibach HI, editors. 21 ed. New York: Marcel Dekker; 2002. pp. 157–61.
57. Franz TJ, Lehman PA, Pochi P, et al. The hamster flank organ model: is it relevant to man? J Invest Dermatol. 1989;93(4):475–9.
58. Weissmann A, Bowden J, Beryn F, et al. Morphometric studies of the hamster flank organ: an improved model to evaluate pharmacologic effects on sebaceous glands. J Invest Dermatol. 1984;82(5):522–5.
59. Uno H. The stumptailed macaque as a model for baldness: effects of minoxidil. Int J Cosmet Sci. 1986;8(2):63–71.
60. Plewig G, Luderschmidt C. Hamster ear model for sebaceous glands. J Invest Dermatol. 1977;68(4):171–6.
61. Mcelwee KJ, Rushton DH, Trachy R, et al. Topical FK506: a potent immunotherapy for alopecia areata? Studies using the dundee experimental bald rat model. Br J Dermatol. 1997;137(4):491–7.
62. Oliver RF, Jahoda CA, Horne KA, et al. The DEBR rat model for alopecia areata. J Invest Dermatol. 1991;96(5):97 S.

63. Sun J, Silva KA, McElwee KJ, et al. The C3 H/HeJ mouse and DEBR rat models for alopecia areata: review of preclinical drug screening approaches and results. Exp Dermatol. 2008;17(10):793–805.
64. Allec J, Chatelus A, Wagner N. Skin distribution and pharmaceutical aspects of adapalene gel. J Am Acad Dermatol. 1997; 36(6 Pt 2):S119–125.
65. Madheswaran T, Baskaran R, Thapa RK, et al. Design and In Vitro evaluation of finasteride-loaded liquid crystalline nanoparticles for topical delivery. AAPS PharmSciTech. 2013;14(1):45–52
66. Parhi R, Suresh P. Production of solid lipid nanoparticles-drug loading and release mechanism. J Chem Pharm Res. 2010;2(1):211–27.
67. Puglia C, Bonina F. Lipid nanoparticles as novel delivery systems for cosmetics and dermal pharmaceuticals. Expert Opin Drug Delivery. 2012;9(4):429–41.
68. Kim BD, Na K, Choi HK. Preparation and characterization of solid lipid nanoparticles (SLN) made of cacao butter and curdlan. Eur J Pharm Sci. 2005;24(2–3):199–205.
69. Uner M. Preparation, characterization and physico-chemical properties of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC): their benefits as colloidal drug carrier systems. Pharmazie. 2006;61(5):375–86.
70. Lademann J, Richter H, Schanzer S, et al. Penetration and storage of particles in human skin: perspectives and safety aspects. Eur J Pharm Biopharm. 2011;77(3):465–8.
71. Liang XW, Xu ZP, Grice J, et al. Penetration of nanoparticles into human skin. Curr Pharm Des. 2013;19(35):6353–66.
72. Prow TW, Grice JE, Lin LL, et al. Nanoparticles and microparticles for skin drug delivery. Adv Drug Delivery Rev. 2011;63(6):470–91.
73. Robertson TA, Sanchez WY, Roberts MS. Are commercially available nanoparticles safe when applied to the skin? J Biomed Nanotechnol. 2010;6(5):452–68.
74. Wang F, Chen Y, Benson HAE. Formulation of nano and micro PLGA particles of the model peptide insulin: preparation, characterization, stability and deposition in human skin. Open Drug Deliv J. 2008;2:1–9.
75. Pitaksuteepong T, Somsiri A, Waranuch N. Targeted transfollicular delivery of artocarpin extract from artocarpus incisus by means of microparticles. Eur J Pharm Biopharm. 2007;67(3):639–45.
76. Morgen M, Lu GW, Du D, et al. Targeted delivery of a poorly water-soluble compound to hair follicles using polymeric nanoparticle suspensions. Int J Pharm. 2011;416(1):314–22.
77. Mittal A, Raber AS, Schaefer UF, et al. Non-invasive delivery of nanoparticles to hair follicles: a perspective for transcutaneous immunization. Vaccine. 2013;31(34):3442–51.
78. Bachhav YG, Mondon K, Kalia YN, et al. Novel micelle formulations to increase cutaneous bioavailability of azole antifungals. J Controlled Release. 2011;153(2):126–32.
79. Lin YK, Al-Suwayeh SA, Leu YL, et al. Squalene-containing nanostructured lipid carriers promote percutaneous absorption and hair follicle targeting of diphencyprone for treating alopecia areata. Pharm Res. 2013;30(2):435–46.
80. Muenster U, Nakamura C, Haberland A, et al. RU 58841-myristate—prodrug development for topical treatment of acne and androgenetic alopecia. Pharmazie. 2005;60(1):8–12.
81. du Plessis J, Egbaria K, Ramachandran C, et al. Topical delivery of liposomally encapsulated gamma-interferon. Antiviral Res. 1992;18(3–4):259–65.
82. Li L Hoffmann RM. Topical liposome delivery of molecules to hair follicles in mice. J Dermatol Sci. 1997;14(2):101–8.
83. Tabbakhian M, Tavakoli N, Jaafari MR, et al. Enhancement of follicular delivery of finasteride by liposomes and niosomes. Int J Pharm. 2006;323(1–2):1–10.
84. Kong M, Chen XG, Kweon DK, et al. Investigations on skin permeation of hyaluronic acid based nanoemulsion as transdermal carrier. Carbohydr Polym. 2011;86(2):837–43.
85. Wu H, Ramachandran C, Weiner ND, et al. Topical transport of hydrophilic compounds using water-in-oil nanoemulsions. Int J Pharm. 2001;220(1–2):63–75.

86. Teichmann A, Heuschkel S, Jacobi U, et al. Comparison of stratum corneum penetration and localization of a lipophilic model drug applied in an o/w microemulsion and an amphiphilic cream. Eur J Pharm Biopharm. 2007;67(3):699–706.
87. Blume-Peytavi U, Massoudy L, Patzelt A, et al. Follicular and percutaneous penetration pathways of topically applied minoxidil foam. Eur J Pharm Biopharm. 2010;76(3):450–3.
88. Abdel-Mottaleb MMA, Neumann D, Lamprecht A. Lipid nanocapsules for dermal application: a comparative study of lipid-based versus polymer-based nanocarriers. Eur J Pharm Biopharm. 2011;79(1):36–42.
89. Han I, Kim M, Kim J. Enhanced transfollicular delivery of adriamycin with a liposome and iontophoresis. Exp Dermatol. 2004;13(2):86–92.
90. Fang JY, Sung KC, Lin HH et al. Transdermal iontophoretic delivery of enoxacin from various liposome-encapsulated formulations. J Controlled Release. 1999;60(1):1–10.
91. Gomaa YA, Garland MJ, McInnes FJ, et al. Microneedle/Nanoencapsulation-mediated transdermal delivery: mechanistic insights. Eur J Pharm Biopharm. 2013. doi:10.1016/j.ejpb.2013.01.026.
92. Zhang W, Ding B, Tang R, et al. Combination of microneedles with PLGA nanoparticles as a potential strategy for topical drug delivery. Curr Nanosci. 2011;7(4):545–51.
93. Zhang Z, Tsai PC, Ramezanli T, et al. Polymeric nanoparticles-based topical delivery systems for the treatment of dermatological diseases. Wiley Interdiscip Rev: Nanomed Nanobiotechnol. 2013;5(3):205–18.
94. Alvarez-Roman R, Naik A, Kalia YN, et al. Skin penetration and distribution of polymeric nanoparticles. J Controlled Release. 2004;99(1):53–62.
95. Lademann J, Richter H, Teichmann A, et al. Nanoparticles—an efficient carrier for drug delivery into the hair follicles. Eur J Pharm Biopharm. 2007;66(2):159–64.
96. Rancan F, Papakostas D, Hadam S, et al. Investigation of polylactic acid (PLA) nanoparticles as drug delivery systems for local dermatotherapy. Pharm Res. 2009;26(8):2027–36.
97. Patzelt A, Richter H, Knorr F, et al. Selective follicular targeting by modification of the particle sizes. J Control Release. 2011;150(1):45–8.
98. Shim J, Seok KH, Park W, et al. Transdermal delivery of mixnoxidil with block copolymer nanoparticles. J Control Release. 2004;97(3):477–84.
99. Vogt A, Combadiere B, Hadam S, et al. 40 nm, but not 750 or 1,500 nm, nanoparticles enter epidermal CD1a + Cells after transcutaneous application on human skin. J Invest Dermatol. 2006;126(6):1316–22.
100. Mahe B, Vogt A, Liard C, et al. Nanoparticle-based targeting of vaccine compounds to skin antigen-presenting cells by hair follicles and their transport in mice. J Invest Dermatol. 2009;129(5):1156–64.
101. Wu X, Griffin P, Price GJ, et al. Preparation and in Vitro evaluation of topical formulations based on polystyrene-poly-2-hydroxyl methacrylate nanoparticles. Mol Pharmaceutics. 2009;6(5):1449–56.
102. Wu X, Biatry B, Cazeneuve C, et al. Drug delivery to the skin from sub-micron polymeric particle formulations: influence of particle size and polymer hydrophobicity. Pharm Res. 2009;26(8):1995–2001.
103. Tsujimoto H, Hara K, Bando Y, et al. Evaluation of the permeability of hair growing ingredient encapsulated plga nanospheres to hair follicles and their hair growing effects. Fragrance J. 2007;35(12):55–62.
104. Dubey A, Prabhu P, Kamath JV. Nano structured lipid carriers: a novel topical drug delivery system. Int J Pharm Tech Res. 2012;4(2):705–14.
105. Castro GA, Orefice RL, Vilela JMC, et al. Development of a new solid lipid nanoparticle formulation containing retinoic acid for topical treatment of acne. J Microencapsulation. 2007;24(5):395–407.
106. Pilcer G, Sebti T, Amighi K. Formulation and characterization of lipid-coated tobramycin particles for dry powder inhalation. Pharm Res. 2006;23(5):931–40.
107. Chen H, Xiao L, Du D, et al. A facile construction strategy of stable lipid nanoparticles for drug delivery using a hydrogel-thickened microemulsion system. Nanotechnology. 2010;21(1). doi: 10.1088/0957–4484/21/1/015101.

108. Abdelbary G, Fahmy RH. Diazepam-loaded solid lipid nanoparticles: design and characterization. AAPS PharmSciTech. 2009;10(1):211–9.
109. du Plessis J, Ramachandran C, Weiner N, et al. The influence of particle size of liposomes on the deposition of drug into skin. Int J Pharm. 1994;103(3):277–82.
110. Desai PR, Shah PP, Hayden P, et al. Investigation of follicular and non-follicular pathways for polyarginine and oleic acid-modified nanoparticles. Pharm Res. 2013;30(4):1037–49.
111. Lim SJ, Kim CK. Formulation parameters determining the physicochemical characteristics of solid lipid nanoparticles loaded with all-trans retinoic acid. Int J Pharm. 2002;243(1–2):135–46.
112. Ridolfi DM, Marcato PD, Justo GZ, et al. Chitosan-solid lipid nanoparticles as carriers for topical delivery of tretinoin. Colloids Surf B 2012;93:36–40.
113. Urban-Morlan Z, Ganem-Rondero A, Melgoza-Contreras LM, et al. Preparation and characterization of solid lipid nanoparticles containing cyclosporine by the emulsification-diffusion method. Int J Nanomed. 2010;5:611–20.
114. Alvarez-Roman R, Naik A, Kalia YN, et al. Enhancement of topical delivery from biodegradable nanoparticles. Pharm Res. 2004;21(10):1818–25.
115. Mak WC, Richter H, Patzelt A, et al. Drug delivery into the skin by degradable particles. Eur J Pharm Biopharm. 2011;79(1):23–27.
116. Levy MY, Schutze W, Fuhrer C, et al. Characterization of diazepam submicron emulsion interface: role of oleic acid. J Microencapsul. 1994;11(1):79–92.
117. Souto EB, Mehnert W, Muller RH. Polymorphic behavior of compritol 888 ATO As bulk lipid and as SLN and NLC. J Microencapsul. 2006;23(4):417–33.
118. Caldorera-Moore M, Guimard N, Shi L, et al. Designer nanoparticles: incorporating size, shape and triggered release into nanoscale drug carriers. Expert Opin Drug Deliv. 2010;7(4):479–95.
119. Baviskar DT, Amritkar AS, Chaudhari HS, et al. Modulation of drug release from nanocarriers loaded with a poorly water soluble drug (Flurbiprofen) comprising natural waxes. Pharmazie 2012;67(8):701–5.
120. Freitas C, Muller RH. Correlation between long-term stability of solid lipid nanoparticles (SLN) and crystallinity of the lipid phase. Eur J Pharm Biopharm. 1999;47(2):125–32.
121. Freitas C, Muller RH. Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticle (SLN) dispersions. Int J Pharm. 1998;168(2):221–9.
122. Teeranachaideekul V, Muller R, Junyaprasert V. Encapsulation of ascorbyl palmitate in nanostructured lipid carriers (NLC)-effects of formulation parameters on physicochemical stability. Int J Pharm. 2007;340(1–2):198–206.
123. Ridolfi DM, Marcato PD, Machado D, et al. In vitro cytotoxicity assays of solid lipid nanoparticles in epithelial and dermal cells. J Phys Conf Ser. 2011;304:012032-1-012032/4.
124. Shah PP, Desai PR, Patel AR, et al. Skin permeating nanogel for the cutaneous co-delivery of two anti-inflammatory drug. Biomaterials. 2012;33(5):1607–17.
125. Bhaskar K, Anbu J, Ravichandiran V, et al. Lipid nanoparticles for transdermal delivery of flurbiprofen: formulation, in vitro, ex vivo and in vivo studies. Lipids Health Dis. 2009;8. doi:10.1186/1476-511X-8-6.
126. Souto EB, Muller R. The use of SLN and NLC as topical particulate carriers for imidazole antifungal agents. Pharmazie 2006;61(5):431–7.
127. ICH. M3(R2) guidance on non-clinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals. Federal Register. 2010.
128. Paul SM, Mytelka DS, Dunwiddie CT, et al. How to improve R&D productivity: the pharmaceutical industry's grand challenge. Nat Rev Drug Discov. 2010;9(3):203–14.
129. Mukherjee S, Date A, Patravale V, et al. Retinoids in the treatment of skin aging: an overview of clinical efficacy and safety. Clin Interv Aging. 2006;1(4):327–48.
130. ICH. Q8(R2) pharmaceutical development. Federal Register. 2009.
131. ICH. Q1A(R2) stability testing of new drug substances and products. Fed Regist. 2003;68:65717–18.
132. USP <1151> Pharmaceutical dosage forms. United States Pharmacopeia (USP 36) 2013.

Chapter 13
Deep Percutaneous Penetration into Muscles and Joints: Update

Vladyslava Doktor, Christine M. Lee and Howard I. Maibach

13.1 Introduction

Percutaneous delivery of drugs into deeper tissues for the treatment of inflammatory muscle, joint, and tendon diseases is a much discussed, but not fully educated topic. With many now living past the 70s, the age when the risk levels for inflammatory diseases, such as osteoarthritis (OA) and rheumatoid arthritis (RA), increase, the need for an efficient and transdermaly delivered medication is substantial [1]. Since oral nonsteroidal anti-inflammatory drugs (NSAIDs) pose a risk of adverse effects such as renal, hepatic, cardiac, and gastrointestinal toxicity, topical use of NSAIDs is necessary. The benefit of a transdermal therapeutic system (TTS) results in a bypass of the gastrointestinal tract and associated side effects. Steps the topical compounds take to reach the joints and underlying muscular tissue are partition and diffusion, primarily through the outermost skin layer, stratum corneum (SC), epidermis, dermis, and subcutaneous fat. The challenge remains to overcome the resistance of SC and of course, to reach the deeper tissue without losing much of the compound's concentration to cutaneous microcirculation. However, earlier work showed that local subcutaneous drug delivery is viable and can be effective [2]. Novel approaches to enhance the penetration of the SC and transdermal drug delivery are now available [3]. Penetration mechanisms and efficacy of absorption of topically applied drugs have reached new heights. Pharmokinetic properties of topical NSAIDS have been studied in length as they are the main focus for the

V. Doktor (✉)
Department of Dermatology, School of Medicine, University of California-San Francisco, San Francisco, CA, USA
e-mail: vladadok@gmail.com

C. M. Lee
Texas Oncology, The Woodlands, TX, USA

H. I. Maibach
Department of Dermatology, School of Medicine, University of California, San Francisco, CA, USA
e-mail: MaibachH@derm.ucsf.edu

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_13

local treatment of several inflammatory diseases of joints and muscles [2]. Some common NSAIDS are salicylic acid (SA), diclofenac, ketoprofen, and naproxen. Diclofenac is the only drug in the group of NSAIDS approved for use in the USA. Available formulations include diclofenac epolamine topical patch 1.3%, diclofenac sodium topical gel 1%, and diclofenac sodium topical solution 1.5% w/w in 45.5% dimethyl sulfoxide (DMSO). The latter two are specifically approved by the US Food and Drug Administration (FDA) for osteoarthritis treatment [4].

Following from a previously published review of percutaneous penetration, new advances in skin barrier function, physicochemical, pharmokinetic, and physiological factors that ground the transdermal administration have been made and are discussed in detail here [5]. The groundwork for determining bioequivalence via kinetics has not been laid.

13.2 Drug Properties Affecting Distribution

As the drug transports through the first barrier, SC, passive diffusion serves as the main mode. Whether it is via intercellular or transcellular pathway, factors mostly involved are lipophilicity and permeability [6]. Other important factors affecting absorption include drug molecular size, water solubility, vehicle, and skin integrity, which mainly is affected by the disease, body site, and age.

13.2.1 Lipophilicity

Lipophilicity is defined by log *P*, the logarithmic octanol-water coefficient [7]. High lipophilicty is needed to overcome SC barrier and to penetrate the deeper tissues. The higher the lipophilicty, the deeper the solute will be transported. It is also directly proportional to increase in log *P* [8]. For example, the lipophilic salt of SA, triethanolamine salicylate (TEA), penetrated more efficiently into muscle compared to ionized SA [9]. Another study performed *in vivo* on male rats demonstrated almost twofold greater penetration of a lipophilic derivative of SA (10% TEA) compared to the less lipophilic derivative than TEA, 10% methyl salicytate, with the topical application of the two compounds on the abdomen of male rats.

If the solution is not sufficiently lipophilic to penetrate the SC enhancers such as propylene glycol, alcohol, dimethyl must be utilized. Diclofenac sodium 1% gel uses isopropyl alcohol, propylene glycol, and water to accelerate drug penetration of the skin. The alcohols typically promote drug solubility and also aid in permeation of the SC. However, higher concentration of alcohol may cause SC dehydration, which will in return impact drug permeability [10].

13.2.2 Permeability

Another drug property contributing to the penetration ability to the underlying muscles and joints is permeability [11], which can be measured by the permeability coefficient, k_p. Permeability coefficient increases with an increase in the fraction of unionized drug [12], and has a parabolic relationship with log *P*, the lipophilicity of a compound [2] for several NSAIDs, as reported in an *in vitro* study of human cadaveric skin from the mid abdomen following delivery of the drug via a donor compartment. An optimal log *P* value will yield a peak permeability coefficient. This observation suggests that a hydrophilic–lipophilic balance is required for deep penetration of drugs [2]. By taking the product of k_p and the solubility of the drug in a given vehicle, the maximum flux, J_{max}, through the skin can be calculated. However, a study showed that molecular weight (MW) is a more significant determinant of maximal flux than k_p [13].

13.2.3 Molecular Weight

Previously, MW was not believed to be a significant determinant of transdermal drug delivery. Even though earlier studies showed that with increasing MW, clearance of the drug from viable skin into the muscle decreases; results were not statistically significant [14]. A review of J_{max} versus several parameters of the drug including MW, solubility in octanol, octanol/water partition coefficient, ultimately showed that MW was the dominant determinant of J_{max}, with the following regression relationship: $\log J_{max} = -3.90 - 0.0190$ MW ($r^2 = 0.847$, $p < 0.001$) [13]. Data were gathered from several experiments performed on human skin *in vitro*. The results changed previous views on which factors affect the maximal flux and transdermal drug distribution.

13.2.4 Fraction Unbound Drug in Viable Skin

Interestingly, unbound fraction of drug in viable skin (fu_{vs}) had statistically significant positive correlation ($p < 0.005$, $R^2 = -0.63$) with the drug clearance from viable skin to the muscle (Table 13.1) [14]. As fu_{vs} increased, an increase in clearance from the viable skin into the muscular layer was observed. The study was performed on stripped-skin rats *in vivo* after application of drug to the rat's abdomen via a donor cell for 0.5, 1, 2, 3, 4, and 7 h. In viable skin, drugs may bind to the cytosolic components, influencing direct penetration [15]. When a drug is not bound to proteins or lipids, the molecule diffuses more easily into deeper tissues including the muscle. However, it is not known why the increase in fraction of unbound drug in viable skin is not an important factor for systemic absorption. Perhaps, the equilibrium between the unbound fraction among the viable skin, muscular layer, and plasma may play a role [14].

Table 13.1 Effect of physiochemical parameters on $CL_{vs\cdot m}$ and peak muscle concentration. (Data from Higaki et al. [14])

Drug	MW (g/mol)	fu_{vs}	$CL_{vs\cdot m}$ (mL/h)	Peak muscle concentration (nmol/g)[a]
Diclofenac	295.1	0.592	0.008 ± 0.000	6.0 ± 0.5
Salicylic acid	138.1	0.870	0.085 ± 0.004	40 ± 5
Ketoprofen	254.3	0.701	0.133 ± 0.069	15 ± 2
Felbinac	212.2	0.667	0.043 ± 0.000	19 ± 2
Flurbiprofen	244.3	0.401	0.032 ± 0.000	33 ± 5

The clearance of a drug from the viable skin into the muscle, $CL_{vs\cdot m}$, and peak muscle concentration are examined in relation to the molecular weight, MW, and the fraction of unbound drug found in viable skin, fu_{vs}. The study was performed on stripped-skin rats *in vivo* after application of the drug to the rat's abdomen via a donor cell. The rats were sacrificed at 0.5, 1, 2, 3, 4, and 7 h

[a] Peak muscle concentration estimated from Higaki et al., Fig. 3 [14]

13.2.5 Relative Importance of the Physicochemical Factors

Among the factors presented above, it seems that the fraction unbound in viable skin is important for the direct penetration of a compound. Penetration rate was characterized by a kinetic parameter, k_{direct}, which was found to correlate with the clearance from the viable skin to muscle [14]. Multiple linear regression analysis was then used to determine the relative contribution of MW, log *P*, and fu_{vs} on the penetration rate. The results were 0.1588 for MW, 0.2686 for log *P*, and 0.5726 for fu_{vs} [14]. This analysis showed that fu_{vs} had the greatest contribution to the penetration rate, as measured by k_{direct}.

13.3 Pharmacokinetic Models

One of the first pharmacokinetic models was developed in 1982, which was a four-compartment model with the epidermis, dermis, capillaries, and urine using first-order kinetics. Two fixed rate constants were used to characterize absorption and elimination, while two variable rate constants helped define the penetration through the viable skin and the competition for the drug between the viable skin and the stratum corneum. Guy et al. found that topical delivery produces high local subcutaneous levels of drug despite reduced blood concentrations [16]. Direct deep penetration is thus the mechanism implicated. The blood flow in skin, particularly in dermis, is a crucial factor in deciding the direct penetration of drugs into muscle. On the one hand, blood vessels in the dermis absorb and dilute most compounds passing the epidermis, keeping a "sink" condition and promoting percutaneous absorption [17]. On the other hand, blood flow prevents drugs from directly penetrating into deeper tissues by removing them to the systemic circulation. Absorption can also be modified by vasoconstrictors. A recent vivo study on rats by Higaki et al.

showed that topical application of a vasoconstrictor, phenylephrine, enhanced the direct penetration of the drugs into viable skin and muscle [18]. Phenylephrine was coadministered with several drugs via a donor cell, and the drugs' concentrations measured after 2 h of application time on the rat's abdomen. Distribution of antipyrine ($p<0.001$), SA ($p<0.001$), and diclofenac ($p<0.01$) into viable skin yielded statistically significant increase in penetration when phenylephrine was coadministered versus control. Similar results were seen for distribution into the muscle layer: antipyrine ($p<0.001$), SA ($p<0.01$), and diclofenac ($p<0.05$) [18].

Multiple groups have analyzed drug distribution kinetics following percutaneous delivery. Singh et al. developed a pharmacokinetic model using multiple differential equations to estimate the drug concentrations found below the epidermis after topical application of a drug [19]. Importance of the pharmacokinetic model is that it can predict the amount of drug that eventually will reach the deep tissue compartments, providing useful information for the development of the controlled dosing methods.

Distribution to underlying tissues was modeled as multiple compartments in series, with the dermis and deep muscle in contact with the systemic circulation. Separate differential equations were developed to characterize drug concentration in four or more compartments: the application cell, the dermis, the underlying tissue, and the systemic circulation [19].

13.3.1 Experimental Methods

Drug solutions were applied to rats and subsequently the underlying tissue concentrations and clearance values were taken. The rat was chosen as an experimental model for these studies since it has been shown to have similar permeability characteristics to human in some studies [20]. However, other studies are not as conclusive and these are discussed in detail in Chap. 2. Solutions were applied to exposed rat epidermis in the absence of SC. A glass cell containing the drug solution was attached to the exposed dermis, and was removed at predetermined times to measure solute concentration. Blood samples were taken from the tail vein. Afterward, the animals were sacrificed and tissues below the treated site were dissected to analyze drug concentrations [7].

13.3.2 Model Predictions

The model predicted tissue concentrations of different NSAIDs, in some cases within 90% of the actual value. With indomethacin, however, there was a higher observed value than predicted, suggesting that indomethacin has poor diffusiblity and accumulates in the dermis [7]. Inaccuracies in the predictions may be due to inherent differences in tissue affinities for different solutes, nonlinearities in tissue binding (although trace concentrations of all solutes were used to avoid the

nonlinearities in plasma and tissue binding), variations in plasma protein binding, dermis water partitioning, tissue-plasma partitioning, and possible drug effects on membrane or blood flow. The apparent tissue–tissue clearances may also vary for different compounds [7].

Higaki et al. also developed a pharmacokinetic model, this time with six compartments including contralateral skin and contralateral muscle, which were not included in the Singh et al. model [14]. Inclusion of the contralateral side allows the contribution of the absorption from the systemic circulation to the muscular disposition to be evaluated more precisely. *In vivo* rat transdermal studies were performed by comparing model predictions to experimental results.

Unlike Singh et al., linear differential equations were used to plot standard curves (drug concentration over time) rather than estimate deep tissue concentrations. For each standard curve, coefficient of variations ranged from 0.1 to 13.0%, and the squared correlation coefficient was over 0.994 for all the model drugs used [14]. The standard curves fit the lines well, and correlate significantly with the observed values for all six drugs. Having the standard curves enable the calculation of the relative contribution of direct penetration of the drug based on the area under the curve values. Thus, the two models appear useful for analyzing and describing the transdermal disposition of drugs after topical application, though there may be slight variations in the way the differential equations are utilized (Table 13.2).

13.3.3 New Physiological Models

Earlier pharmokinetic models created by Singh and Roberts and Higaki et al. assumed that solute is transported to deeper layers by molecular diffusion only. Kretsos et al. reanalyzed the data and confirmed the consistency with their own similar distributed diffusion-clearance model [21]. Following the previous research, Kretsos and Kasting created a new model to explain the dermal capillary clearance process based on the assumed periodic microscopic distribution of dermal capillaries in three-dimensional space. The downfall of this model is that it only applies to the steady-state scenario and explains localized concentration in dermis [22]. Bound by the inability to recreate *in vivo* conditions using *in vitro* experiments and invasiveness associated with the collection of such data via biopsy, the contributory research is difficult to carry out. However, a breakthrough came when Anissimov and Roberts determined that the deep percutaneous transport of drug cannot be attributed to diffusion alone and offered a new two-compartment model that considers blood and/or lymphatics, in addition to molecular diffusion to be involved in the transport to deeper tissues. More specifically, they focused on the effect of blood flow, blood protein binding, and dermal binding exert on the rate and depth of percutaneous penetration of topical drugs. Unlike Singh and Roberts, Higaki et al. used the combination of human biopsy data collected by Schaefer et al. [23–27] in applying their model and their own human *in vitro* dermis penetration experiments to obtain dermal diffusion/dispersion coefficient, and dermal blood clearance rate of six solutes.

Table 13.2 Comparison of two different pharmacokinetic models

	Singh and Roberts [7]	Higaki et al. [14]
No. of compartments	9	6
Model arrangement	Donor cell and deep tissue compartments in series arranged in parallel to plasma	Donor cell, viable skin, and muscle in series arranged in parallel to both plasma nad contralateral skin, muscle
Experimental studies	*In vitro* human study on epidermal penetration	*In vitro* human study on epidermal penetration
	In vivo rat study on transdermal absorption	*In vivo* rat study on transdermal absorption
Parameters from *in vitro* studies	k_p (epidermal permeability coefficient) Log p (lipophilicity)	k_{direct} (penetration rate constant from viable skin to muscular layer) fu_{vs}
Application	To predict deep tissue concentrations and compare with observed values	To formulate standard curves to approximate the decreasing concentrations in deep tissues
Statistical significance	N/A	CV ranges from 0.1 to 13 %, R^2 greater than 0.994 for standard curves

Two of the pharmacokinetic models are used to approximate deep tissue drug concentrations with differential equations and nonlinear regression analysis. The experimental methods, applications, and statistical significance of the results are examined

Schaefer et al. collected human tissue concentration-depth profile of drugs *in vivo* after topical application.

The term dispersion implies transport of solute by both blood and diffusion in the dermis. They recognized that in order for convective blood flow transport to significantly impact the transport to deeper tissues, there must be sufficient binding to plasma proteins and blood flow, as the surface area of blood vessels is much less than that of the dermal matrix through which diffusion transport will occur. Therefore, the contribution of dermal blood-flow transport is likely to be noticeably decreased when there is vasoconstriction [28]. They analyzed human dermal distribution data from previous microdialysis experiments and recorded similar findings. One limitation of their analysis was the assumption of no contribution of topically absorbed drug into the systemic circulation contributing to the underlying tissue concentration on recirculation. It is considered to occur at long times and most considerable contribution to tissue concentrations deep below the treated site [28].

Dancik et al. further expanded on the findings by Anissimov and Roberts' physiological pharmokinetic model in that it recognized the interstitial convection associated with capillary flow and draining of the interstitial space. Their new comprehensive model described drug diffusion in the extravascular tissue space and vascular binding, axial (into the tissue) vascular, lymphatic, and interstitial convection transport and constant radial (clearance) vascular transport, with the assumption of high-capillary permeability. This was done by comparing the *in vitro* penetration lag

times of diclofenac and nicotine gathered from their *in vitro* experiments to those reported *in vivo* penetration lag times in the dermis and deeper tissues [29].

They concluded that the transport of highly plasma protein-bound drugs into deeper tissues increases by several orders of magnitude faster than predicted by passive dermal diffusion. Although considerable concentration-depth gradient is evident for poor protein-bound drugs, it is nonexistent for highly bound drugs in the papillary dermis and small in the reticular dermis [29]. High protein-bound drugs bind to collagen and albumin in the dermis. The convective transport of albumin into lymphatic vessels that run deeper in subcutaneous tissue encourage deeper transport.

13.4 Distribution in Deeper Tissues

13.4.1 Direct Versus Indirect Penetration

NSAIDs directly penetrate to a depth of 3–4 mm, with the systemic blood supply accounting for penetration into the deeper, underlying tissues [19]. Drug levels peaked between 2 and 4 h due to direct NSAID penetration. At around 10 h, drug levels peaked again, this time due to the systemic blood supply redistributing the drug [19].

Higaki et al. also examined drug penetration distribution after topical application [14]. Similar to Singh and Roberts' findings, also performed *in vivo* on rats [19], they determined that direct penetration was the predominant mechanism during the early period after starting the absorption study. There is a variability between the drugs in regards to the concentration of the direct penetration into muscle after topical application. For instance, the muscular disposition of diclofenac was almost all attributed to the direct penetration (90.8%), but felbinac was distributed to the muscle via the systemic circulation (a > 50%; Table 13.2) [14].

Contrary to Singh and Roberts' conclusions, Higaki et al. found that SA in the muscle layer was mainly from direct penetration (72.0%) and less from systemic distribution (28%) [14]. This is almost the reverse of Singh and Roberts' observations in1993 (80% due to systemic blood supply, 20% due to direct penetration) [19]. Subsequent research confirmed that most of the drug in muscle is due to direct migration and not from the systemic circulation following topical application [30].

13.4.2 Penetration Efficacy

SA yielded the highest local tissue levels, followed by piroxicam, naproxen, indomethacin, and diclofenac (Table 13.3). Note that aqueous solutions were used for estimating tissue concentrations, but in clinical practice, NSAIDs may be admin-

Table 13.3 Estimated concentrations of various NSAIDs found in muscle at maximal flux

Drug	Fraction of initial concentration[a]
Salicylic acid	10^{-6}
Piroxicam	10^{-7}
Naproxen	$10^{-7.8}$
Indometacin	$10^{-9.5}$
Diclofenac	$10^{-9.7}$

Drug concentration found in muscle at maximal flux after topical, aqueous drug delivery. Singh and Roberts' estimated concentrations were obtained using pharmokinetic equations and experimental k_p values

[a] Data from Singh and Roberts, Fig. 6 [7]

Table 13.4 Comparison of NSAID concentrations 2 h following topical application[a]

	Reference	Donor cell	Viable skin	Plasma	Muscle
Diclofenac	13	1	0.50 ± 0.02	$(1.70 \pm 0.09) \times 10^{-3}$	$(1.00 \pm 0.05) \times 10^{-3}$
	2	1	0.10 ± 0.01	$(4.5 \pm 0.2) \times 10^{-3}$	$(1.00 \pm 0.05) \times 10^{-3}$
Salicylic acid	13	1	0.22 ± 0.01	$(2.3 \pm 0.1) \times 10^{-2}$	$(1.00 \pm 0.08) \times 10^{-2}$
	2	1	0.080 ± 0.004	$(1.00 \pm .05) \times 10^{-2}$	$(2.50 \pm 0.12) \times 10^{-3}$
Ketoprofen	13	1	0.20 ± 0.01	$(5.7 \pm 0.3) \times 10^{-3}$	$(1.00 \pm 0.15) \times 10^{-3}$
Naproxen	2	1	0.08 ± 0.004	$(2.5 \pm 0.1) \times 10^{-2}$	$(4.00 \pm 0.15) \times 10^{-3}$
	26[b]	1 (epidermis)	0.22 ± 0.01 (dermis)	$(1.3 \pm 0.1) \times 10^{-3}$	$(4.60 \pm 0.23) \times 10^{-3}$

The drug concentration profiles from the donor cell to the deep tissues from several experimental findings are compared

[a] Results are expressed as a fraction of initial donor cell or epidermis concentration

[b] Concentration profile taken 3 h post application

istered as partially nonaqueous creams, ointments, or gels. The observed differences may also reflect the differences in formulation, patches, application method (solution, ointment, or cream with or without rubbing), duration, application site, or species studied [7].

Table 13.4 examines the relative drug concentrations below the site of application and in the plasma of different studies. In general, the results agreed with one another, though the relative concentration observed may be different. This may be attributed to variations in topical application methods and the tissue location where the sample was taken. Diclofenac, SA, ketoprofen, all have a greater concentration found in plasma than in muscle, whereas naproxen has a higher drug amount in the muscle than in plasma.

13.5 Distribution into Joints and Surrounding Soft Tissues

13.5.1 Diclofenac Controversy

In a human study of percutaneous penetration into the joints, diclofenac gel was applied to one knee and a placebo gel to the other knee of patients with bilateral knee joint effusions [31]. Drug distribution through synovial fluid was mainly through the systemic blood supply. Direct penetration, if at all, was minimal [31]. These results differ from other studies involving diclofenac, including the aforementioned study by Higaki et al., which found that 90.8% of the diclofenac's distribution to muscle was due to direct penetration [14].

However, Higaki et al. used a six-compartment model based on steady-state kinetics in collecting their data. Other literature supports direct penetration as the main delivery mechanism for diclofenac including the one which observed 219.68 mg/mL of diclofenac in skeletal muscle allowing topical administration, whereas only 18.75 mg/mL of the drug was found in plasma where the study was also done *in vivo* on patients, but diclofenac was applied to the anterior thigh [32]. A recent study of diclofenac concentration in soft tissues after oral versus topical diclofenac administration in 14 patients prior to knee orthoplasty reported that the diclofenac concentration was significantly lower in synovial membrane and synovial fluid in topically applied diclofenac than in oral administration ($p = 0.0181$ for topical diclofenac group and $p = 0.004$ for oral diclofenac group). The authors could not identify the difference as the concentration time curves of plasma and synovial tissues or the peak values after administration were not determined [33]. On the contrary, using equivalent study methods and materials on 16 human subjects prior to ACL reconstruction surgery with the same study participation exclusion criteria, Kai et al. have found no significant difference in flurbiprofen concentration in synovial tissue after oral and topical administration of NSAID. They postulated that the role of dermal vessels in the delivery of NSAID to the bone is reduced since the bone tissue is surrounded by a dense calcified matrix [33, 34].

The minimal concentration of NSAID formulation that has anti-inflammatory effects in the synovial tissue remains unknown. In addition, the question of whether injection site and/or injection needle serve as contaminants in the studies ought to be given special attention. Precision of measurement of diclofenac concentration inside the synovial tissue and plasma is imperative in drawing the appropriate conclusion of studies.

Further investigations into this apparent contradiction of results are required, including whether the topical application site would make a difference as this has not been assessed properly to date to the best of our knowledge. In addition, note that higher relative drug concentrations in the synovial fluid were measured in the smaller joints, such as finger and wrist joints, after topical delivery, which probably reflects the shorter diffusion distance that is needed to reach the deeper tissues.

Difference in drug prescription, formulation, and measurement play a role in the outcomes of all studies. It is vital to collect more human data and develop more standardized animal/human models that will take into account not just one dimensional kinetics but the blood flow and lymphatic distribution of the microcirculation in the dermal layers of skin.

13.5.2 Salicylic Acid

SA penetrates into the synovial fluid. Rabinowitz et al. found high levels of salicylate in local tissues after transdermal application of its triethanolamine salt to knees of dogs. After a 60 min administration period, 1.18 ± 0.84 mmol/g was found in the synovial fluid, significantly more than the 0.0094 mmol/g observed in serum [35]. The greater tissue penetration of salicylate (used as a triethanolamine salt) is probably the result of the more lipophilic nature of TEA compared with SA. Similarly, Mills et al. also demonstrated higher concentrations of SA and methyulsalicylate (MeSA), its commercial ester, in the synovial fluid after topical drug application to affected joints in greyhound dogs. They used combined experimental procedure of microdialysis and direct tissue concentrations to measure penetration of a commercial SA ester. This technique allowed plasma drug concentration measurement from both systemic circulation and regional vascular drainage. Mills et al. credited direct diffusion and local blood redistribution to be responsible for the results [36].

13.6 Modes of Delivery

In recent years, the delivery of SA following intracutaneous (IC), subcutaneous (SC), intramuscular injections (IM), and also after topical application were examined in rats [37]. A pharmacokinetic model was employed to calculate the rate constants between the skin, muscle, central, and peripheral compartments of the systemic circulation. For calculations of intramuscular drug delivery, a two-muscle-compartment model was used, with the skin compartment eliminated. The first-order differential equations were also fitted to the concentration data of SA using the nonlinear least-square method. However, for the IC and SC injection data, the fitted lines were a little bit higher than observed for early period measurements in the skin [37]. From this observation, it appears that the first-order kinetics model for the drug migration may be too simple to express the complex migration process of SA. However, the model should be sufficient to illustrate the general differences in skin dispositions of SA following IC, SC, and IM, injections, as well as after topical application. The group concluded that IC injection was the best for localizing the drug to the muscle while maintaining an effective drug concentration (Table 13.5) [37]. Their results corresponded with previous studies where most of the SA in the muscle following cutaneous injections was due to direct delivery from the injection site, and not from systemic redistribution.

Table 13.5 Salicylic acid clearance into muscle for various delivery routes

Delivery rate	% Injected dose	$K_{s\cdot m}$
IC injection	10 ± 1	8.24×10^{-3}
SC injection	9 ± 1	7.58×10^{-3}
IM injection	2.0 ± 0.1	2.94×10^{-3}
Topical application	1.0 ± 0.1	4.95×10^{-3}

The percent of the injected drug concentration found in the muscle 2 h following delivery for different delivery routes are compared. K_{sm} is the first-order rate constant from skin to muscle, obtained through curve fitting using values obtained from several experiments [37]

13.7 Transdermal Drug Delivery Enhancements

Benefits of transdermal delivery of drugs include the following:

- Presystemic metabolism is eliminated, which allows for reduction in the daily dosage levels.
- Blood or plasma levels of the drug can be retained within the therapeutic window for prolonged periods of time.
- Patient's compliance is improved and the drug administration can be aborted by removal of the patch.

On the other hand, the limitation is that the transdermal delivery only works for potent drugs with daily dose of the order of 10 mg or less. They must be "small" lipophilic molecules with molecular weight no greater than 500 Da. The drug must also be free of local irritation. Therefore, it comes as no surprise that noninvasive approaches to enhance and control the drug transport in transdermal manner has been a hot topic for some time now.

Those drug molecules that exceed the size of 500 Da need enhancements to aid in their passing through the main security gate, SC barrier. Most current tactics encompass: chemical penetration enhancers, iontophoresis, transporter carriers, ultrasound/microneedles/thermal poration.

13.7.1 Transporter Proteins

Transporter mechanisms have been implicated in affecting the drug absorption, disposition, and elimination. One such is a P-glycoprotein (P-gp), a member of the ABC transporter family, was recently found expressed in human skin in addition to already known expression in other human tissues (liver, blood–brain barrier). By comparing mRNA, protein expression, and localization of P-gp in several skin tissues to human liver tissues, Skazik et al. determined strong P-gp protein expression within sweat ducts, vessels, nerve sheaths, and most importantly muscles of human skin [38]. P-gp appears capable of transporting itraconazole within dermal tissue.

This new knowledge can possibly play a role in drug development and increasing the efficiency of delivery to specific sites in deep layers of skin such as muscles and joints. Data on this mechanism and deep penetration are in its early stage.

13.7.1.1 Ultrasound Guided Percutaneous Drug Delivery

High-frequency sonophoresis, HFS, (≥0.7 MHz) has been used for five decades to aid percutaneous delivery of corticosteroids. With the discovery of the cavitation effect within the skin that can impact skin permeability, thermal, and convective effects can also play a role in increasing solute partitioning into the SC. Most compounds delivered by HFS are small molecules, with only a handful of drugs having molecular weights greater than 1000 Da tested. As HFS is safe and FDA is approved, many studies that include treatment protocols have been done testing the use of various drugs including NSAIDs and the efficacy of transdermal delivery [39].

13.7.1.2 Combination of Iontophoresis, Terpene, and Hypothermia

Kigasawa et al. showed that using new iontophoresis device with terpene, like geraniol, in rats the percutaneous penetration of diclofenac was amplified. Plasma concentration of diclofenac was increased 20-fold based on the time-dependent delivery. This new device uses an ion-exchange membrane that when combined with geraniol improves the penetration of diclofenac into the stratum corneum. Overall, there was no report of skin irritation [40]. Another study demonstrated the synergistic effect of iontophoresis and regional cutaneous hypothermia on trandermal delivery of diclofenac and prednisone to synovial fluid in rats. The study showed three-fold increase in biovailability of both drugs by decreasing the dermal clearance of the drug via vasoconstrictive effects of hypothermic reaction [41]. The results of both studies are promising and warrant further investigation to determine the mechanism and efficacy in human subjects.

13.7.2 Efficacy and Safety of Percutaneous Drug Delivery in Humans

While research in humans regarding percutaneous drug delivery to muscle, tendon, and joint is limited, more than a dozen studies assess the efficacy and safety of topical NSAIDs in several pain conditions. Two randomized double-blinded controlled studies by Tugwell et al. and Simon et al. assessed efficacy and safety of a topical diclofenac solutions compared to an oral diclofenac solution, as well as the ability to alleviate the primary osteoarthritis symptoms in the knee in a 12-week period. The first study by Tugwell et al. was performed on 622 female and male patients pre-

sented with radiographic evidence of knee OA. Three efficacy measures were pain, physical function, and patient global assessment, which were measured on a nominal index scale. Results demonstrated no clinically significant difference between the two treatment arms in treating pain associated with osteoarthritis ($p=0.10$), asserting that a topical diclofenac solution is as effective as an oral diclofenac treatment [42]. In the second study, Simon et al. assessed pain scale, physical function as well as patient overall health assessment on ordinal scale. The patient population consisted of 775 females and males also presented with radiographic evidence of knee OA. However, in addition to topical and oral diclofenac solutions, the subjects were blindly given either placebo solution or a diclofenac solution in DMSO vehicle or a mixture of topical and oral diclofenac solutions. The results were similar to the first study in that the efficacy of topical diclofenac was comparable to that of oral diclofenac treatment ($p=0.429$) [43].

Overall both studies recommended that topical NSAIDs, specifically daclofenac, are indeed able to provide the therapeutic relief of osteoarthritis pain. Moreover, when comparing the safety of diclofenac in both studies, it is clear that topical diclofenac treatment demonstrated a lower incidence of GI side effects such as dyspepsia, diarrhea, abdominal distention, abdominal pain and nausea. Treatment with oral diclofenac showed an association with significantly greater increases in liver enzymes and creatinine, and greater decreases in creatinine clearance and hemoglobin ($p<0.001$ for all). The most common adverse effect associated with topical diclofenac solution was dry skin. In fact, the dryness and irritation of the skin was similar in patients receiving the vehicle alone and topical solution with DMSO vehicle. This finding might be possibly explained by the fact that vehicle dissolved lipids on the skin surface [44]. Typically, in clinical setting, the use of emollients parallel to the main treatment of topical NSAID is encouraged but was not allowed in the trials. Also, it was reported previously that DMSO can cause halitosis and body order in some patients as a result of its metabolite dimethyl sulfide producing a garlic-like order [45].

Elderly patients exhibit predisposition for topical NSAIDs based on the perception that a lower dose of the medication would result in less toxicity and provides quick effect without affecting the rest of the body based on localized application [46]. In order to elicit the best advice more longitudinal studies (>12 weeks) are needed with participants that have nonlimited comorbid conditions and concomitant medications [47].

13.8 New and Future Research Direction

13.8.1 Photoacoustic Spectroscopy

One limitation in human studies on topical penetration of anti-inflammatory drugs is the inability to quantitatively and noninvasively measure the formulation penetra-

tion rate through the tissue where the topical compound is applied. Photo acoustic Spectroscopy (PAS) is one technique used in dermatological research to analyze the depth of penetration of a compound. It has been shown to measure accurately penetration and distribution of various compounds through skin *in vitro, ex vivo,* and *in vivo* [48]. PAS measures the effect of absorbed light through the skin layers with and without the applied compounds. Oliveira et al. demonstrated with PAS that deep percutaneous infiltration of Helicteresgardneriana (EEHg) crude extract reduced significantly the croton oil-induced auricle inflammation in mice. Doses of 5.0 and 7.5 mg of the EEHg created 61 and 75% decrease in edema of the auricle ($p < 0.001$). High resolution and the low cost and effectiveness of PAS might be the future of instrumentalization of human dermatological research studies [49].

13.8.2 Compound Transdermal Patch

Xi et al. explored the potential of compound transdermal patch containing teriflunomide (TEF) and lornoxicam (LOX), both recommended for treatment of RA. TEF is an active metabolite of leflunomide, a disease modifying antirheumatic drug (DMARD) and lornoxicam is a NSAID. This is the first of its kind study examining compound topical agent for RA as it has not been investigated before in either animals or humans. The challenge is the delivery of drugs into the synovial tissues. Xi et al. first used *in vitro* permeation animal experiments to optimize the formulation of the compound patch and then delivered it to the inflammation-induced two knee joints of rabbits. The goal was to observe whether the drug released into the articular cavity via direct diffusion on the application or via systemic circulation. After applying the transdermal compound patch, authors noted the isochronous rates of penetration for TEF and LOX. Moreover, direct diffusion of transdermal application of the patch with the medicine was more successful for the superficial joint tissues than for the deeper tissue synovial fluid as correlated to the drug concentration measured in the extracellular synovial fluid at 2 and 6 h. Additionally, drug concentrations were detected in the contralateral skin that did not receive the direct application of the transdermal patch. This signified that the systemic blood supply played part in it. As with any topical application of patches, there is a risk for the local area irritation. This experiment also showed that applying the compound patch at noninflammatory skin of the rats' knee and abdominal skin was sufficient to have drug's concentration detected in the inflamed bilateral hind paws of each rat [50]. These findings are important for future research as they continue to contribute to the next generation of researchers who like the idea of combining treatment methods to increase the efficacy for a given disease. The query of topical site application remains to be explored further with the next way of research studies.

13.9 Improvements to Future Research

Unequivocally, all the studies presented above continue propelling forward the research on percutaneous drug penetration into deeper underlying tissues. Recent findings of involvement of dermal blood flow, lymphatic flow, and convective transport in the transdermal delivery mechanism of NSAIDs added another dimension to consider for researchers. It is possible that the newly formulated physiological pharmokinetic model represents more sound explanation for variations of transdermal transport of NSAIDs to muscle, tendon, and joint in handful human studies that have been completed, so far. Perhaps, the questions of direct versus indirect drug penetration should be attributed to percutaneous drug penetration into muscles, tendon, and periosteal tissues rather than all the way to bone tissue. A new classification including the contribution of local blood flow system might be useful in applying to mechanism of delivery of NSAIDs into bone tissues.

Questions raised by the studies primarily focus on the need of standardization of study procedures and increase the number of human studies to reciprocate the findings from *in vivo* and *in vitro* animal studies. It cannot be emphasized properly the imperative nature of development not only for longitudinal studies in studying the safety and efficacy of topical NSAIDs, but also appropriate study protocols that will focus on standardization of anatomical sites and techniques used to collect the data. For instance, clinical techniques routinely used to clean or prepare skin can significantly affect the rate and extent of penetration of a topically applied drug. This may sway the results and affect our precise understanding of the percutaneous drug delivery into deeper tissues. Further investigations into the NSAIDs, diclofenac specifically, deep penetration needed in order to resolve the conflicting results concerning the drug distribution mechanisms.

13.10 Conclusion

The current focus in research of transdermal drug transport remains on finding ways to effectively control drug dosing, as well as targeting and retention into the site of interest. Taken together, topical therapy for deep tissues has demonstrated efficacy and safety advantage. Yet much remains to be done to clarify the mechanisms so as to permit further clinical development of treatments of various chronic local conditions in muscles and joints. Confirmation of the clinical relevance of animals will be a major step forward.

13.11 Declaration of Interest

No conflict of Interest

References

1. Prieto-Alhambra D, Judge A, Javaid MK, Cooper C, Diez-Perez A, Arden NK. Incidence and risk factors for clinically diagnosed knee, hip and hand osteoarthritis: influences of age, gender and osteoarthritis affecting other joints. Ann Rheum Dis. 2013;73(9):1659–64. doi:10.1136/annrheumdis-2013–203355.
2. Singh P. Percutaneous penetration as a method of delivery to skin and underlying tissues. In: Bronaugh RL, Maibach HI, editors. Percutaneous absorption: drugs-cosmetics-mechanisms-methodology. 7th ed. New York: Marcel Dekker Inc.; 1999. pp. 597–613.
3. Guy RH. Transdermal drug delivery. In: Schafer-Korting M, editor. Drug delivery. 197th ed. Vol. 197. Berlin: Springer; 2010. pp. 399–410.
4. Stanos SP. Osteoarthritis guidelines: a progressive role for topical nonsteroidal anti-inflammatory drugs. J Multidiscip Healthc. 2013;6:133–7.
5. Lee CM, Maibach HI. Deep percutaneous penetration into muscles and joints. J Pharm Sci. 2006;95(7):1405–13.
6. Jepps OG, Dancik Y, Anissimov YG, Roberts MS. Modeling the human skin barrier—towards a better understanding of dermal absorption. Adv Drug Deliv Rev. 2013;65(2):152–68.
7. Singh P, Roberts MS. Skin permeability and local tissue concentrations of nonsteroidal anti-inflammatory drugs after topical application. J Pharmacol Exp Ther. 1994a;268(1):144–51.
8. Singh P, Roberts MS. Deep tissue penetration of bases and steroids after dermal application in rat. J Pharm Pharmacol. 1994b;46(12):956–64.
9. Baldwin JR, Carrano RA, Imondi AR. Penetration of trolamine salicylate into the skeletal muscle of the pig. J Pharm Sci. 1984;73(7):1002–4.
10. Herndon CM. Topical delivery of nonsteroidal anti-inflammatory drugs for osteoarthritis. J Pain Palliat Care Pharmacother. 2012;26(1):18–23.
11. Roberts MS, Anderson RA, Swarbrick J. Permeability of human epidermis to phenolic compounds. J Pharm Pharmacol. 1977;29(11):677–83.
12. Swarbrick J, Lee G, Brom J, Gensmantel NP. Drug permeation through human skin II: permeability of ionizable compounds. J Pharm Sci. 1984;73(10):1352–5.
13. Magnusson BM, Anissimov YG, Cross SE, Roberts MS. Molecular size as the main determinant of solute maximum flux across the skin. J Invest Dermatol. 2004;122(4):993–9.
14. Higaki K, Asai M, Suyama T, Nakayama K, Ogawara K, Kimura T. Estimation of intradermal disposition kinetics of drugs: II. factors determining penetration of drugs from viable skin to muscular layer. Int J Pharm. 2002;239(1–2):129–41.
15. Yagi S, Nakayama K, Kurosaki Y, Higaki K, Kimura T. Factors determining drug residence in skin during transdermal absorption: studies on beta-blocking agents. Biol Pharm Bull. 1998;21(11):1195–201.
16. Guy RH, Hadgraft J, Maibach HI. A pharmacokinetic model for percutaneous absorption. Int J Pharm. 1982;11(2):119–129.
17. Barry B. Transdermal drug delivery. In: Aulton M, editor. Pharmaceutics: the science of dosage form design. London: Harcourt; 1988. p. 499–533.
18. Higaki K, Nakayama K, Suyama T, Amnuaikit C, Ogawara K, Kimura T. Enhancement of topical delivery of drugs via direct penetration by reducing blood flow rate in skin. Int J Pharm. 2005;288(2):227–33.
19. Singh P, Roberts MS. Dermal and underlying tissue pharmacokinetics of salicylic acid after topical application. J Pharmacokinet Biopharm. 1993a;21(4):337–73.
20. Singh P, Roberts MS. Iontophoretic transdermal delivery of salicylic acid and lidocaine to local subcutaneous structures. J Pharm Sci. 1993b;82(2):127–31.
21. Kretsos K, Kasting GB, Nitsche JM. Distributed diffusion-clearance model for transient drug distribution within the skin. J pharm Sci. 2004;93(11):2820–35.
22. Kretsos K, Kasting GB. A geometrical model of dermal capillary clearance. Math Biosci. 2007;208(2):430–53.

23. Schaefer H, Stüttgen G. Absolute concentrations of an antimycotic agent, econazole, in the human skin after local application. Arzneimittelforschung. 1976;26(3):432–5.
24. Schaefer H, Zesch A. Penetration of vitamin A acid into human skin. Acta Derm Venereol Suppl (Stockh). 1975;74:50–5.
25. Zesch A, Schaefer H. [Penetration of radioactive hydrocortisone in human skin from various ointment bases. II. In vivo-experiments (author's transl)]. Arch Dermatol Forsch. 1975;252(4):245–56.
26. Schaefer H, Zesch A, Stüttgen G. Penetration, permeation, and absorption of triamcinolone acetonide in normal and psoriatic skin. Arch Dermatol Res (Archiv für dermatologische Forschung). 1977;258(3):241–9.
27. Schaefer H, Stüttgen G, Zesch A, Schalla W, Gazith J. Quantitative determination of percutaneous absorption of radiolabeled drugs in vitro and in vivo by human skin. Curr Probl Dermatol. 1978;7:80–94.
28. Anissimov YG, Roberts MS. Modelling dermal drug distribution after topical application in human. Pharm Res. 2011;28(9):2119–29.
29. Dancik Y, Anissimov YG, Jepps OG, Roberts MS. Convective transport of highly plasma protein bound drugs facilitates direct penetration into deep tissues after topical application. Br J Clin Pharmacol. 2012;73(4):564–78.
30. Yanagimoto G, Hayashi T, Hasegawa T, Seki T, Juni K, Sugibayashi K, Morimoto Y. Skin disposition of drugs after topical application in hairless rats. Chem Pharm Bull (Tokyo). 1999;47(6):749–54.
31. Radermacher J, Jentsch D, Scholl MA, Lustinetz T, Frölich JC. Diclofenac concentrations in synovial fluid and plasma after cutaneous application in inflammatory and degenerative joint disease. Br J Clin Pharmacol. 1991;31(5):537–41.
32. Müller M, Rastelli C, Ferri P, Jansen B, Breiteneder H, Eichler HG. Transdermal penetration of diclofenac after multiple epicutaneous administration. J Rheumatol. 1998;25(9):1833–6.
33. Miyatake S, Ichiyama H, Kondo E, Yasuda K. Randomized clinical comparisons of diclofenac concentration in the soft tissues and blood plasma between topical and oral applications. Br J Clin Pharmacol. 2009;67(1):125–9.
34. Kai S, Kondo E, Kawaguchi Y, Kitamura N, Yasuda K. Flurbiprofen concentration in soft tissues is higher after topical application than after oral administration. Br J Clin Pharmacol. 2013;75(3):799–804.
35. Rabinowitz JL, Baker D. Absorption of labeled triethanolamine salicylate in human and canine knee joints. II. J Clin Pharmacol. 1984;24(11–12):532–9.
36. Mills PC, Magnusson BM, Cross SE. Penetration of a topically applied nonsteroidal anti-inflammatory drug into local tissues and synovial fluid of dogs. Am J Vet Res. 2005;66(7):1128–32.
37. Yoshida D, Hasegawa T, Sugibayashi K. Targeting of salicylate to skin and muscle following topical injections in rats. Int J Pharm. 2002;231(2):177–84.
38. Skazik C, Wenzel J, Marquardt Y, Kim A, Merk HF, Bickers DR, Baron JM. P-glycoprotein (ABCB1) expression in human skin is mainly restricted to dermal components. Exp Dermatol. 2011;20(5):450–2.
39. Polat BE, Hart D, Langer R, Blankschtein D. Ultrasound-mediated transdermal drug delivery: mechanisms, scope, and emerging trends. J Control Release. 2011;152(3):330–48.
40. Kigasawa K, Kajimoto K, Watanabe M, Kanamura K, Saito A, Kogure K. In vivo transdermal delivery of diclofenac by ion-exchange iontophoresis with geraniol. Biol Pharm Bull. 2009;32(4):684–7.
41. Sammeta SM, Murthy SN. "ChilDrive": a technique of combining regional cutaneous hypothermia with iontophoresis for the delivery of drugs to synovial fluid. Pharm Res. 2009;26(11):2535–40.
42. Tugwell PS, Wells GA, Shainhouse JZ. Equivalence study of a topical diclofenac solution (pennsaid) compared with oral diclofenac in symptomatic treatment of osteoarthritis of the knee: a randomized controlled trial. J Rheumatol. 2004;31(10):2002–12.

43. Simon LS, Grierson LM, Naseer Z, Bookman AAM, Zev Shainhouse J. Efficacy and safety of topical diclofenac containing dimethyl sulfoxide (DMSO) compared with those of topical placebo, DMSO vehicle and oral diclofenac for knee osteoarthritis. Pain. 2009;143(3):238–45.
44. Roth SH, Fuller P. Diclofenac topical solution compared with oral diclofenac: a pooled safety analysis. J Pain Res. 2011;4:159–67.
45. Bookman AAM, Williams KS, Shainhouse JZ. Effect of a topical diclofenac solution for relieving symptoms of primary osteoarthritis of the knee: a randomized controlled trial. CMAJ. 2004;171(4):333–8.
46. Carnes D, Anwer Y, Underwood M, Harding G, Parsons S. Influences on older people's decision making regarding choice of topical or oral NSAIDs for knee pain: qualitative study. BMJ. 2008;336(7636):142–5.
47. Makris UE, Kohler MJ, Fraenkel L. Adverse effects of topical nonsteroidal antiinflammatory drugs in older adults with osteoarthritis: a systematic literature review. J Rheumatol. 2010;37(6):1236–43.
48. Haisch C. Photoacoustic spectroscopy for analytical measurements. Meas Sci Technol. 2012;23:12001.
49. De Melo JO, Pedrochi F, Baesso ML, Hernandes L, Truiti MC, Barons S, Bersani-Amado CA. Evidence of deep percutaneous penetration associated with anti-inflammatory activity of topically applied Helicteres gardneriana extract: a photoacoustic spectroscopy study. Pharm Res. 2011;28(2):331–6.
50. Xi H, Cun D, Xiang R, Guan Y, Zhang Y, Li Y, Fang L. Intra-articular drug delivery from an optimized topical patch containing teriflunomide and lornoxicam for rheumatoid arthritis treatment: does the topical patch really enhance a local treatment? J Control Release. 2013;169(1–2):73–81.

Chapter 14
Efficacy and Toxicity of Microneedle-Based Devices

Michael S. Leo, Hadar A. Lev-Tov, Faranak Kamangar, Howard I. Maibach and Raja K. Sivamani

14.1 Introduction

The field of transdermal drug delivery has experienced significant innovative advancements [1]. Traditional modes of drug delivery include oral, parenteral, or cutaneous delivery via a hypodermic needle. Oral drug delivery is affected by the acidity of the stomach, poor intestinal absorption, and first-pass hepatic metabolism, which all contribute to lower bioavailability [2]. Hypodermic needles are painful and associated with needle phobia and apprehension [3]. Transdermal drug delivery is an alternative delivery method that introduces drugs by bypassing the skin barrier to allow for either systemic or local drug delivery.

The major barrier in transdermal drug delivery is the stratum corneum, the outermost layer of skin, ranging from 15 to 170 μm in thickness depending on the anatomical site [4, 5], and composed of keratinized dead cells. Transdermal drug delivery systems bypass the stratum corneum, which is usually the rate-limiting barrier. The stratum corneum is lipophilic in nature and is resistant to the passage of hydrophilic substances or the passage of molecules that are larger than a few hundred Daltons [6]. In general, multiple different approaches are taken to bypass the stratum corneum [1]. Microneedles enhance transdermal drug delivery through the creation of physical conduits or channels in the stratum corneum. Because microneedles typically penetrate over 200 μm, anatomical changes in the thickness

R. K. Sivamani (✉) · F. Kamangar
Department of Dermatology, University of California-Davis, 3301 C Street, Suite 1400, Sacramento, CA 95816, USA
e-mail: rksivamani@ucdavis.edu

M. S. Leo
Department of Dermatology, University of California-San Francisco, San Francisco, CA 94143, USA

H. I. Maibach
Department of Dermatology, School of Medicine, University of California, San Francisco, CA, USA

H. A. Lev-Tov
Division of Dermatology, Albert Einstein College of Medicine, Bronx, NY 10467, USA

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_14

Table 14.1 List of microneedle patents by company and year

Company	Year
Regents of the University of California	2012
Industry-academic corporation foundation Yonsei University	2013
Therajet Inc.	2013
Clinical resolution laboratory Inc.	2008
Alza corporation	2005
Alza corporation	2006
Corium International Ltd.	2008
3M Innovative Properties company	2005
3M Innovative Properties company	2008
3M Innovative Properties company	2008
3M Innovative Properties company	2008
3M Innovative Properties company	2008
NanoBioSciences LLC	2007 and 2010
BD & Company	2009

Details about the microneedle designs can be found in [63]

Table 14.2 Current microneedle based devices on the market

Name	Manufacturer	Description
Skin microchannel system®	3M	Solid medical grade polymer material. Solid microneedle array with 351 microneedles/cm
Dermaroller	Dermaroller GMBH	Solid microneedle roller
MTS-roller	Clinical Resolution Laboratory, Inc.	Solid microneedle roller

of stratum corneum are unlikely to alter the efficacy of microneedle penetration past the stratum corneum. This field is growing as evidenced by the number of microneedle-based patents (Table 14.1). Several marketed microneedle devices are outlined in Table 14.2.

14.2 Microneedle Fabrication

Microneedles are miniature needles created using the lithographic techniques and are designed to penetrate the stratum corneum to enter the epidermis without piercing the underlying dermis. By doing so, they avoid nerve endings in the dermis to render painless insertions [7–11]. However, longer microneedles are available when dermal remodeling is desired such that the needles enter the dermis and thus are painful with application. The main feature of microneedles that contributes to pain is their length [12]. Shorter microneedles do not reach the dermis and do not come into contact with nerves or blood vessels; they either elicit little to no pain, thereby making short microneedles highly desirable for drug delivery and they are the focus of this chapter.

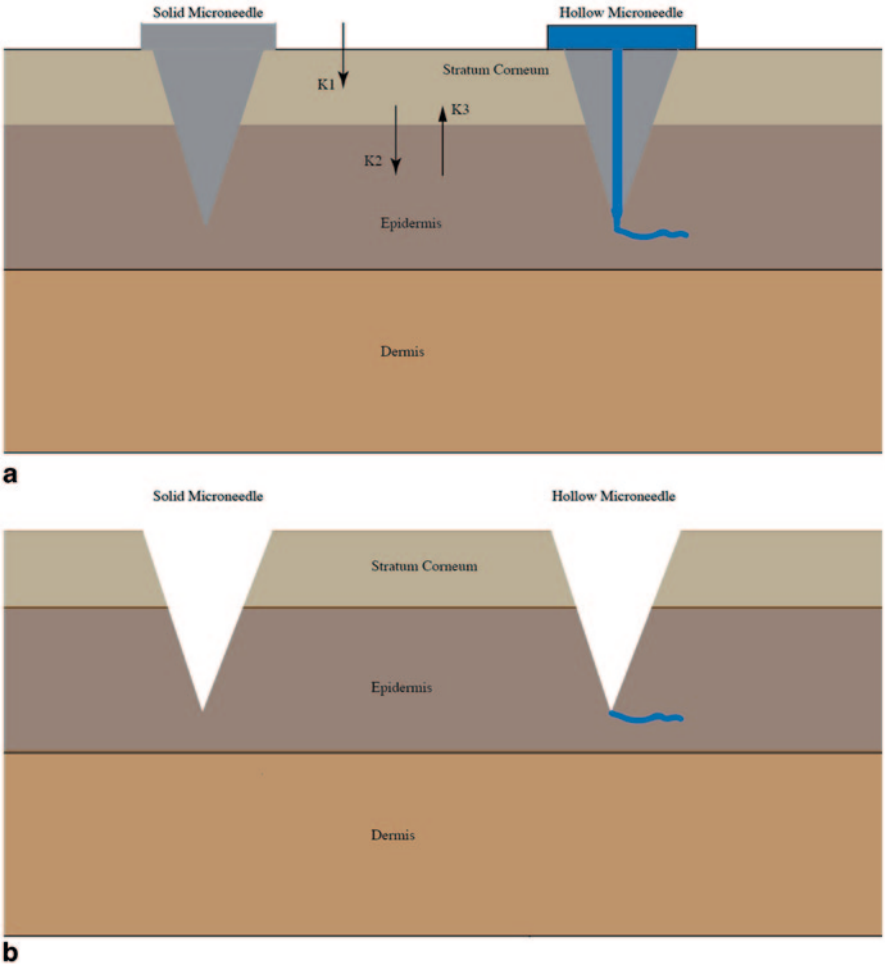


Fig. 14.1 Schematic of microneedle penetration of solid and hollow microneedles. **a** During insertion the microneedles penetrate to the epidermis. The partition coefficients k1 and k2 promote penetration into the epidermis and into the dermis, respectively. The penetration coefficient k3 promotes retention within the stratum corneum. **b** The microneedles leave behind conduits into the epidermis after they are removed. The conduits allow topically applied substances to have direct access to the epidermis. The hollow microneedles allow for intraepidermal injection as depicted

Microneedles have been developed in several different designs, including out-of-plane and in-plane microneedles [13]. Out-of-plane needles are designed such that the microneedle is perpendicular to the surface (Fig. 14.1), whereas in-plane microneedles are parallel to the surface but are more difficult to place into arrays [14]. As most of the research and applications have focused on the out-of-plane microneedle, this chapter will review their use in transdermal drug delivery. Out-of-plane microneedles are subdivided into solid and hollow microneedles. Solid

microneedles are used to create temporary physical holes in the stratum corneum or are coated such that the drug is delivered from the surface of the microneedle upon insertion (Fig. 14.1). Hollow microneedles consist of a conduit (Fig. 14.1) allowing for either bolus or continuous infusion after the microneedle is inserted (Fig. 14.1). A variety of materials have been used to make microneedles including metal [15], titanium [16, 17], glass [18–20], polymers [19, 21–25], and sugars [8, 21, 26].

There are many factors that can impact the efficacy of microneedles including its design: e.g., radius of the microneedles, microneedle shape, the quantity of microneedles used, and microneedle thickness [27].

14.3 Microneedle Strategies for Drug Delivery

The strategy for drug delivery depends on the design of the microneedle. Solid microneedles cannot infuse drugs through the needle. Instead, they are used in three different strategies. The first strategy is to create micropores in the stratum through insertion and removal of the microneedle array. These pores remain open for as little as a few hours before closure when left uncovered to a range of 48 h when covered with a drug-delivered patch [11, 28]. Drug solutions can then be applied topically to traverse these transiently open channels to bypass the stratum corneum, and some studies have utilized iontophoresis to accelerate movement through these channels [29–31]. Because solid microneedle pretreatment of the skin create transient pores with direct access past the stratum corneum, this can enhance the penetration of a topical formulation without necessitating changes to the formulation.

Another strategy is to coat microneedles with a drug prior to insertion. The third strategy is to create dissolving microneedles that dissolve upon insertion to deliver a drug payload. The latter two strategies are limited to drugs that are stable enough to be coated onto or encapsulated during the microneedle fabrication process [32]. Hollow microneedles can be used to infuse drugs as a bolus [33, 34] or as a continuous infusion [35] after insertion.

Utilizing these various strategies, microneedles have been employed to deliver vaccines [36–38], insulin [15, 19, 35, 39, 40], erythropoietin [21], desmopressin [16], and methotrexate [41]. Testing in humans with *in vivo* studies have shown that microneedles can inject nicotinic acid derivatives through hollow silicon out-of-plane microneedles [33, 34] and insulin through a hollow glass micropipette tip [42].

14.4 Transdermal Drug Delivery Applications

Microneedles are versatile and have numerous dermatologic applications. Short (70–80 μm) solid microneedle treatment has been shown to significantly increase the permeation of the small hydrophilic compound galathamine *in vivo* in mice

[43]. Hollow 500 μm microneedles delivering a 2% dose of lidocaine, have been shown to provide anesthesia as quickly and effectively as a hypodermic needle [44]. Solid microneedle generated micropores have been demonstrated to accelerate the delivery of topical dyclonine [45] and topical aminolevulinic acid [46]. A study in mice evaluated the use of a polymer-based microneedle roller to enhance the topical delivery of l-ascorbic acid for hair growth. This study employed microneedle rollers to create microchannels before topically applying l-ascorbic acid *in vivo* in mice. Pretreatment with the microneedle rollers significantly reduced skin pigmentation in melasma patients compared to depigmentation serums alone [47].

The applications of microneedles expand to enhancing photodynamic therapy (PDT) for the treatment of actinic keratoses. PDT requires the local application of a photosensitive drug combined with incident light to selectively cause cell death, presumably through locally generated singlet oxygen [48]. In one study, silicon microneedle patches were used to improve the efficiency of administering topical photosensitive drugs by creating transient microchannels in nude mice before delivering one of two preformed photosensitizer, either 5-ALA [49] or meso-tetra (N-methyl-4-pyridyl) porphine tetra tosylate [50]. A transdermal patch was used to deliver the photosensitizing drug over the skin perforated by a microneedle. The results revealed that the photosensitizing drug was delivered in greater quantities and in a shorter time in comparison to sites that were not perforated with the microneedle patch. The greater depth of penetration is likely the result of faster penetration past the stratum corneum, but this was not specifically studied. As such, one potential advantage of microneedle based therapy would be to reduce incubation times for PDT.

Indeed, clinical studies have demonstrated that channels produced by solid microneedles enhanced the delivery of both aminolevulinic acid (ALA) and methyl-aminolevulinic acid (mALA) [51]. Solid microneedle pretreatment decreased the requisite incubation time of 5-ALA from 60 to 20 min without affecting the efficacy of PDT treatment on actinic keratoses as measured by complete response rate [46]. Furthermore, combining mALA with microneedle pretreatment resulted in the effective treatment of actinic keratosis while preventing most reoccurrences [52, 53]. One clinical study evaluated the role of short microneedles with PDT for photorejuvenation and noted clinical improvement of photodamage [54]. Of note, this latter trial was limited by a lack of a control group.

14.4.1 Hollow Microneedles

Only a few animal [35, 55] and human [33, 34, 42] studies of hollow microneedles have been performed. *In vivo* human experiments revealed that hollow microneedles significantly hastened penetration of methyl nicotinate compared to topical administration [7]. Hollow microneedles were shown to deliver insulin either through a transdermal patch that can actively release insulin *in vivo* in mice [35] or deliver a bolus of insulin in humans without the pain associated with hypodermic needles [42].

14.5 Safety of Microneedles

One of the initial concerns with microneedles was the biocompatibility of silicon or glass, since there are reports of silicon and glass related granulomas [56, 57]. Short microneedles are developed to only penetrate into the epidermis, and not into the dermis. Therefore, any remaining foreign body produced by fragments of the microneedle will likely be contained in the epidermis and discarded within the regular turnover time of 2–4 weeks. Silicon is expensive when considering mass production for commercialization, and manufacturing has moved away from silicon and toward polymer and sugar based synthesis. The polymers and sugars are biocompatible and many are designed to biodegrade. As microneedle technology continues to move toward biocompatibility, microneedle material related adverse events will likely become less of a concern.

The stratum corneum is also a formidable barrier against infection. Microneedles physically breach this barrier, raising the possibility for cutaneous infections. Channels created by solid microneedles are open transiently for a few hours or less [11], and microneedles create a lower bacterial burden than injection with traditional hypodermic needles [58]. Furthermore, facial transepidermal water loss (TEWL) after treatment with short microneedles of 150 and 250 μm recovered to near baseline levels after 8 h [59]. Considering that TEWL is a measurement of epidermal skin barrier function, this further suggests that the skin recovers quickly after a treatment to recover the protective skin barrier. Transient insertions of short solid microneedles of 70–80 μm did not affect the skin's capability to resist *Staphylococcus aureus* infection despite incubation with the bacteria *in vivo* in mice [43]. However, the use of longer microneedles that reach the dermis increased the infection rates compared to the untreated control [43]. In comparison, the use of hollow microneedles for extended infusions may elevate the risk for infection. There are no studies of the infectious risk of long-term infusions with hollow microneedles and the risk of cutaneous infections will need to be further studied.

14.6 Pharmacokinetics and Pharmacodynamics

Comparative studies between oral and transdermal drug delivery have shown some similarities and differences. Studies in estrogen delivery have shown that transdermal delivery may mimic physiological levels more closely [60]. However, another study in subjects with Turner syndrome showed no difference when evaluating the metabolic effects of oral and transdermal estrogen [61]. However, few articles have compared microneedle based transdermal drug delivery to oral or intravenous based drug delivery.

Microneedle based delivery of desmopressin was found to have similar elimination kinetics compared to intravenous delivery in guinea pigs [16]. Short microneedle based drug delivery will require drugs to traverse the interstitial fluid

of the epidermis and dermis prior to enter the blood stream but it is unknown how different or similar this may be in comparison to oral, intravenous, intramuscular, or subcutaneous delivery. Further studies in animals and in humans will be needed to better assess if microneedle-assisted delivery results in similar or different pharmacokinetic and pharmacodynamic profiles. These studies will need to take into account the 15 factors of transcutaneous penetration [62].

14.7 Conclusion

Microneedle use in transdermal drug delivery is an innovative and practical technology with a bright future. Microneedles now make it possible to deliver agents through the stratum corneum that were previously impermeable. Microneedles enhance patient comfort by decreasing and often eliminating pain. The pharmacokinetics and pharmacodynamics will need to be further evaluated, especially when comparing one microneedle design against another. Collaboration between physicians and engineers will continue to drive the evolution and growth of microneedles and their use for cutaneous drug delivery.

References

1. Prausnitz MR, Langer R. Transdermal drug delivery. Nat Biotech. 2008;26:1261–68.
2. Fasinu P, Pillay V, Ndesendo VMK, du Toit LC, Choonara YE. Diverse approaches for the enhancement of oral drug bioavailability. Biopharm Drug Dispos. 2011;32:185–209.
3. Nir Y, Paz A, Sabo E, Potasman I. Fear of injections in young adults: prevalence and associations. Am J Trop Med Hyg. 2003;68:341–4.
4. Koehler MJ, Vogel T, Elsner P, König K, Bückle R, et al. In vivo measurement of the human epidermal thickness in different localizations by multiphoton laser tomography. Skin Res Technol. 2010;16:259–64.
5. Egawa M, Hirao T, Takahashi M. In vivo estimation of stratum corneum thickness from water concentration profiles obtained with Raman spectroscopy. Acta Derm Venereol. 2007;87:4–8.
6. Bos JD, Meinardi MM. The 500 Dalton rule for the skin penetration of chemical compounds and drugs. Exp Dermatol. 2000;9:165–9.
7. Sivamani RK, Stoeber B, Wu GC, Zhai H, Liepmann D, et al. Clinical microneedle injection of methyl nicotinate: stratum corneum penetration. Skin Res Technol. 2005;11:152–6.
8. Miyano T, Tobinaga Y, Kanno T, Matsuzaki Y, Takeda H, et al. Sugar micro needles as transdermic drug delivery system. Biomed Microdevices. 2005;7:185–8.
9. Kaushik S, Hord AH, Denson DD, McAllister DV, Smitra S, et al. Lack of pain associated with microfabricated microneedles. Anesth Analg. 2001;92:502–4.
10. Bal SM, Caussin J, Pavel S, Bouwstra JA. In vivo assessment of safety of microneedle arrays in human skin. Eur J Phar Sci. 2008;35:193–202.
11. Haq MI, Smith E, John DN, Kalavala M, Edwards C, et al. Clinical administration of microneedles: skin puncture, pain and sensation. Biomed Microdevices 2009;11:35–47.
12. Gill HS, Denson DD, Burris BA, Prausnitz MR. Effect of microneedle design on pain in human volunteers. Clin J Pain. 2008;24:585–94.

13. Sivamani RK, Liepmann D, Maibach HI. Microneedles and transdermal applications. Expert Opin Drug Deliv. 2007;4:19–25.
14. Reed ML, Lye WK. Microsystems for drug and gene delivery. Proc IEEE. 2004;92:56–75.
15. Davis SP, Martanto W, Allen MG, Prausnitz MR. Hollow metal microneedles for insulin delivery to diabetic rats. IEEE Trans Biomed Eng. 2005;52:909–15.
16. Cormier M, Johnson B, Ameri M, Nyam K, Libiran L, et al. Transdermal delivery of desmopressin using a coated microneedle array patch system. J Control Release. 2004;97:503–11.
17. Tsuchiya K, Nakanishi N, Uetsuji Y, Nakamachi E. Development of blood extraction system for health monitoring system. Biomed Microdevices. 2005;7:347–53.
18. Lee S, Jeong W, Beebe DJ. Microfluidic valve with cored glass microneedle for microinjection. Lab Chip. 2003;3:164–7.
19. McAllister DV, Wang PM, Davis SP, Park JH, Canatella PJ, et al. Microfabricated needles for transdermal delivery of macromolecules and nanoparticles: fabrication methods and transport studies. Proc Natl Acad Sci U S A. 2003;100:13755–60.
20. Wang PM, Cornwell M, Prausnitz MR. Minimally invasive extraction of dermal interstitial fluid for glucose monitoring using microneedles. Diabetes Technol Ther. 2005;7:131–41.
21. Ito Y, Yoshimitsu J, Shiroyama K, Sugioka N, Takada K. Self-dissolving microneedles for the percutaneous absorption of EPO in mice. J Drug Target. 2006;14:255–61.
22. Matsuda T, Mizutani M. Liquid acrylate-endcapped biodegradable poly(epsilon-caprolactone-co-trimethylene carbonate). II. Computer-aided stereolithographic microarchitectural surface photoconstructs. J Biomed Mater Res. 2002;62:395–403.
23. Park JH, Allen MG, Prausnitz MR. Biodegradable polymer microneedles: fabrication, mechanics and transdermal drug delivery. J Controlled Release. 2005;104:51–66.
24. Park JH, Allen MG, Prausnitz MR. Polymer microneedles for controlled-release drug delivery. Pharm Res. 2006;23:1008–19.
25. Han M, Hyun D-H, Park H-H, Lee SS, Kim C-H, et al. A novel fabrication process for out-of-plane microneedle sheets of biocompatible polymer. J Micromec Microeng. 2007;17:1184–91.
26. Kolli CS, Banga AK. Characterization of solid maltose microneedles and their use for transdermal delivery. Pharm Res. 2008;25:104–13.
27. Davidson A, Al-Qallaf B, Das DB. Transdermal drug delivery by coated microneedles: geometry effects on effective skin thickness and drug permeability. Chem Eng Res Des. 2008;86:1196–206.
28. Enfield J, O'Connell ML, Lawlor K, Jonathan E, O'Mahony C, et al. In-vivo dynamic characterization of microneedle skin penetration using optical coherence tomography. J Biomed Opt. 2010;15:046001.
29. Chen H, Zhu H, Zheng J, Mou D, Wan J, et al. Iontophoresis-driven penetration of nanovesicles through microneedle-induced skin microchannels for enhancing transdermal delivery of insulin. J Control Release. 2009;139:63–72.
30. Katikaneni S, Badkar A, Nema S, Banga AK. Molecular charge mediated transport of a 13 kD protein across microporated skin. Int J Pharm. 2009;378:93–100.
31. Wu XM, Todo H, Sugibayashi K. Enhancement of skin permeation of high molecular compounds by a combination of microneedle pretreatment and iontophoresis. J Control Release. 2007;118:189–95.
32. Gill HS, Prausnitz MR. Coating formulations for microneedles. Pharm Res. 2007;24:1369–80.
33. Sivamani RK, Stoeber B, Liepmann D, Maibach HI. Microneedle penetration and injection past the stratum corneum in humans. J Dermatol Treat. 2009;20:156–9.
34. Sivamani RK, Stoeber B, Wu GC, Zhai HB, Liepmann D, et al. Clinical microneedle injection of methyl nicotinate: stratum corneum penetration. Skin Res Technol. 2005;11:152–6.
35. Roxhed N, Samel B, Nordquist L, Griss P, Stemme G. Painless drug delivery through microneedle-based transdermal patches featuring active infusion. IEEE Trans Biomed Eng. 2008;55:1063–71.

36. Kim YC, Quan FS, Yoo DG, Compans RW, Kang SM, et al. Improved influenza vaccination in the skin using vaccine coated microneedles. Vaccine. 2009;27(49):6932–8.
37. Zhu Q, Zarnitsyn VG, Ye L, Wen Z, Gao Y, et al. Immunization by vaccine-coated microneedle arrays protects against lethal influenza virus challenge. Proc Natl Acad Sci U S A. 2009;106:7968–73.
38. Ding Z, Verbaan FJ, Bivas-Benita M, Bungener L, Huckriede A, et al. Microneedle arrays for the transcutaneous immunization of diphtheria and influenza in BALB/c mice. J Control Release. 2009;136:71–8.
39. Gardeniers H, Luttge R, Berenschot EJW, de Boer MJ, Yeshurun SY, et al. Silicon micromachined hollow microneedles for transdermal liquid transport. J Microelectromech Syst. 2003;12:855–62.
40. Ito Y, Hagiwara E, Saeki A, Sugioka N, Takada K. Feasibility of microneedles for percutaneous absorption of insulin. Eur J Pharm Sci. 2006;29:82–8.
41. Vemulapalli V, Yang Y, Friden PM, Banga AK. Synergistic effect of iontophoresis and soluble microneedles for transdermal delivery of methotrexate. J Pharm Pharmacol. 2008;60:27–33.
42. Gupta J, Felner EI, Prausnitz MR. Minimally invasive insulin delivery in subjects with type 1 diabetes using hollow microneedles. Diabetes Technol Ther. 2009;11:329–37.
43. Li WZ, Huo MR, Zhou JP, Zhou YQ, Hao BH, et al. Super-short solid silicon microneedles for transdermal drug delivery applications. Int J Pharm. 2010;389:122–9.
44. Gupta J, Denson DD, Felner EI, Prausnitz MR. Rapid local anesthesia in humans using minimally invasive microneedles. Clin J Pain. 2012;28:129–35.
45. Li X, Zhao R, Qin Z, Zhang J, Zhai S, et al. Microneedle pretreatment improves efficacy of cutaneous topical anesthesia. Am J Emerg Med. 2010;28:130–4.
46. Lev-Tov H, Larsen L, Zackria R, Chahal H, Eisen D, et al. Microneedle pretreatment may reduce incubation time in photodynamic therapy; 2013; International Investigative Dermatology Meeting Edinburgh, UK.
47. Fabbrocini G, De Vita V, Fardella N, Pastore F, Annunziata MC, et al. Skin needling to enhance depigmenting serum penetration in the treatment of melasma. Plast Surg Int. 2011;2011:158241.
48. Kennedy JC, Pottier RH, Pross DC. Photodynamic therapy with endogenous protoporphyrin IX: basic principles and present clinical experience. J Photochem Photobiol B 1990;6:143–8.
49. Donnelly RF, Morrow DI, McCarron PA, Woolfson AD, Morrissey A, et al. Microneedle-mediated intradermal delivery of 5-aminolevulinic acid: potential for enhanced topical photodynamic therapy. J Control Release. 2008;129:154–62.
50. Donnelly RF, Morrow DI, McCarron PA, David Woolfson A, Morrissey A, et al. Microneedle arrays permit enhanced intradermal delivery of a preformed photosensitizer. Photochem Photobiol. 2009;85:195–204.
51. Mikolajewska P, Donnelly RF, Garland MJ, Morrow DI, Singh TR, et al. Microneedle pretreatment of human skin improves 5-aminolevulininc acid (ALA)- and 5-aminolevulinic acid methyl ester (MAL)-induced PpIX production for topical photodynamic therapy without increase in pain or erythema. Pharm Res. 2010;27:2213–20.
52. Bencini PL, Galimberti MG, Pellacani G, Longo C. Application of photodynamic therapy combined with pre-illumination microneedling in the treatment of actinic keratosis in organ transplant recipients. Br J Dermatol. 2012;167:1193–94.
53. Clementoni MT, M BR, Munavalli GS. Photodynamic photorejuvenation of the face with a combination of microneedling, red light, and broadband pulsed light. Lasers Surg Med. 2010;42:150–9.
54. Clementoni MT, B-Roscher M, Munavalli GS. Photodynamic photorejuvenation of the face with a combination of microneedling, red light, and broadband pulsed light. Lasers Surg Med. 2010;42:150–9.
55. Hafeli UO, Mokhtari A, Liepmann D, Stoeber B. In vivo evaluation of a microneedle-based miniature syringe for intradermal drug delivery. Biomed Microdevices. 2009;11(5):943–50.
56. Millard DR Jr., Maisels DO. Silicon granuloma of the skin and subcutaneous tissues. Am J Surg. 1966;112:119–23.

57. Finley J, Knabb J. Cutaneous silica granuloma. Plast Reconstr Surg. 1982;69:340–3.
58. Donnelly R, Singh T, Tunney M, Morrow D, McCarron P, et al. Microneedle arrays allow lower microbial penetration than hypodermic needles in vitro. Pharm Res. 2009;26(11):2513–22.
59. Han TY, Park KY, Ahn JY, Kim SW, Jung HJ, et al. Facial skin barrier function recovery after microneedle transdermal delivery treatment. Dermatol Surg. 2012;38:1816–22.
60. Taboada M, Santen R, Lima J, Hossain J, Singh R, et al. Pharmacokinetics and pharmacodynamics of oral and transdermal 17beta estradiol in girls with Turner syndrome. J Clin Endocrinol Metab. 2011;96:3502–10.
61. Mauras N, Shulman D, Hsiang HY, Balagopal P, Welch S. Metabolic effects of oral versus transdermal estrogen in growth hormone-treated girls with turner syndrome. J Clin Endocrinol Metab. 2007;92:4154–60.
62. Ngo MA, Maibach HI. 15 Factors of percutaneous penetration of pesticides. Parameters for pesticide QSAR and PBPK/PD models for human risk assessment. American chemical society: Washington, DC; 2012. p. 67–86.
63. Singh TR, Dunne NJ, Cunningham E, Donnelly RF. Review of patents on microneedle applicators. Recent Pat Drug Deliv Formul. 2011;5:11–23.

Part V
Transdermal Drugs and Modelling

Chapter 15
Mathematical Models for Topical and Transdermal Drug Products

Yuri G. Anissimov and Michael S. Roberts

15.1 Introduction

An understanding of the time course of solutes penetrating through the skin is crucial in defining both the acute and chronic exposure an individual may have to a desirable topically applied medicine or undesirable absorption of a potentially toxic exogenous solute. The description of this time course is best achieved by expressing the process as a mathematical model. Those that have been used to describe drug penetration through and distribution in skin are based on either diffusion or compartmental equations. The object of any mathematical model is to (a) be able to represent the processes associated with absorption and distribution accurately, (b) be able to describe/summarise experimental data with parametric equations or moments and (c) predict processes under varying conditions. However, in describing the processes involved, some developed models often suffer from being too complex to be practically useful. In this chapter, we have attempted to update our previous work [1] in which we approached the issue of mathematical modelling in

Y. G. Anissimov (✉)
Queensland Micro- and Nanotechnology Centre, Griffith University,
Brisbane, QLD 4111, Australia
e-mail: Y.Anissimov@Griffith.edu.au

School of Biomolecular and Physical Sciences, Griffith University,
Gold Coast, QLD 4222, Australia.

M. S. Roberts
Therapeutics Research Unit, School of Medicine, The University of Queensland,
Princess Alexandra Hospital, Woolloongabba, QLD 4102, Australia

School of Pharmacy & Medical Sciences, University of South Australia,
Adelaide, SA 5001, Australia

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_15

percutaneous absorption and distribution from four perspectives. These are to (a) describe simple practical models, (b) provide an overview of the more complex models, (c) summarise some of the more important/useful models used to date and (d) examine some practical applications of the models. This chapter revises an earlier one [1] incorporating some of the more recent findings and expanding discussion of drug distribution in skin.

The range of processes involved in percutaneous absorption and considered in developing the mathematical models in this chapter are shown in Fig. 15.1. We initially address *in vitro* skin diffusion models and consider (a) constant donor concentration and receptor conditions, (b) the corresponding flux, donor, skin and receptor amount-time profiles for solutions and (c) amount and flux-time profiles when the donor phase is removed. More complex issues such as finite volume donor phase, finite volume receptor phase, the presence of an efflux rate constant at the membrane-receptor interface, two layer diffusion and drug transport and distribution in deeper skin layers are then considered. We then look at specific models and issues concerned with (a) release from topical products, (b) use of compartmental models as alternatives to diffusion models, (c) concentration-dependent absorption, (d) modelling of skin metabolism (e) role of solute-skin-vehicle interactions (f) effects of vehicle loss, (g) shunt transport and (h) *in vivo* diffusion, compartmental, physiological and deconvolution models. We conclude by examining topics such as (a) deep tissue penetration, (b) pharmacodynamics, (c) iontophoresis, (d) sonophoresis and (e) pitfalls in modelling.

Each model is described in diagrammatic and equation form. Given that the analytical solution to most models is in the form of infinite series, often involving solutions to transcendental equations, we have emphasised the Laplace domain and steady state solutions. Many nonlinear regression programs such as MULTI FILT, MINIM and SCIENTIST enable analysis of concentration-time data using numerical inversion of Laplace domain solutions and avoid some of the computational difficulties associated with series solutions especially those involving solving transcendental equations. The steady state solutions describing the linear portion of a cumulative amount versus time profile for a constant donor concentration are of great practical use, being described by a linear equation with lag time and steady state flux as the intercept and the slope, respectively. In order to make equations in this chapter as useable as possible, each equation has been presented in a form which preserves dimensions of all variables. Simulations and nonlinear regressions presented in this review were undertaken using either SCIENTIST 2.01 or Python 2.7.

15.2 *In Vitro* Skin Diffusion Models in Percutaneous Absorption

We consider first mathematical models associated with solute penetration through excised skin. The simplest of these models is when a well-stirred vehicle of infinite volume is applied to the stratum corneum (SC) and the solute passes into a receptor

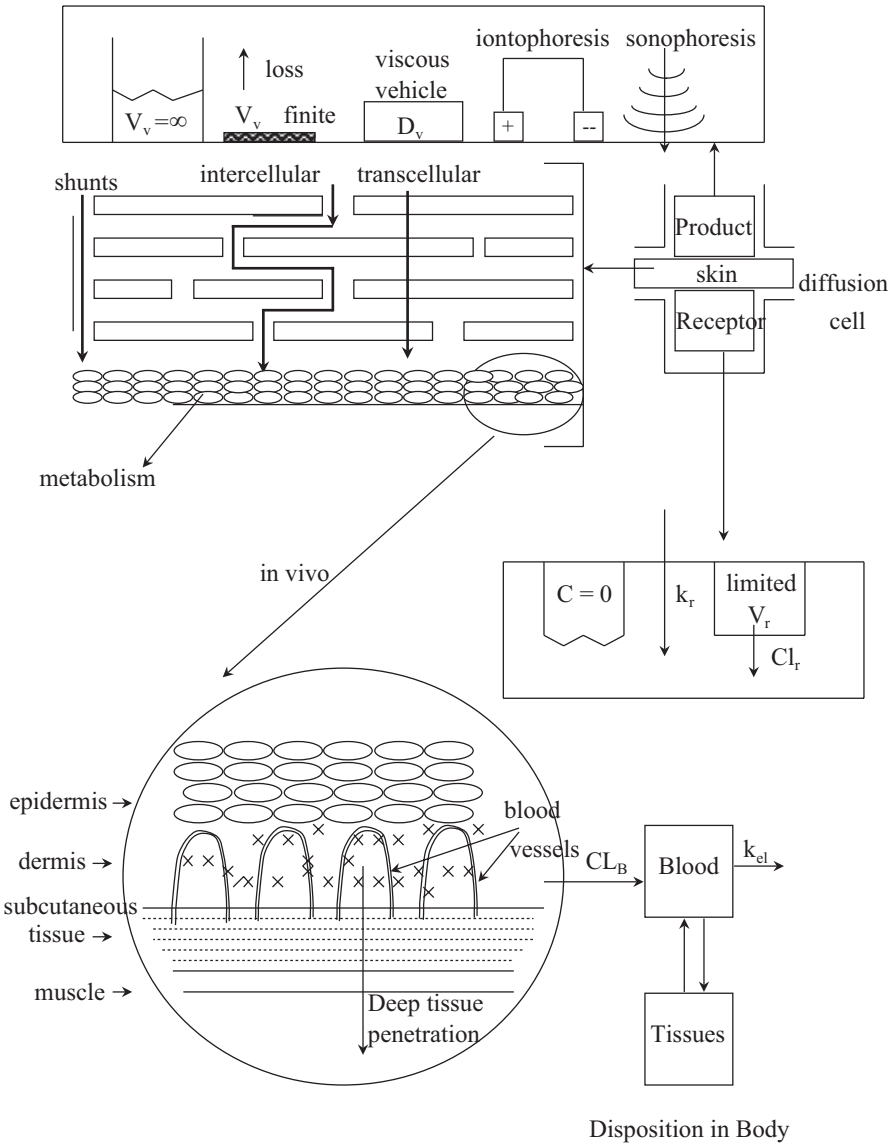


Fig. 15.1 Diagrammatic overview of percutaneous processes associated with mathematical models

sink (Fig. 15.2a). Increasing complexity of the models arises when the vehicle volume is finite (Fig. 15.2b), when the receptor is no longer a sink (Fig. 15.2c) and when the vehicle cannot be considered well-stirred (Fig. 15.2d). We examine each of these models in terms of expressions for amount penetrating, flux and, where possible, summary parameters such as mean absorption time, normalised variance, peak time for flux and peak flux.

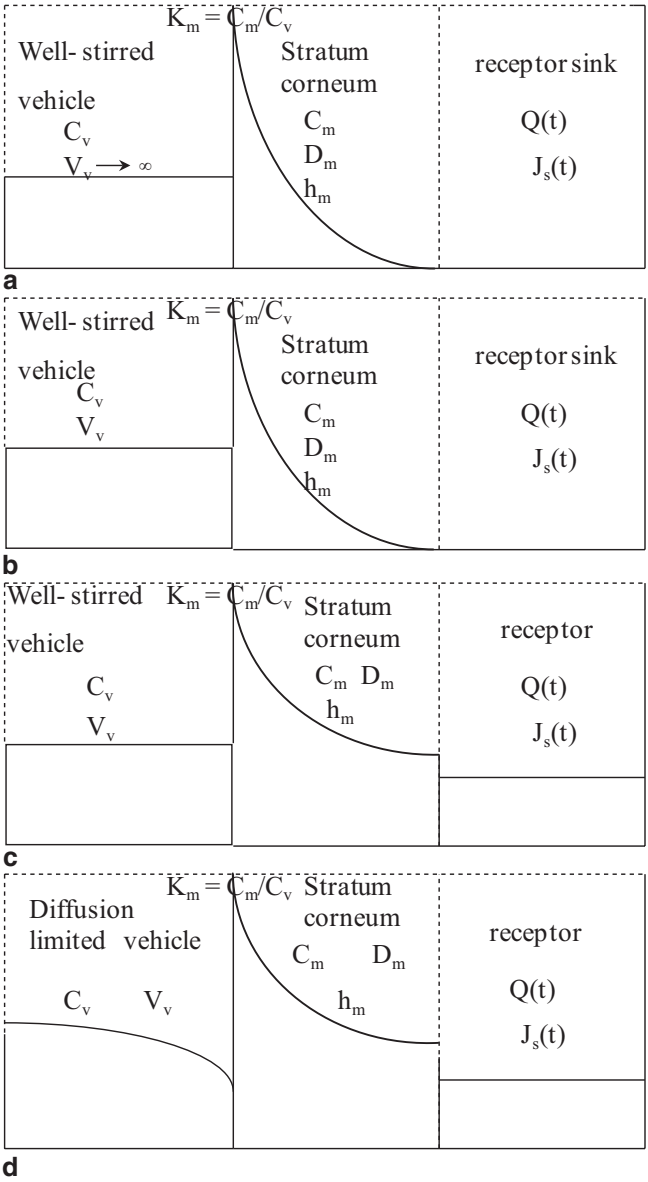


Fig. 15.2 *In vitro* skin models of transport. **a** Well-stirred vehicle containing solute concentration C_v in volume V_v (where $V_v = \infty$) adjacent to assumed homogenous stratum corneum with solute concentration C_m at distance x from applied vehicle. Solute moves with a diffusion coefficient D_m over an effective path length h_m and penetrates into a receptor sink to give an amount penetrated $Q(t)$ in time t or flux $J(t)$. **b** As for A but with V_v finite. **c** As for B but the receptor is not a sink and **d** as for B but the vehicle is not well-stirred.

15.2.1 In Vitro *Skin Permeability Studies with a Constant Donor Concentration and Sink Receptor Conditions*

Most *in vitro* skin permeability studies are carried out assuming that both (a) the concentration of solute in a vehicle applied to the skin and (b) the sink conditions provided by the receptor remain constant over the period of the study. Significant depletion of solute in the donor vehicle or an inadequate receptor sink requires more complex modelling as discussed later. When transport through the SC is rate limiting, the most widely used steady-state approximation of amount of solute absorbed ($Q(t)$) at time t when a concentration of solute in vehicle (C_v) is applied to an area of application (A) for an exposure time (T) is given by Eq. (15.1) [2]:

$$Q(t) \approx k_p A C_v (t - \text{lag}), \quad \text{for: } 2\text{lag} \le t \le T + \text{lag} \tag{15.1}$$

where k_p is the permeability coefficient (units: distance/time) of the SC. The lag time (*lag*) reflects the delay taken for a solute to cross a membrane. The effect of this lag also is, as shown in Eq. (15.1), for absorption to continue at about the same rate after removal of the vehicle for an additional lag time, so that the overall absorption at $t = T + \text{lag}$ equals $k_p A C_v T$. This equation can be shown to have been derived from Fick's first law of diffusion, which relates the steady state flux of a solute through a membrane (J_{ss}), which is achieved roughly after about two lag times, to the concentration gradient across that membrane ΔC_m, the thickness of that membrane h_m and the diffusivity of the solute in the membrane, D_m, i.e.

$$J_{ss} = \frac{D_m \Delta C_m}{h_m}. \tag{15.2}$$

With sink conditions, ΔC_m approximates to the membrane concentration at the surface next to the vehicle C_m and can, in turn, be related to C_v by the dimensionless partition coefficient between the SC and vehicle (K_m), defined as the ratio of solute concentrations in the SC (C_m) and vehicle (C_v) under equilibrium, i.e. $K_m = C_m / C_v$. Hence, the flux J_{ss} can also be written as $J_{ss} = K_m D_m C_{v/h}$ equilibrium. Hence, the flux J_{ss} can also be 2 or

$$J_{ss} = \frac{Q}{A(t - \text{lag})} = k_p C_v \tag{15.3}$$

where the permeability coefficient term is normally defined as:

$$k_p = \frac{K_m D_m}{h_m}. \tag{15.4}$$

In practice, the permeability coefficient k_p is a composite parameter. When solute transport occurs via both a lipid pathway of permeability coefficient $k_{p.\text{lipid}}$ and a polar pathway of permeability coefficient $k_{p.\text{polar}}$ and an aqueous boundary layer of the epidermis provides a rate limiting permeability coefficient $k_{p.\text{aqueous}}$, k_p is more properly expressed as

$$k_p = \left(\frac{1}{k_{p.\text{lipid}} + k_{p.\text{polar}}} + \frac{1}{k_{p.\text{aqueous}}} \right)^{-1}. \tag{15.5}$$

As discussed by Roberts and Walters [2], for most solutes, $k_p \approx k_{p.\text{lipid}}$. However, as Scheuplein [3] has recently pointed out Eq. (15.5) really describes the main resistance barriers to transport rather than pathways for transport. For instance, he asserts that a key pathway for penetration for many solutes is via diffusion across the corneocytes as well as across the main lipid barrier, defined by $k_{p.\text{lipid}}$.

Although the amount of solute absorbed is most often described by Eq. (15.1) in the skin literature, it is based on the permeability coefficient (k_p), which for the same solute is different for different vehicles. An alternative approach, pioneered by Higuchi, recognises that all saturated solutions are the same for various vehicles providing the vehicle does not affect the skin [4, 5]. Hence, the maximum flux is defined by Eq. (15.2), in which C_m is now the solubility of the solute in the stratum corneum (S_m) so that $J_{\max} = D_m S_m / h_m$. Provided Raoult's law holds, the flux through the skin can be related to the fractional solubility of the solute in the skin:

$$J_{\max} \frac{C_m}{S_m} = J_{\max} \frac{C_v}{S_v} \tag{15.6}$$

which in turn equals the fractional solubility in the vehicle, so that Eq. (15.1) can also be written in the form:

$$Q(t) = J_{\max} A \frac{C_v}{S_v} (t - \text{lag}), \tag{15.7}$$

where $J_{\max}$ is the maximum flux of a solute through SC, realised when maximum possible concentration (= solubility in the vehicle, S_v) for a given vehicle is used. However, a number of deviations from Raoult's law can occur [6] and high concentrations of certain solutes may also dehydrate the skin [7–9]. In general, fluxes derived using high solute concentrations should be based on mole fraction rather than molarity concentrations.

Equations (15.1), (15.3), and (15.7) are the simplified forms of a more complex expression based on the solution of the diffusion equation for transport of solute in the skin:

$$\frac{\partial C_m}{\partial t} D_m \frac{\partial^2 C_m}{\partial x^2}, \tag{15.8}$$

the initial condition:

$$C_m(x,0) = 0, \tag{15.9}$$

and boundary conditions:

$$C_m(0,t) = K_m C_v, \tag{15.10}$$

$$C_m(h_m,t) = 0. \tag{15.11}$$

Traditionally Eq. (15.8) is solved in terms of the amount of solute $Q(t)$ exiting from the membrane in time t and expressed as a series solution [10]:

$$Q(t) = -D_m A \int_0^t \left.\frac{\partial C_m}{\partial x}\right|_{x=h_m} dt = K_m A C_v h_m \left(\frac{t}{t_d} - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \exp(-\frac{t}{t_d} \pi^2 n^2) \right) \tag{15.12}$$

where the diffusion time is given by:

$$t_d = \frac{h_m^2}{D_m} \tag{15.13}$$

It should be noted that as the exponent of a very large negative number approaches zero, the summation term in Eq. (15.12) can be ignored at long times so that Eq. (15.12) reduces to the form of Eq. (15.1):

$$Q(t) = K_m A C_v h_m \left(\frac{D_m t}{h_m^2} - \frac{1}{6} \right) = k_p A C_v \left(t - \frac{h_m^2}{6 D_m} \right) = k_p A C_v (t - \text{lag}) \tag{15.14}$$

where *lag* is given by

$$\text{lag} = \frac{h_m^2}{6 D_m} = \frac{t_d}{6} \tag{15.15}$$

Given the advent of numerical Fast Inverse Laplace Transforms (FILT) [11–13], with non-linear regression modelling, it is convenient to analyse cumulative amount versus time data numerically inverting from the Laplace domain using Eq. (15.16) where s is the Laplace variable:

$$\hat{Q}(s) = -D_m A \frac{1}{s} \left.\frac{\partial \hat{C}_m}{\partial x}\right|_{x=h_m} = \frac{k_p A C_v}{s^2} \frac{\sqrt{s t_d}}{\sinh(\sqrt{s t_d})}. \tag{15.16}$$

The mathematical steps required to derive Eq. (15.16), as well as an overview of the Laplace transform application in the skin literature is given in [14]. Figure 15.3 shows a plot of the cumulative amount penetrated for the diffusion (Eq. (15.16), curve 2)

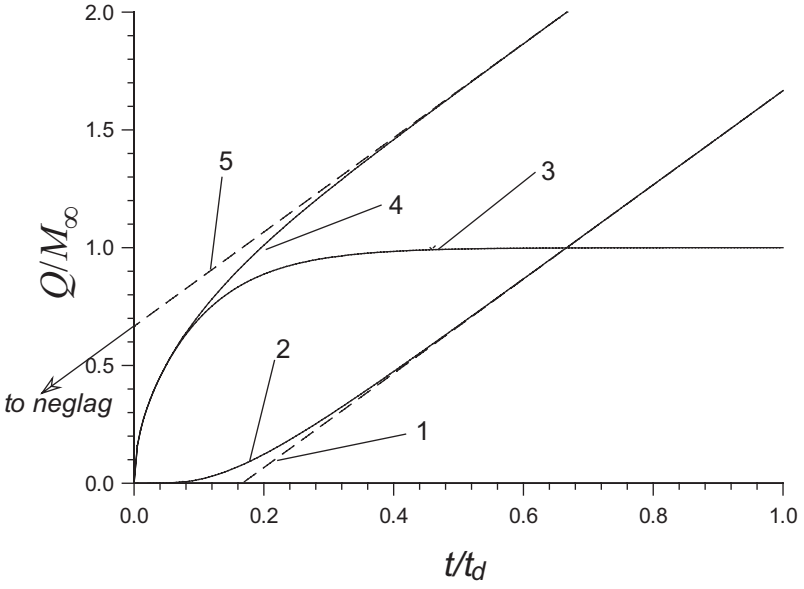


Fig. 15.3 Normalised cumulative amount of solute penetrating Q/M_{∞} (curve 2, Eq. (15.16)); taken up by the stratum corneum (curve 3, Eq. (15.18)); and leaving vehicle (curve 4, Eq. (15.21)) with normalised time. Curves 1–5 represent steady state approximations of the cumulative amount penetrating the stratum corneum (Eq. (15.14)) and leaving the vehicle (Eq. (15.20)) with normalised time

and steady state (Eq. (15.14), curve 1) models versus time. Equations (15.12) or (15.16) can be used to analyse *in vitro* experimental data by nonlinear regression as shown in Fig. 15.4.

Figure 15.3 (curve 3) also shows the amount of solute taken up by the SC with time. These profiles are of interest for those solutes which may be targeted for retention in this tissue, e.g. sunscreens or which may be sequestered in this tissue, e.g. steroids. The time domain and Laplace domain solutions for the amount of solute $M(t)$ taken up into an assumed homogeneous SC with time are:

$$M(t) = M_{\infty}\left(1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n=1)^2}\exp\left(-\frac{t}{t_d}\pi^2(2n+1)^2\right)\right) \tag{15.17}$$

$$\hat{M}(s) = M_{\infty}\frac{2}{s}\frac{\cosh(\sqrt{st_d})-1}{\sqrt{st_d}\sinh(\sqrt{st_d})}, \tag{15.18}$$

where M_{∞} is the amount of solute in the skin at steady state and is given by $K_m C_v h_m A/2$ when a linear concentration gradient is assumed.

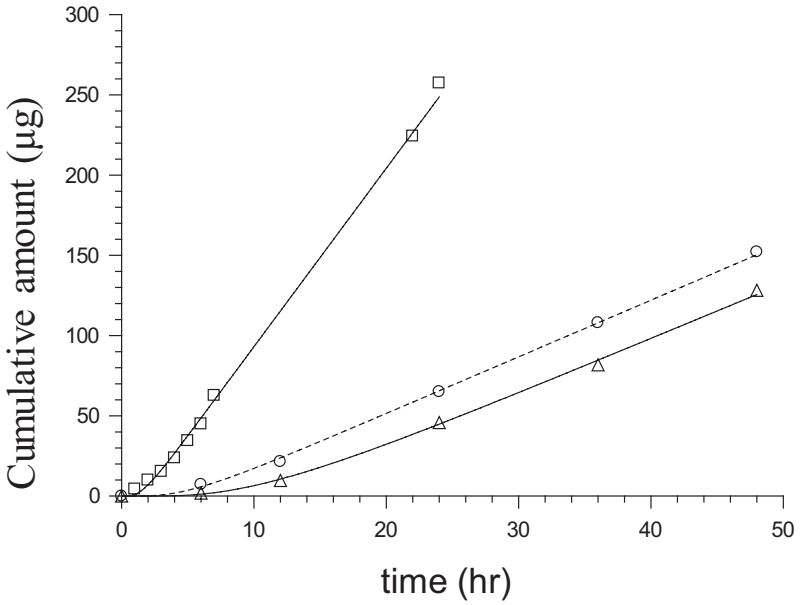


Fig. 15.4 Nonlinear regressions of cumulative amount penetrating human epidermis with time using Eq. (15.16) and a weighting of $1/y_{obs}$. Data corresponds to triethanolamine salicylate (□, $t_d = 9.8$ hr, $J_{ss} = 11.1\mu g/\text{hr}$, diclofenac skin 1(○, $t_d = 32.7$ hr, $J_{ss} = 3.5\mu g/\text{hr}$) and diclofenac skin 2 (Δ, $t_d = 68.0$ hr, $J_{ss} = 3.8\mu g/\text{hr}$)

The summation of $Q(t)$ and $M(t)$ yields the expression for the amount which leaves the vehicle $Q_{in}(t)$ (the profile shown in Fig. 15.3 curve 4):

$$Q_{in}(t) = K_m A C_v h_m \left(\frac{t}{t_d} + \frac{1}{3} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left(-\frac{t}{t_d} \pi^2 n^2 \right) \right) \tag{15.19}$$

When $t \to \infty$, Eq. (15.19) reduces to:

$$Q_{in}(t) = K_m A C_v h_m \left(\frac{t}{t_d} + \frac{1}{3} \right) = k_p A C_v \left(t + \frac{t_d}{3} \right) = k_p A C_v (t + neglag). \tag{15.20}$$

Hence, the linear portion of $Q_{in}(t)$ versus t has a slope of $k_p AC_v$ and intercepts on the negative side of the time axis at a point of $neglag = t_d/3 = h_m^2/(3D_m)$ (Fig. 15.3 curve 5).

The corresponding Laplace domain expression for $Q_{in}(t)$ is:

$$\hat{Q}_{in}(s) = \frac{k_p A C_v}{s^2} \sqrt{s t_d} \operatorname{cotanh}(\sqrt{s t_d}). \tag{15.21}$$

The absorption rate or flux of solutes in the period before steady state is important for many ointments applied topically for local effects and in the toxicology of ointments applied to the skin. The flux of solutes exiting membrane per unit area of membrane, $J_s(t)$, is defined by $J_s(t) = (1/A)\partial Q/\partial t$ or $\hat{J}_s(s) = s\hat{Q}(s)/A$ in the Laplace domain. Using Eqs. (15.12) and (15.16) we find therefore

$$J_s(t) = -D_m \frac{\partial C_m}{\partial x}_{|x=h_m} = k_p C_v \left(1 + 2\sum_{n=1}^{\infty}(-1)^n \exp\left(-\frac{t}{t_d}\pi^2 n^2\right)\right) \tag{15.22}$$

$$\hat{J}_s(s) = \frac{k_p C_v}{s} \frac{\sqrt{st_d}}{\sinh(\sqrt{st_d})}. \tag{15.23}$$

The corresponding equation for the flux of solute from the vehicle into the membrane, $J_{in}(t)$, is given as

$$J_{in}(t) = k_p C_v \left(1 + 2\sum_{n=1}^{\infty}\exp\left(-\frac{t}{t_d}\pi^2 n^2\right)\right) \tag{15.24}$$

$$\hat{J}_{in}(s) = \frac{k_p C_v}{s}\sqrt{st_d}\,\mathrm{cotanh}(\sqrt{st_d}). \tag{15.25}$$

Figure 15.5 shows the flux-profiles for solutes leaving the membrane and vehicle, respectively.

The concentration in the membrane in the Laplace domain is [14]:

$$\hat{C}_m(x,s) = \frac{K_m C_v}{s} \frac{\sinh\left[\sqrt{st_d}(1 - x/h_m)\right]}{\sinh(\sqrt{st_d})} \tag{15.26}$$

This equation may be important as illustrated in the use of *in vivo* ATR-FTIR to examine the kinetics of solute uptake into human SC *in vivo* [15].

15.2.2 Amount and Flux-Time Profiles on Removing the Donor Phase After Reaching the Steady State for Conditions Described in Section 15.1.1

We now consider the amount- and flux-time profiles for the specific case in which the donor phase has been removed after a steady state has been reached. This equates to a number of practical cases of interest such as patch removal, sunscreen and other products being washed of and removal of toxins from the skin, when the assumption can be made that there has not been a significant (>10%) depletion

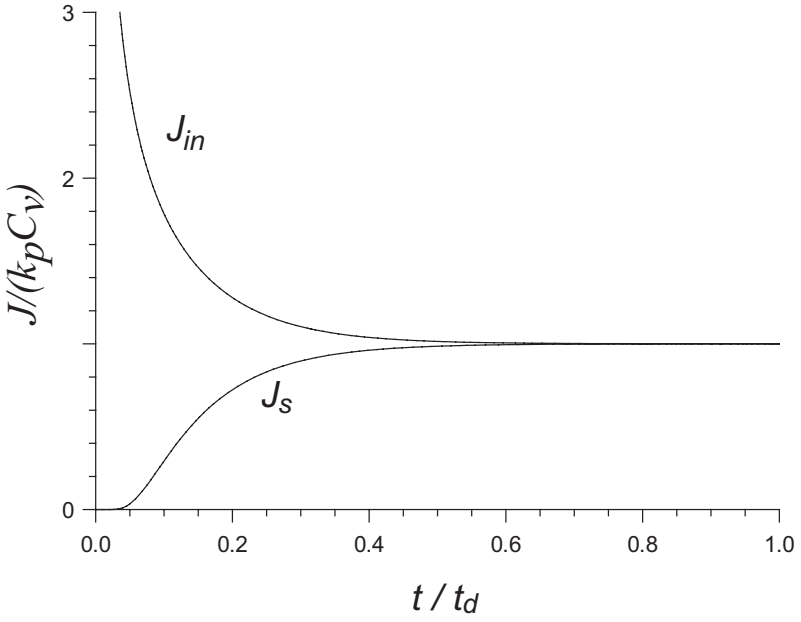


Fig. 15.5 Normalised flux $J/(k_pC_v)$ against normalised time (t/t_d) for flux of solutes penetrating the stratum corneum (J_s, Eq. (15.23)) and entering the stratum corneum (J_{in}, Eq. (15.25))

in the concentration of solute at the surface. The amount absorbed into a systemic circulation across the skin from the time the dosage form is removed is given by

$$Q(t) = M_\infty \left(1 - \frac{4}{\pi^3} \sum_{n=0}^{\infty} \frac{(-1)^n}{\left(n+\frac{1}{2}\right)^3} \exp\left(-\frac{t}{t_d} \pi^2 \left(n+\frac{1}{2} \right)^2 \right) \right), \tag{15.27}$$

where $M_\infty = K_m C_v A h_m / 2 = k_p A C_v t_d / 2$ is amount of solute present in the skin before removal of the vehicle. The Laplace domain equivalent of this expression is

$$\hat{Q}(s) = \frac{M_\infty}{s^2} \frac{2}{t_d} \left(1 - \frac{1}{\cosh\left(\sqrt{s t_d}\right)} \right). \tag{15.28}$$

The corresponding equations for flux are

$$J_s(t) = k_p C_v \frac{2}{\pi} \sum_{n=0}^{\infty} \frac{(-1)^n}{n+\frac{1}{2}} \exp\left(-\frac{t}{t_d} \pi^2 \left(n+\frac{1}{2} \right)^2 \right), \tag{15.29}$$

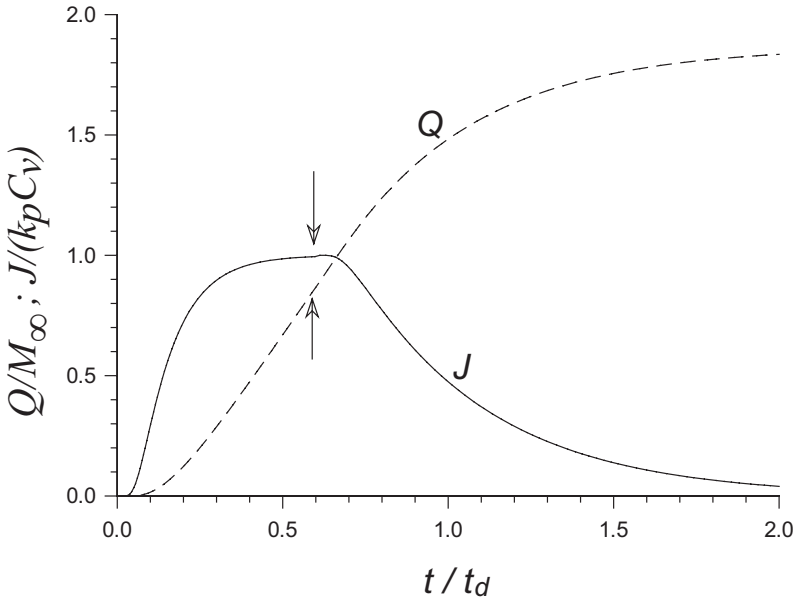


Fig. 15.6 Changes in normalised cumulative amounts penetrating (Q/M_∞) and flux ($J/(k_pC_v)$) when the vehicle is removed (as indicated by arrows) at a specific normalised time (t/t_d) after application

$$\hat{J}_s(s) = \frac{k_p C_v}{s}\left(1 - \frac{1}{\cosh\left(\sqrt{st_d}\right)}\right). \tag{15.30}$$

Figure 15.6 shows the amount and flux-time profiles associated with donor phase removal.

The mean time for absorption of solute from the skin in this case is given by:

$$MAT = \frac{\int_0^\infty J_s(t)t\,dt}{\int_0^\infty J_s(t)dt} = -\lim_{s\to 0}\frac{d}{ds}\ln(\hat{J}_s) = \frac{5h_m^2}{12D_m} = \frac{5}{12}t_d, \tag{15.31}$$

and the amount absorbed after infinite time is the amount in the skin before the vehicle is removed, which is equal to M_∞.

Frasch and Barbero [16] considered a case where exposure to the solute is not long enough for steady state concentration to form in the SC for volatile and non-volatile solutes.

15.2.3 In Vitro *Permeability Studies with a Constant Donor Concentration and Finite Receptor Volume*

In most *in vitro* studies, it is assumed that sink conditions apply in the receptor phase. However, the receptor phase is a finite volume and solute accumulation may be possible if there is an inadequate removal rate of the solute penetrating through. Siddiqui et al. [17] related the steady state flux of steroids through human epidermis to the differences in concentrations between donor (C_v) and receptor (C_{ss}) concentrations. In the present notation, this equation is:

$$J_{ss} = k_p \left(C_v - \frac{C_{ss}}{K_r} \right), \tag{15.32}$$

where K_r is the partition coefficient between receptor and vehicle $(K_r = C_r / C_v)$ and K_m is the partition coefficient between the membrane and vehicle $(K_m = C_m / C_v)$. Siddiqui et al. [17] assumed that $K_r = 1$.

Implicit in the underlying boundary conditions for the receptor phase is a constant clearance of solute Cl_r, due to repeated sampling or use of a flow through cell. If such a clearance was absent, C_{ss} would continually increase and approach $C_v K_r$. The value of C_{ss} is defined by the relative magnitudes of the clearance (Cl_r) and $k_p A / K_r$:

$$C_{ss} = \frac{k_p A C_v}{Cl_r + k_p A / K_r}. \tag{15.33}$$

Siddiqui et al. [17] also applied this equation and the dermal clearance of solutes (Cl_r) to predict the steady state epidermal concentrations of solutes C_{ss}. Roberts [18] considered the limits of large $k_p A$ as exists for phenols absorption and low $k_p A$ as exists for steroid absorption. He suggested that, when $k_p A >> K_r Cl_r$, C_{ss} would eventually approach the donor concentrations used $(C_v K_r)$. In contrast, when $k_p A << K_r Cl_r$, C_{ss} approaches $k_p A C_v / Cl_r$.

The derivation of the full equation, from which steady state Eqs. (15.32) and (15.33) arise, needs to take into account a finite receptor or epidermis volume. The boundary condition at $x = h_m$ in this case is $C_m(h_m, t) / K_m = C_r(t) / K_r$, together with: [19]

$$V_r \frac{dC_r}{dt} = -AD_m \frac{\partial C_m}{\partial x}_{|x=h_m} - Cl_r C_r, \tag{15.34}$$

where Cl_r is the clearance (ml/min) of solution containing solute from the receptor phase, V_r is the volume of the receptor and C_r is the concentration in the receptor.

Using this boundary condition together with boundary condition (10) yields for the amount of solute which penetrated the skin into the receptor (= amount in receptor + amount cleared from receptor) and for the flux of solute into the receptor: [19]

$$\hat{Q}(s) = \frac{k_p A C_v}{s^2} \frac{\sqrt{st_d}}{\sinh(\sqrt{st_d}) + \dfrac{\sqrt{st_d}}{st_d V_{rN} + Cl_{rN}} \cosh(\sqrt{st_d})}, \tag{15.35}$$

$$\hat{J}_s(s) = \frac{k_p C_v}{s} \frac{\sqrt{st_d}}{\sinh(\sqrt{st_d}) + \dfrac{\sqrt{st_d}}{st_d V_{rN} + Cl_{rN}} \cosh(\sqrt{st_d})} \tag{15.36}$$

where dimensionless parameter $Cl_{rN} = Cl_r K_r / (k_p A)$ is a measure of the magnitude of the removal rate from the receptor phase (Cl_r) relative to transport through the membrane ($k_p A$) and $V_{rN} = V_r K_R / (V_m K_m)$ is the dimensionless receptor volume defined as the ratio of the amount of drug in the receptor phase and membrane ($C_r V_r / [C_m V_m]$) assuming equilibrium exists between phases.

Figure 15.7 shows the effect of receptor volume (as defined by V_{rN}) and clearance of solution from the receptor phase (as defined by Cl_{rN}) on $J_s(t)$-time profile.

The steady state approximation of Eq. (15.35) is

$$Q(t) \approx A J_{ss}(t - \text{lag}), \tag{15.37}$$

where

$$J_{ss} = k_p C_v \frac{Cl_r}{Cl_r + k_p A / K_r} = \frac{k_p C_v}{1 + 1 / Cl_{rN}}, \tag{15.38}$$

and

$$\text{lag} = \frac{t_d}{6}\left(1 + \frac{2Cl_{rN} - 6V_{rN}}{Cl_{rN}\left(Cl_{rN} + 1\right)}\right). \tag{15.39}$$

We note that if Eq. (15.33) is substituted into (15.32), the expression for J_{ss} is identical to Eq. (15.38). We also note that when $Cl_r \to \infty$ (infinite sink), J_{ss} and *lag* reduce to Eqs. (15.3) and (15.15), respectively.

The corresponding expression for the receptor/epidermal concentration with the above boundary conditions is

$$\hat{C}_r(s) = \frac{k_p A C_v}{s} \frac{\sqrt{st_d}}{(V_r s + Cl_r)\left((\sinh\sqrt{st_d}) + \dfrac{\sqrt{st_d}}{st_d V_{rN} + Cl_{rN}} \cosh(\sqrt{st_d})\right)}. \tag{15.40}$$

At long times $(t \to \infty)$, C_r is defined by Eq. (15.33).

Parry et al. [20] has described a percutaneous absorption model in which both the donor and receptor compartments for an *in vitro* membrane study were well-stirred

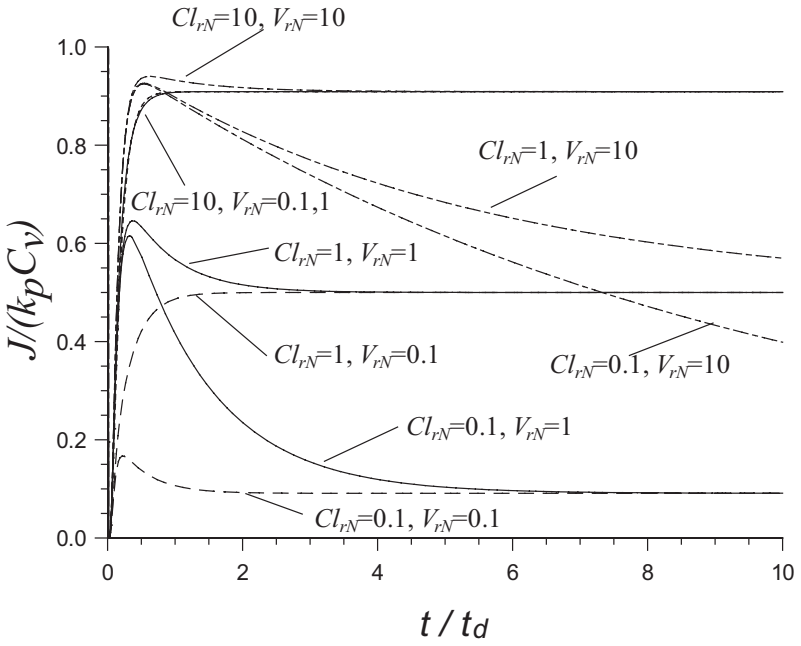


Fig. 15.7 Normalised flux ($J / (k_p C_v)$) versus normalised time (t / t_d) for a finite receptor volume and limited clearance (Eq. (15.36)), $Cl_{rN} = Cl_r K_r / (k_p A)$, $V_{rN} = V_r K_R / (V_m K_m)$

and finite. Boundary conditions similar in form to that defined by Eqs. (15.34) and (15.46), but with $Cl_r = 0$, were used to describe the disappearance of solute from the donor chamber into the membrane and efflux of solute from the membrane into the receptor chamber. The resultant expression included a complex function requiring the solution of transcendental equations. It should be emphasised that this model differs from others described in this section in that it does not have a clearance term to account for sampling.

15.2.4 In Vitro *Permeability Studies with a Constant Donor Concentration or Defined Input Flux and Finite Clearance of Solute from the Epidermis*

The importance of receptor conditions on epidermal transport has been the subject of various studies over the last 30 years. Two models are widely used. In the first model, it is assumed that the viable epidermis or aqueous diffusion layer below the SC can exert a significant influence on skin penetration [21, 22]. The second model is one where there is a rate limited step in partitioning from the membrane to the next phase (eg epidermis → receptor solution, SC → epidermis, epidermis →

dermis). This rate constant of dimension time^{-1}, which we will define as k_c, and the interfacial barrier rate constant are identical if the lag time for the interfacial barrier is negligible compared to the lag time in the SC. In the specific case of an aqueous diffusion layer being a barrier, $k_c = D_{aq} / l_{aq}^2$ where l_{aq} is the thickness of the layer and D_{aq} is the diffusion coefficient in the layer [22].

Guy and Hadgraft [23] developed a pharmacokinetic model for skin absorption based on the diffusion model with the boundary conditions defined by (a) the influx into the membrane being related to an assumed exponential decline in vehicle donor concentration and (b) the efflux from the membrane being related to first order removal at a rate constant k_c. These authors went on to examine short and long-time approximations. Kubota and Ishizaki [24] presented a more generalised diffusion model for drug absorption through excised skin by using the boundary conditions of the fluxes (a) into the skin being defined by an arbitrary function $f(t)$ and (b) out of the skin being defined by $ClC(h_m)$, where Cl is the clearance from the skin and $C(h_m)$ is the concentration of solute at the depth h_m, that is the skin–system interface. They considered a boundary condition at the membrane–vehicle interface defined by an input rate into the membrane $f(t)$ together with a first order rate constant k_c determined efflux from the membrane. Accordingly, the amount of solute absorbed across the skin $Q(t)$ at various times t is defined in the Laplace domain as:

$$\hat{Q}(s) = \frac{A}{s} \frac{k_c t_d \hat{f}(s)}{\sqrt{st_d}\,\sinh(\sqrt{st_d}) + k_c t_d \cosh(\sqrt{st_d})}. \tag{15.41}$$

Of particular interest in this overview is the case of a constant donor concentration (infinite donor) and sink receptor. $\hat{Q}(s)$ is then defined by:

$$\hat{Q}(s) = \frac{k_p C_v A}{s^2} \frac{k_c t_d \sqrt{st_d}}{\sqrt{st_d}\,\cosh(\sqrt{st_d}) + k_c t_d \sinh(\sqrt{st_d})} \tag{15.42}$$

Figure 15.8a shows the effect of k_c (as defined by $\alpha = k_c t_d$) on $Q(t)$ versus time profile.

It is to be noted that, at long times, the linear portion of $Q(t)$ (defined by Eq. (15.42)) versus t profile describes a steady state flux J_{ss} and lag time (lag):

$$Q(t) = J_{ss} A(t - \text{lag}) = C_v k_p A \frac{k_c t_d}{1 + k_c t_d}(t - \text{lag}), \tag{15.43}$$

$$\text{lag} = \frac{t_d}{6}\left(1 + \frac{2}{1 + k_c t_d}\right). \tag{15.44}$$

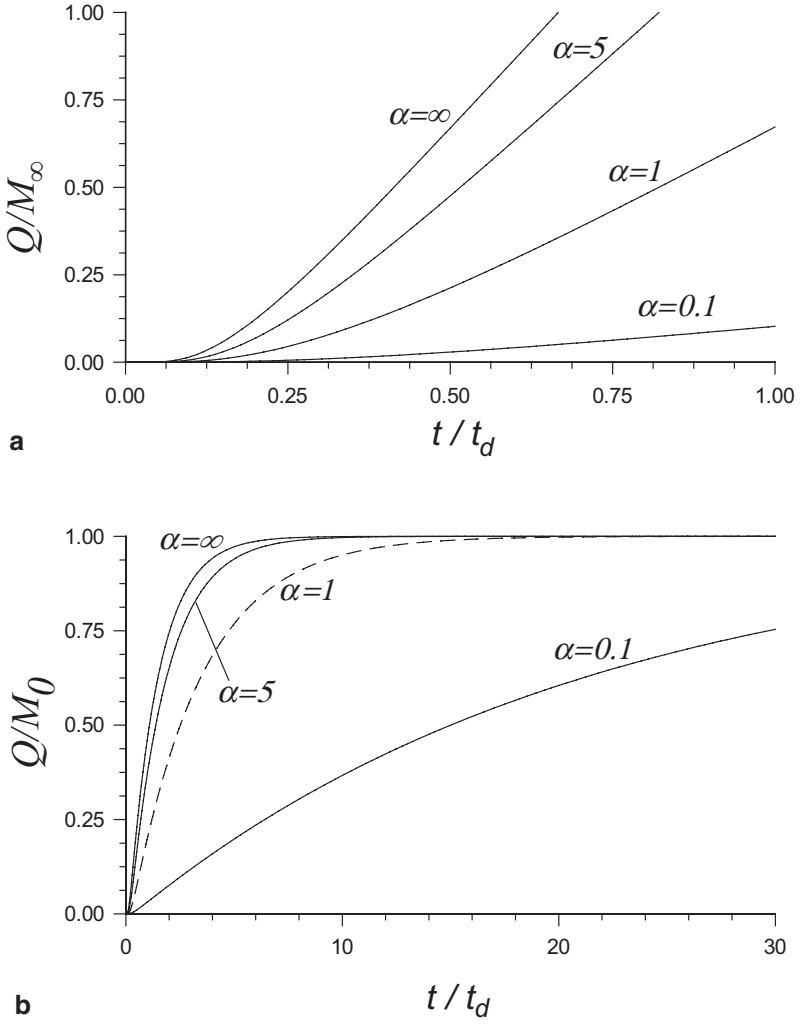


Fig. 15.8 Effect of interfacial barrier rate constant (expressed as $\alpha = k_c t_d$) on exit from stratum corneum on normalised amount penetrating the epidermis with normalised time (t/t_d). **a** Constant donor concentration and **b** a finite dose in well stirred vehicle, where $V_{rN}(=V_r K_R/(V_m K_m))=1$ for time normalised to diffusion time

Thus, both the slope and lag of the steady state portion of a $Q(t)$ versus t plot depend on k_c.

J_{ss} in Eq. (15.43) can be re-expressed as:

$$J_{ss} = C_v k_p \frac{1}{1+1/k_c t_d}, \tag{15.45}$$

which is identical to Eq. (15.38), if $k_c t_d$ is replaced with Cl_{rN}.

Elsewhere we have analysed a more general case with simultaneous rate limitations due to clearance from receptor (Cl_r), finite receptor volume (V_r), finite permeability through viable epidermis $\left(k_p^{ve}\right)$ and finite permeability through unstirred donor layer $\left(k_p^d\right)$ [19]. Eqs. (15.35) and (15.42) for $\hat{Q}(s)$ are limiting cases of this more general solution.

15.2.5 In Vitro *Skin Permeability Studies with Finite Donor Volume and Receptor Sink Conditions*

In practice, the solute concentration applied to the skin does not remain constant but declines owing to the finite volumes of vehicles applied to the skin. We therefore need to examine solutions for Eq. (15.8) in which the boundary condition allows for a depletion in solute concentration. We assume, initially, the simplest boundary conditions applying to the sorption of solutes into a membrane from the well-stirred vehicle at $x = 0$ and from membrane into a systemic circulation at $x = h_m$ (Eqs. (15.10) and (15.11)) together with a condition of depletion of concentration in the vehicle: [10]

$$V_v \frac{dC_v}{dt} = AD_m \left.\frac{\partial C_m}{\partial x}\right|_{x=0}, \tag{15.46}$$

where $V_v = Ah_v$ is the volume of the vehicle applied to the skin. Solution of Eq. (15.8) with (15.10), (15.11) and (15.46) as boundary conditions gives: [25]

$$Q(t) = M_0 \left(1 - \sum_{n=1}^{\infty} \frac{2\exp\left(-\frac{t}{t_d}\gamma_n^2\right)}{\cos\gamma_n \left(1 + V_{vN}\gamma_n^2 + 1/V_{vN}\right)} \right), \tag{15.47}$$

where $M_0 = C_{v0}V_v = C_{v0}Ah_v$ is the initial amount of solute in the vehicle, C_{v0} is the initial concentration in the vehicle, h_v is the effective thickness of the vehicle, V_{vN} is a dimensionless parameter defined by:

$$V_{vN} = \frac{V_v}{K_m V_m},$$

and γ_n are positive roots of transcendental equation:

$$\gamma \tan\gamma = \frac{1}{V_{vN}}.$$

The Laplace transform of $Q(t)$ is given by: [26]

$$\hat{Q}(s) = \frac{M_0}{s} \frac{1}{V_{vN}\sqrt{st_d}\sinh(\sqrt{st_d}) + \cosh(\sqrt{st_d})}. \tag{15.48}$$

The corresponding expressions for flux are given by Eqs. (15.49) and (15.50):

$$J_s(t) = V_{vN} C_{v0} k_p \sum_{n=1}^{\infty} \frac{2\gamma_n^2 \exp\left(-\frac{t}{t_d}\gamma_n^2\right)}{\cos\gamma_n \left(1 + V_{vN}\gamma_n^2 + 1/V_{vN}\right)}, \tag{15.49}$$

$$\hat{J}_s(s) = V_{vN} C_{v0} k_p \frac{t_d}{V_{vN}\sqrt{st_d}\sinh(\sqrt{st_d}) + \cosh(\sqrt{st_d})}. \tag{15.50}$$

Equation identical to Eq. (15.49) was used by Kasting [27] for analysis of the *in vitro* absorption rates of varying finite doses of vanillylnonamide applied to excised human skin from propylene glycol.

Figure 15.9 shows the predicted profiles for the flux of solute (Eq. (15.50)) with varying $V_{vN}=V_v/(K_m V_m)$. It is apparent that both the peak time and area under the curve decrease with the decreasing V_{vN}. The longer peak time with increasing V_v reflects the movement from a finite to an infinite donor source. The larger area under the curve reflects the higher dose associated with an increase in V_v.

Two summary parameters can be derived from Eq. (15.50):

1. Mean absorption time measuring from systemic side of the skin is

$$MAT_s = -\frac{d\ln\hat{J}_s(s)}{ds}_{|s=0} = \frac{1}{2}t_d + V_{vN}t_d \tag{15.51}$$

It needs to be emphasised that MAT_s differs from MTT, which is mean transit time through SC. MTT can be calculated as

$$MTT = MAT_s - MAT_v,$$

where MAT_v is the mean absorption time from the vehicle:

$$MAT_v = -\frac{d\ln\hat{J}_v(s)}{ds}_{|s=0},$$

where $\hat{J}_v(s)$ is the Laplace transform of the flux from the vehicle into the skin. It can be found that

$$\hat{J}_v(s) = V_{vN} C_{v0} k_p \frac{t_d}{V_{vN}\sqrt{st_d}\tanh(\sqrt{st_d}) + 1},$$

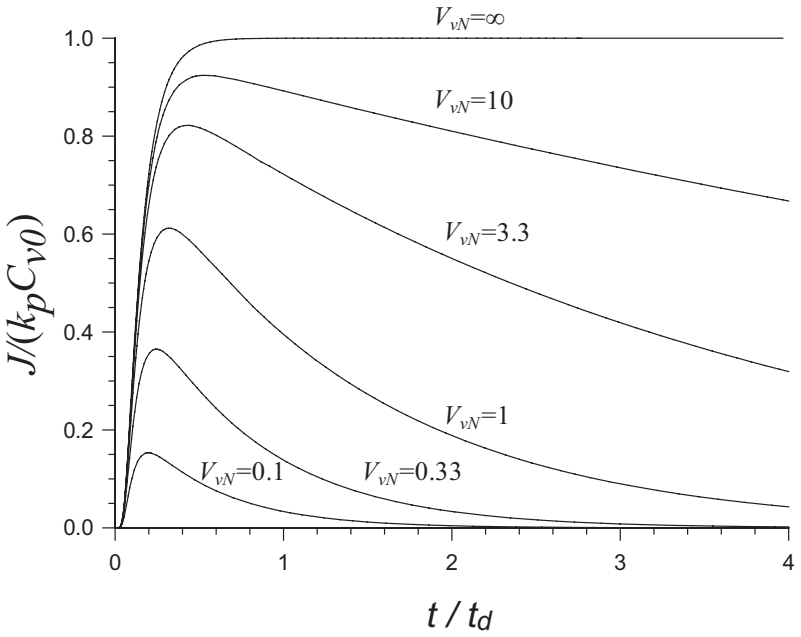


Fig. 15.9 Normalised flux for penetration of a solute from a finite dose in a well stirred vehicle ($J/(k_pC_{vo})$) against normalised time (t/t_d) for diffusion time with varying $V_{rN} = (V_rK_R/(V_mK_m))$

and $MAT_v = V_{vN}t_d$. We therefore have $MTT = t_d/2$.

2. CV^2 for absorption:

$$CV^2 = \frac{\frac{d^2}{ds^2}\ln \hat{J}_s(s)}{\frac{d}{ds}\ln \hat{J}_s(s)_{|s=0}} = \frac{2}{3} + \frac{4}{3(2+1/V_{vN})^2}. \tag{15.52}$$

Another two summary parameters can be derived from Eq. (15.50) when $V_{vN} << 1$ ($V_v << V_mK_m$). This applies in a specific case of solvent deposited solids. Scheuplein and Ross [28] described the absorption of drugs when 25 μl of acetone is applied to 2.54 cm^2 of skin and allowed to evaporate, leaving a very thin layer of solid material. When $V_{vN}<<1$ Eq. (15.50) reduces to:

$$\hat{J}_s(s) = V_{vN}C_{v0}k_p \frac{t_d}{\cosh(\sqrt{st_d})},$$

and therefore $J(t)$ can be written as

$$J(t) = V_{vN}C_{v0}k_p f(t/t_d) = V_{vN}C_{v0}k_p f(\tau),$$

where $\tau = t/t_d$, $f(\tau)$ is a function independent of V_{vN} and whose Laplace transform is $\hat{f}(s) = 1/\cosh\sqrt{s}$. It can be shown by the numerical inversion of $\hat{f}(s)$ that the maximum of the function $f(\tau)$ occurs at $\tau = 1/6$ with the value $f(\tau_{\max}) = 1.850$. The peak flux, J_p, and for the time of peak flux, t_p, for finite dose absorption solvent deposited solutes are therefore described by the simple equations:

$$J_p = 1.85 V_{vN} C_{v0} k_p = \frac{1.85 C_{v0} D_m h_v}{h_m^2}, \quad t_p = \frac{t_d}{6} = \frac{h_m^2}{6 D_m}. \tag{15.53}$$

Hence, the peak time corresponds to the lag time observed after application of a constant donor solution (Eq. (15.15)). Scheuplein and Ross [28] provided experimental data to show: (a) J_p is proportional to C_{v0} for benzoic acid, (2) t_p for different solutes is inversely related to their D_m values and (3) penetration was facilitated by hydrating the SC.

15.2.6 In Vitro *Permeability Studies with a Finite Donor Volume and a Finite Clearance from the Epidermis into the Receptor*

Another case of particular practical interest is when the donor phase is assumed to be well-stirred and finite in volume and there is limiting clearance from the epidermis to the receptor phase. Applying the boundary condition defined by Eq. (15.46), together with boundary condition for $x = h_m$:

$$D_m \left.\frac{\partial C_m}{\partial x}\right|_{x=h_m} = h_m k_c C_m(h_m, t), \tag{15.54}$$

yields for $\hat{Q}(s)$:

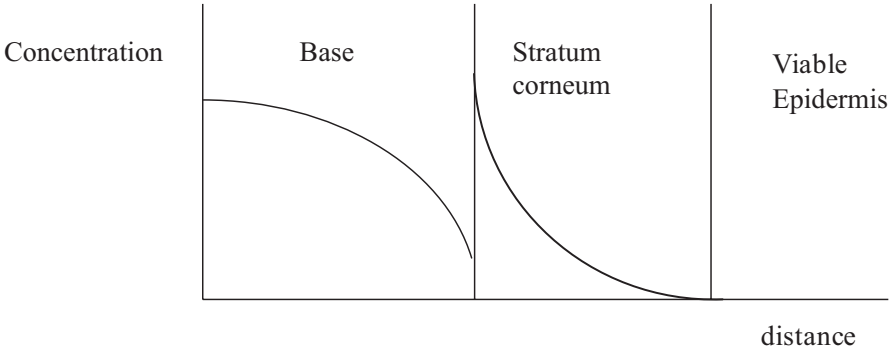
$$\hat{Q}(s) = \frac{M_0}{s} \frac{1}{\sqrt{st_d}\sinh(\sqrt{st_d})\left(V_{vN} + \dfrac{1}{t_d k_c}\right) + \cosh(\sqrt{st_d})\left(1 + V_{vN}\dfrac{s}{k_c}\right)} \tag{15.55}$$

The profiles for $Q(t)$ versus t defined by Eq. (15.55) for different values of k_c ($\alpha = k_c t_d$) and V_{vN}=1 are shown in Fig. 15.8b.

A case of finite volume of the vehicle with simultaneous rate limitations due to clearance from receptor (Cl_r), finite receptor volume (V_r), finite permeability through viable epidermis k_p^{ve} and finite permeability through unstirred donor layer $\left(k_p^d\right)$ were analysed by Anissimov and Roberts [26]. Equations (48) and (55) presented here for $\hat{Q}(s)$ are limiting cases of their more general solution.

15.2.7 In Vitro *Skin Permeability Studies with Diffusion Limited Finite Donor, and Sink Receptor Conditions*

One of the first attempts at modelling percutaneous absorption with diffusion limiting uptake from both the vehicle and the skin was made by Kakemi et al. [29]. The schematics of their one dimensional model is shown in Fig. 15.2d. Guy and Hadgraft [30] used a similar model with sink receptor conditions as shown in Fig. 15.10.



	Base	Stratum corneum	Viable Epidermis
Diffusion coefficient	D_o	D_s	---
Concentration	$C_{o,o}$ at t=0	C_s	0
Partition Coefficient	$K = C_o/C_s$ (at X = X = 0) and zero flux		
Distance	$l_o \leftarrow 0$	$0 \rightarrow l_s$	
Normalised distance	$X` = X` / l_o$; $1 \leftarrow 0$	$X = X / l_s$; $0 \rightarrow 1$	
Area	A	A	

Fig. 15.10 Two layer diffusion model for *in vitro* percutaneous absorption kinetics as defined by Guy and Hadgraft [30]

In the latter model, the solute has a diffusivity D_v in a finite vehicle of volume V_v which is in contact with SC in which a solute has a diffusivity D_m down a path-length h_m. The Laplace transform for the amount penetrating the epidermis $\hat{Q}(s)$ into an absorbing "sink" is:

$$\hat{Q}(s) = \frac{M_0}{s} \frac{\sinh(\sqrt{st_{dv}})}{\sqrt{st_{dv}}\left(V_{vN}\sqrt{\frac{t_d}{t_{dv}}}\sinh(\sqrt{st_{dv}})\sinh(\sqrt{st_d}) + \cosh(\sqrt{st_{dv}})\cosh(\sqrt{st_d})\right)}, \tag{15.56}$$

where as usual $M_0 = C_{v0}V_v$ and t_{dv} is the diffusion time in the vehicle, $t_{dv} = h_v^2 / D_v$.

When the transport across the epidermis is also dependent on a first order rate constant k_c for removal from the epidermis, the Laplace transform becomes:

$$\hat{Q}(s) = \frac{M_0}{s} \frac{\sinh\left(\sqrt{st_{dv}}\right)}{\sqrt{st_{dv}}}\left[V_{vN}\sqrt{\frac{t_d}{t_{dv}}}\sinh\left(\sqrt{st_{dv}}\right)\sinh\left(\sqrt{st_d}\right) + \cosh\left(\sqrt{st_{dv}}\right)\cosh\left(\sqrt{st_d}\right) + \frac{\left(\sqrt{st_d}\right)}{t_d k_c}\left(V_{vN}\sqrt{\frac{t_d}{t_{dv}}}\cosh\left(\sqrt{st_d}\right)\sinh\left(\sqrt{st_{dv}}\right) + \cosh\left(\sqrt{st_{dv}}\right)\sinh\left(\sqrt{st_d}\right)\right)\right]^{-1} \tag{15.57}$$

Figure 15.11 shows profiles of $Q(t)$ versus t as defined by Eq. (15.57) for different vehicle diffusivities $(\gamma = t_{dv} / t_d)$ and $k_c (\alpha = k_c t_d)$ for V_{vN}=1.

In the particular cases, when $t_{dv} \gg t_d$ and $t_d \gg t_{dv}$ Eq. (15.56) reduces to Eqs. (15.69) and (15.48), respectively.

15.2.8 In Vitro *Permeability Studies with Two Layer Diffusion Limitations in Transport*

The complex cases of diffusion being a limitation in the transport through both the SC and epidermis have been considered by Hadgraft [31]. He considered the case when solute exists as a reservoir in the SC. In his approach, the solute initially present in the SC diffuses from it into and through the epidermis. The case of rate limiting removal from the epidermis (k_c) was considered.

Cleek and Bunge [32] considered similar two phase in series model for the amount of solute entering skin $(Q_{\text{in}}(t))$ and determined it both as an analytical solution and simulations. This model was then extended to include solute properties as a determinant of uptake [33]. They suggested that steady state permeability will be under estimated if not corrected for the relative permeabilities of the SC and epidermis. The result of these considerations is a steady state Eq. (15.58) similar to Eqs. (15.43) and (15.44):

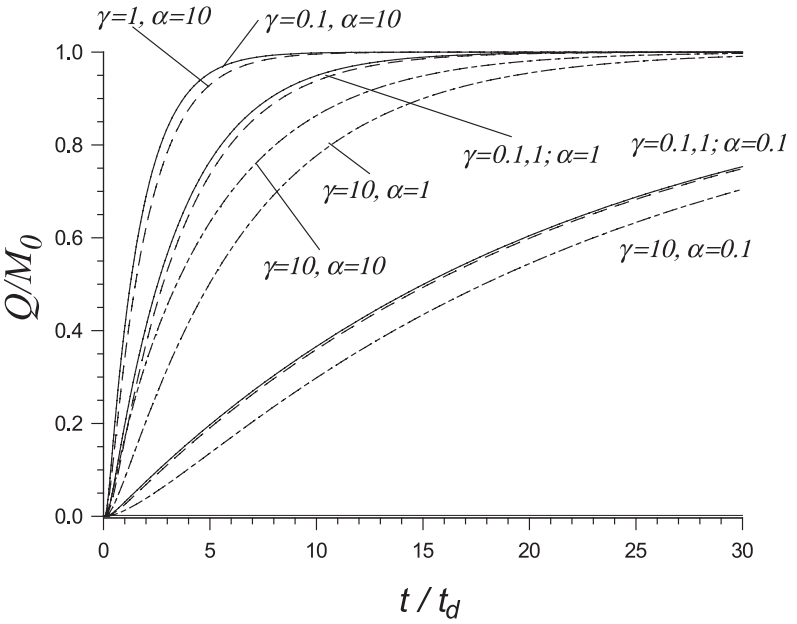


Fig. 15.11 Cumulative amount penetrated Q normalised for amount applied M_o versus time normalised for diffusion time t_d for the case of both vehicle and stratum corneum limited diffusion. The two parameters varied define the relative diffusion time in vehicle relative to that in stratum corneum $\gamma(= t_{dv} / t_d)$ and the interfacial barrier rate constant effect on the exit of solutes from the stratum $\alpha(= k_e t_d)$ (Eq. (15.57))

$$Q_{\text{in}}(t) = C_{v0} k_p A \frac{1}{1+B}\left(t + t_{ds} \frac{G(1+3B)+(1+3BG)}{3G(1+B)} \right), \tag{15.58}$$

where $k_p = K_{sv} D_{sc} / h_{sc}$, $G = t_{ds} / t_{de}$, $B = D_{sc} h_e K_e / (D_e h_{sc})$ and K_{sv} is the partition coefficient between SC and the vehicle $K_{sv} = C_{sc} / C_v$.

Seko et al. [34] considered similar model with the solute metabolism in the second phase (viable epidermis). The resulting equations for the amount exiting the epidermis for drug Q_d and metabolite Q_m are:

$$\hat{Q}_d(s) = \frac{A K_{sd} C_v h_s \sqrt{s t_{sd}}}{s^2 t_{sd}\left(\cosh\sqrt{s t_{sd}} \sinh\sqrt{(s+k_m) t_{ed}} + \frac{K_{sd} h_s \sqrt{s t_{ed}}}{K_{ed} h_e \sqrt{s t_{sd}}} \sinh\sqrt{s t_{sd}} \cosh\sqrt{(s+k_m) t_{ed}} \right)} \tag{15.59}$$

$$\hat{Q}_m(s) = \frac{k_m t_{ed}}{\left(\sqrt{(s+k_m)t_{ed}} - \sqrt{st_{em}}\right)}\left(\frac{\cosh\sqrt{(s+k_m)t_{ed}}}{\cosh\sqrt{st_{em}}} - 1\right)\hat{Q}_d(s) \qquad (15.60)$$

where k_m is the rate of metabolism in the viable epidermis, $t_{ed} = h_e^2 / D_{ed}$, $t_{sd} = h_s^2/D_{sd}$; $t_{em} = h_e^2/D_{em}$ and first subscripts (s and e) denote the SC and the viable epidermis, and second subscripts (d and m) denote the drug and its metabolite, respectively. We note that boundary conditions used in this work excluded the metabolite from diffusing back into SC and the donor. This simplifies the solution for the metabolite, but could in some cases slightly overestimate its amount penetrating into the receptor.

15.2.9 Desorption

Although penetration (absorption) experiments are most common in studying percutaneous kinetics, desorption experiments have been used to study SC solute transport [35, 36]. In these desorption experiments, the membrane is initially saturated with the solute so that it is at equilibrium with the donor phase with concentration C_d. The membrane is then immersed into the receptor phase with no solute present at time $t = 0$. Assuming sink conditions in the receptor, the initial and boundary conditions for this case are:

$$C_m(x,0) = K_m C_d \qquad (15.61)$$

$$C_m(0,t) = 0 \qquad (15.62)$$

$$C_m(h_m,t) = 0 \qquad (15.63)$$

Solving Eq. (15.8) with initial and boundary conditions (61–63) in the Laplace domain yields for the amount of solute desorbed into receptor phase:

$$\hat{Q}(s) = \frac{1}{s}\frac{AK_m C_d h_m}{\sqrt{st_d}} 2\tanh\left(\frac{\sqrt{st_d}}{2}\right) \qquad (15.64)$$

Equation (64) could be inverted to time domain to yield infinite series solution:

$$Q(t) = M_\infty\left(1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{\exp\left(-\frac{t}{t_d}\pi^2(2n+1)^2\right)}{(2n+1)^2}\right) \qquad (15.65)$$

where $M\infty$ (= $C_d K_m A h_m$) is the total amount of solute absorbed by the membrane. Equation (65) was used by Roberts et al. [36] to fit experimental desorption profiles for some solutes to yield t_d and K_m. These parameters could then be used to calculate permeability coefficient:

$$k_p = \frac{K_m h_m}{t_d} \tag{15.66}$$

15.2.10 SC Heterogeneity

A homogeneous membrane model is assumed in this chapter and in most of the literature for the majority of the mathematical analysis of solute transport in SC. In this model, SC is represented as a membrane with constant diffusion and partition coefficients. However, the SC morphology is that of a heterogeneous multiphase tissue consisting of intracellular lipids and corneocytes hold together by desmosomes [37]. One way to model this is by applying finite-difference or finite-element schemes with an appropriate spatial mesh over spatially organised lipid (mortar) and corneocyte (bricks) domains. This in-silico modelling can yield important insights to the solute transport in SC and has been recently reviewed in detail by Mitragotri et al. [38]. Nevertheless, even with the constant increase in the amount of available computational power, it is not feasible yet to fit experimental data using this in-silico approach, in part due to the fact that such models have very large number of parameters. A different approach has been proposed to address heterogeneity of the SC for modelling water transport in SC [39] in which slow equilibration between different phases of the SC is added to the diffusion equation. This slow equilibration model was successful in fitting water desorption and penetration data simultaneously [39].

Another potential source of discrepancy of SC transport from a homogeneous membrane model is the presence of two spatially distinct regions in SC: the stratum dysjunctum and stratum compactum. The applicability of the homogeneity assumption to SC has therefore been questioned [40]. It has been experimentally shown that the partitioning coefficient of clobetasol propionate in SC is highly variable and decreases exponentially from outer SC layers (stratum dysjunctum) to the inner layers (stratum compactum) [41]. Solutions for variable diffusion and partition coefficients in the SC have been presented [42] and based on this model analysis. It was found that penetration flux experiments are relatively insensitive even to extreme values of diffusion and partition coefficient heterogeneity, whereas for desorption experiments, using Eq. (15.66) may lead to a misinterpretation when the SC is heterogeneous [42]. It was also found that concentration–distance profiles are the most sensitive to SC heterogeneity and ignoring partition coefficient heterogeneity in using tape-stripping data to predict penetration flux-time profiles may result in a significant miscalculation of the steady state flux and lag time values [42].

15.3 Release Profiles from Topical Products

A number of transdermal systems are now available for clinical use. Hadgraft [43] considered the solutions for release from patches for a range of boundary conditions. When a drug is contained in both the contact adhesive (priming dose) and patch, the release rate R_s approximates to [44]:

$$R_s = R_0 + H\exp(-at) \tag{15.67}$$

where R_o is the zeroth order flux from the patch assuming no depletion, and H and a are constants defining the release kinetics of the priming dose. Lordanski et al. [45] simulated factors such as matrix diffusion, partition coefficient, and polymer membrane thickness in the modelling of drug delivery kinetics from the adhesive of transdermal delivery device into skin imitating membranes.

15.3.1 Diffusion Controlled Release

Of practical interest is a homogenous phase in which the drug is released by diffusion. The expression for the drug release from slabs is well known to be that of the "burst" effect [10]:

$$Q(t) = M_0\left[1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left(-\frac{t}{4t_{dv}}\pi^2(2n+1)^2\right)\right], \tag{15.68}$$

where again $M_0 = C_{v0}V_v$ and $t_{dv} = h_v^2 / D_v$. The Laplace expression for Eq. (15.69) is:

$$\hat{Q}(s) = \frac{M_0}{s}\frac{\tanh(\sqrt{st_{dv}})}{\sqrt{st_{dv}}}. \tag{15.70}$$

At short times when the amount released is less than 30%, Eq. (15.68) can be approximated to:

$$Q(t) = 2M_0\left(\frac{t}{\pi t_{dv}}\right)^{\frac{1}{2}}. \tag{15.71}$$

15.3.2 Release of a Suspended Drug by Diffusion

Another special case is that for a vehicle or patch containing a suspended drug. In this case, the amount of solute released into a perfect sink is given by [46]:

$$Q(t) = A\sqrt{tD_v(2C_{v0} - C_s)C_s} \tag{15.72}$$

where C_{v0} in this context have the meaning of the total amount of drug (soluble and suspended) in the vehicle per unit volume, and C_s is the saturation concentration of the drug in the vehicle.

15.4 Compartmental Models as an Alternative to Diffusion Models in Percutaneous Absorption

Riegelman [47] analysed a range of *in vivo* skin absorption data using a unidirectional absorption and simple compartment-based pharmacokinetic models (Fig. 15.12a). A more complex series of models (Fig. 15.12b, c, d) were used by Wallace and Barnett [48] to describe the *in vitro* methotrexate absorption across the skin. A discussion of the various models and their approximations is given in our earliest version of this chapter [49]. More recently, a comprehensive review of compartmental models has been presented by McCarley and Bunge [50].

The key models developed [51] match (a) steady state SC concentration and penetration rate with an assumption of equilibrium at the two boundaries of the membrane (equilibrium model); (b) steady state SC concentration, penetration rate, and lag time for all blood and vehicle concentration ratios, and for large blood concentrations (general time lag model); (c) as in (b) but for low blood concentrations (simplified time lag model); and (e) the traditional model. The real contribution of this work has been a systematic derivation of equations for coefficients of compartmental models, expressed in terms of physicochemical parameters, which are documented in the paper in a readily accessible form.

It could be argued that the model representing SC as five compartments (Fig. 15.12e) [52] corresponds best to the diffusion model and its rate constants can be easily related to the diffusion coefficient and the membrane thickness [53]. This model was developed to account for potential binding of solute to SC proteins for finite vehicle volume. A more general, *n*-compartmental model has been considered in detail and compared with the diffusion model in [53]. It has been argued that the exact number of compartments can be dictated by experimental conditions (e.g how many tape-strips are required to remove SC), but should normally not exceed 20–25, the number of corneocyte layers in human SC [53].

15.5 Other Processes Affecting *In Vitro* Percutaneous Absorption

15.5.1 Concentration-Dependent Diffusive Transport Processes

It is well known that the permeability of solutes through the skin may be affected by their concentration-dependent interaction with the skin as shown for the alcohols

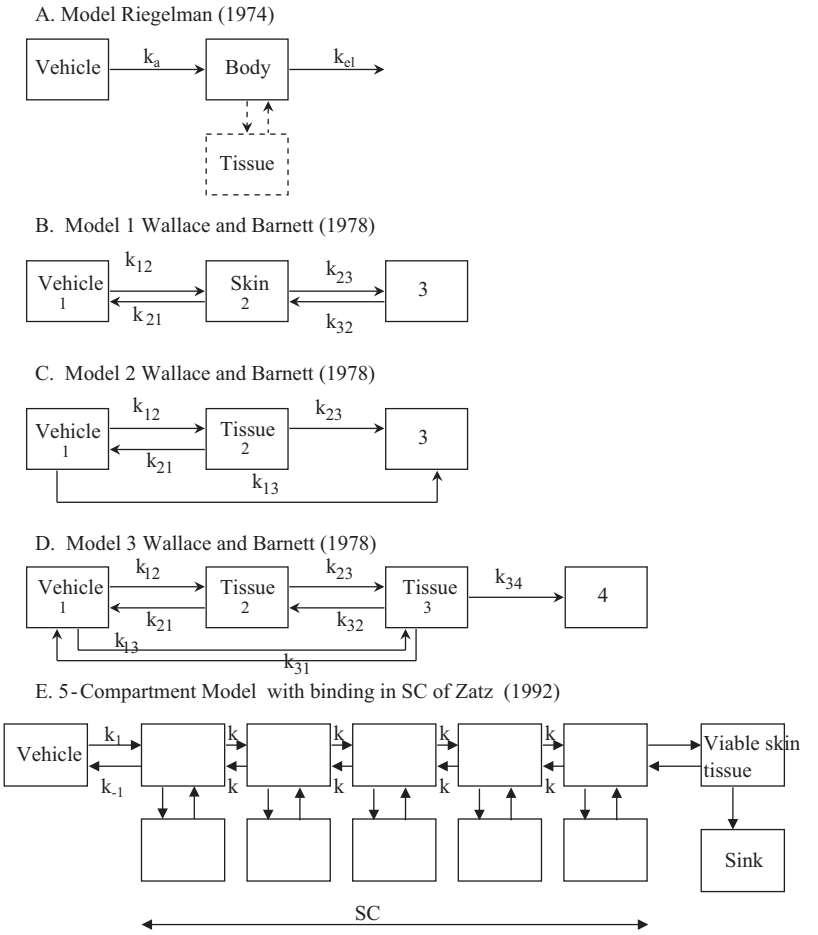


Fig. 15.12 Examples of compartmental models of skin penetration

[21] and phenols [54]. At this time, relatively little work has been published on mathematical models for diffusion in a swelling or denaturing skin environment. Wu [55] has attempted to relate water diffusivity $D(C)$ as a function of its water concentration (C) in a keratinous membrane. The expression $D(C) = D_o + AC^B$, where D_o, A, and B are constants, best described the results. Giengler et al. [56] have described the numerical solution of solute concentrations in a system consisting of a polymer film, microporous membrane, adhesive, skin layer, and capillary sink and in which the diffusion coefficients were time dependent.

Higuchi and Higuchi [57] summarised the theory associated with diffusion through heterogeneous membranes and vehicles. They suggested that transport through a two phase system was a function of the volume fraction and permeability of each phase. They also derived an expression for lag time across a membrane when simultaneous diffusion and Langmuir adsorption has occurred. The lag time across the membrane is increased by binding of solute to membrane's binding sites:

$$lag = \frac{h_m^2}{4D_m} + \frac{h_m^2 A}{2D_m K_1 V_1 C_v}, \tag{15.73}$$

where D_m is effective diffusion constant in heterogeneous membrane, A is amount of solute taken up by filler per unit volume of membrane material, K_1 is the partition coefficient of the solute in vehicle and phase 1 in the membrane, V_1 is volume fraction of phase 1, and C_v is concentration of solute in the vehicle.

When the solute concentration in the vehicle increases, the second term in Eq. (15.72) decreases and the overall lag time is shorter.

Chandrasekaran et al. [44, 58] assumed that uptake of solutes by skin was described by a dual-sorption model

$$Z\frac{dC}{dt} = D\frac{d^2C}{dx^2}, \tag{15.74}$$

where

$$Z = B\left(1 + \frac{C_i b / k_D}{\left(1 + C_D b / kD\right)^2}\right).$$

However, these authors then assumed that Z was a constant. This model reduces to the conventional diffusion model in which the effective diffusivity is the diffusion coefficient of free solute modified by the instantaneous partitioning of solute into immobile sites in the diffusion path [6]. Expressions were presented for plasma concentrations and urinary excretion rate profiles after single and multiple patch applications of scopolamine to humans. Kubota et al. [59] applied the dual sorption model to account for the nonlinear percutaneous absorption of timolol. The model accounted for the prolongation in timolol lag time associated with the decrease in applied concentration.

15.5.2 Bioconversion/Metabolism of Solutes in the Skin

Roberts and Walters [2] related the *in vitro* (metabolically inactive) skin flux $J_{s,\text{in vitro}}$ to that *in vivo* $J_{s,\text{in vivo}}$ by a first pass bioavailability F_s and a fraction released from the product into the skin F_R :

$$J_{s,\text{in vitro}} = F_s F_R J_{s,\text{in vitro}}. \tag{15.75}$$

The importance of recognising F_s is illustrated by methylsalicylate where $F_s < 0.05$ [2]. Caution must therefore be applied in extrapolating *in vitro* data into likely *in vivo* absorption.

The modelling of percutaneous absorption kinetics in the epidermis when diffusion and metabolic processes occur simultaneously leads to relatively complex solutions. Ando et al. [60] examined the diffusive transport of a solute through a

metabolically inactive SC and hence through the epidermis, where it was assumed that there was homogeneous distribution of metabolising enzymes. Subsequent work developed this model to examine the bioconversion prodrug $\rightarrow$ drug $\rightarrow$ metabolite [61, 62]. The work applied the diffusion equation and derived expressions for the steady state fluxes and cutaneous concentration–distance relationships for each of the species. Yu et al. [63] then solved this model for nonuniform enzyme distribution in the skin. Fox et al. [64] considered Michaelis–Menten kinetics in their examination of prodrug, drug and metabolite concentrations in the epidermis and dermis. More recently Seko et al. [34] used a two-layer skin diffusion/metabolism model to describe parabens cutaneous metabolism after topical application (see also Eqs. (15.59) and (15.60)). Analysis involved nonlinear regression of numerical inversion of Laplace transform solutions. Approximations to diffusion-based models were applied by Hadgraft [65], Guy and Hadgraft [66] and Kubota et al. [67] to describe the effect of linear and saturable (Michaelis–Menten) epidermal metabolism on percutaneous absorption.

Guy and Hadgraft [68] adapted their compartmental pharmacokinetic model to include a metabolic conversion of the solute with consequent removal of the metabolite. Linear kinetics was assumed to enable solution in the Laplace domain and inversion to give analytical solutions. A number of theoretical plasma concentration profiles were then constructed. In reality, Michaelis–Menten kinetics may be operative for a number of solutes.

15.5.3 Solute–Vehicle, Vehicle–Skin and Solute–Skin Interactions

The practical application of mathematical models in percutaneous absorption to therapeutics or risk assessment is dependent on an understanding of solute–skin, solute–vehicle and vehicle–skin interactions. Some aspects of each of these areas have been discussed by Roberts and Walters [2], Hadgraft and Wolff [69], Robinson [70] and Roberts et al. [6].

The present analysis has generally been limited to percutaneous absorption kinetics in which the underlying physicochemical parameters are time-independent. In practice, the application of a vehicle to the skin will lead to a time-dependent change in permeability due to either a solute–skin or a vehicle–skin interaction. The solutions of the resultant concentration-dependent diffusion processes also lead to a time- and space-dependent change in solute diffusivity, and are relatively complex and felt to be beyond the scope of this overview.

Of critical importance in both therapeutics and toxicology is the maximum flux of a solute $J_{\max}$. This flux is normally attained at the solubility of the solute in the given vehicle S_v, consistent with the solubility of the solute in the SC transport pathway S_m

$$J_{\max} = \frac{D_m K_m S_v}{h_m} = k_p S_v = \frac{D_m S_m}{h_m}. \tag{15.76}$$

The importance of J_{max} as a parameter describing penetration through the skin is in its invariance for a given solute transport from different vehicles, unlike k_p, which is vehicle-dependant. This invariance holds unless the vehicle affects either D_m or S_m [6]. J_{max} (in mol/cm^2 h) may be expressed in terms of their molecular weight (MW), melting point (Mpt) and hydrogen bonding acceptor ability (Ha) [4]:

$$\log J_{max} = -4.350 - 0.0154\mathrm{MW} - 0.293\mathrm{Mp}^* + 0.371\mathrm{Ha}, \quad n = 87, \quad r^2 = 0.937 \tag{15.77}$$

where J_{max} is in mol/cm^2 h and the dependence on Mpt being described using the Mpt term: Mp*= $\Delta S_f (Mpt - T)u(Mpt - T)/T$, from Yalkowsky's solubility equation [71], where T is the temperature and $\triangle S_f$ is the entropy of fusion of a solute, $u(x)$ is the unit step function (i.e. $u(x)$=1 for x > 0 and $u(x)$=0 for x < 0)). Most of the regression variance for J_{max} is defined by MW, showing the dominance of size as a determinant of maximum flux [4]:

$$\log J_{max} = -3.90 - 0.0190\mathrm{MW}, \qquad n = 87, \qquad r^2 = 0.847 \tag{15.78}$$

15.5.4 *Effect of Surface Loss Through Processes Such as Evaporation and Adsorption to Skin Surface*

There is a potential change in solute concentration as a consequence of surface loss during percutaneous absorption. The loss may result in (a) an effective reduction in the volume of the vehicle alone due to evaporation and an increase in solute concentration as a consequence, (b) a reduction in both solute and vehicle due to a removal process and (c) a loss of solute only due to volatilisation or adsorption to skin surface. For instance, Reifenrath and Robinson [72] have shown that mosquito repellents may be lost due to evaporation at a rate comparable to their percutaneous absorption. Guy and Hadgraft [73, 74] examined the first and zero order loss of solute from the vehicle surface using diffusion and compartment models, respectively. More recently Saiyasombati and Kasting [75] examined disposition of benzyl alcohol after topical application to human skin *in vitro* and showed that evaporation plays a significant role. They found that two-compartment models were adequate to describe first order loss of benzyl alcohol from the vehicle surface.

Kasting and Miller [76] considered a case of solvent deposited solutes with simultaneous evaporation of the solute from the surface of the SC. Infinite series solutions for diffusion model for various limiting cases were presented and later applied to the experimental data for penetration of benzyl alcohol (BA) through split-thickness cadaver skin [77]. Similar modelling for volatile solutes was considered for finite volume vehicle and resulted in the amount absorbed expressed in Laplace domain as [53]:

$$\hat{Q}(s) = \frac{k_p A C_{v0} V_{vN} t_d}{s\left(\cosh\sqrt{st_d} + \dfrac{V_{vN} st_d + \kappa_{ev}}{\sqrt{st_d}}\sinh\sqrt{st_d}\right)} \tag{15.79}$$

where κ_{ev} is the dimensionless parameter describing the ratio of the rate of evaporation (k_{ev}, volume time^{-1}) to the rate of absorption $\kappa_{ev} = k_{ev} / (Ak_p)$.

15.5.5 Shunt Transport

The importance of shunt transport by appendages has been well recognised. Scheuplein [78] and Wallace and Barnett [48] assumed a parallel pathway with a minimal lag time relative to transepidermal transport for diffusion and compartmental models, respectively. In our attempted modelling of epidermal and shunt diffusion, we assumed that the overall amount penetrating was the sum of the amounts penetrating through independent epidermal and shunt pathways [17]. The amount penetrating through each pathway was assumed to be defined by Eq. (15.12) in which K_m and D_m were defined in terms of the corresponding constants for the two pathways.

More recently, the presence of polar and nonpolar pathways through the intercellular region of the SC has been recognised as described in Eq. (15.5). Mathematical models described include those for steady state conditions [79, 80], and an infinite dosing condition [81]. Yamashita et al. [82] have presented the Laplace solution for a well-stirred finite donor phase in contact with SC in which solutes can diffuse through both polar and nonpolar routes. The solute can then diffuse through the epidermis into a sink. Numerical inversion of the Laplace transform with FILT was then undertaken to generate real time profiles. Edwards and Langer [83] have derived expressions for a range of conditions and suggested their theory confirmed the importance of shunt and intercellular transport for small ions and uncharged solutes, respectively.

15.5.6 Reservoir Effect

It is well recognised that significant amounts of solute can accumulate in the SC and be released into lower tissues on rapid skin hydration, the so called “reservoir” effect [84, 85]. Modelling of this process including the effects of desquamation has been undertaken using both diffusion [86] and compartmental models [87]. In the latter model, the kinetics of reservoir depletion was shown to be dependent on solute diffusivity in the SC solute clearance from the underlying tissue and the rate of epidermal turnover.

15.6 Simple *In Vivo* Models in Percutaneous Absorption

15.6.1 Compartmental Pharmacokinetic Models

One of the first evaluations of the pharmacokinetics of skin penetration was reported by Riegelman [47]. Absorption of solutes through the skin was generally

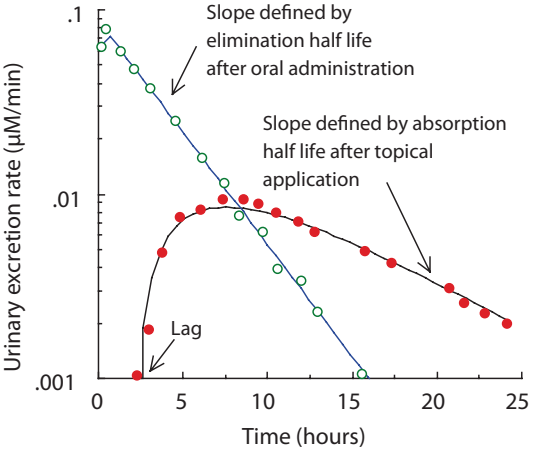


Fig. 15.13 Simultaneous nonlinear regression of urinary excretion rate-time data for norephedrine hydrochloride administered orally (○) and free base applied topically (●) using a weighting of $1/y_{obs}$ and a common elimination half life for norephedrine. The regression yielded an absorption half-life of 0.09 h for oral administration. A lag of 2.2 h and an absorption half-life of 6.0 h for topical application and an elimination half-life of 2.5 h ($r^2 = 0.999$). Data from Beckett et al [145].

assumed to follow first order kinetics with a rate constant k_a (units: time^{-1}). Much of the data analysed appeared to be characterised by "flip-flop" kinetics where the absorption halftime is much longer than the elimination half-time, as illustrated later in Fig. 15.13. This modelling approach has been used by a number of authors including the work of Rohatagi et al. [88]. This work described an integrated pharmacokinetic-metabolic model for selegiline after application of a transdermal system for 24 h. A series of differential equations and nonlinear regressions were then used to solve drug and metabolite concentrations.

Roberts and Walters [2] have suggested that four processes are commonly used to describe plasma concentrations C_{plasma} with time after topical application when the body is assumed to be a single compartment, with an elimination rate constant k_{el} and apparent distribution volume V_B.

1. Depletion of the applied dose at a first order rate constant k_a

$$C_{\text{plasma}} = k_a F \text{ Dose} \left(\exp\left[-k_a(t-\text{lag})\right] - \exp[-k_a(t-\text{lag})]\right), \quad t > \text{lag}, \qquad (15.80)$$

where lag is the lag time for absorption through the skin and F is the fraction that would be absorbed if the product were applied for an infinite time.

Figure 15.13 shows the nonlinear regression of norephedrine urinary excretion rate after topical application using Eq. (15.79). Also shown in Fig. 15.13 is the profile for an equimolar dose given orally. It is apparent that the topical application is associated with a lag of 2 h and an absorption half-life (the terminal phase) of 8 h $(0.693/k_a)$. A common elimination half-life $(0.693/k_{el})$ of 3.3 h is found for both routes of administration.

Cross et al. [89, 90] related the cumulative amount-eluted versus time profiles $(Q(t)-t)$, after dermal absorption of solutes into a perfused limb preparation, to Eq. (15.80)

$$Q(t) = M_0\left[1+\frac{k_a}{k_{el}-k_a}\exp\left(-k_{el}t\right)-\frac{k_{el}}{k_{el}-k_a}\exp\left(-k_a t\right)\right], \qquad (15.81)$$

where M_o is the initial amount applied to the dermis, k_a is the absorption rate constant for solute absorption from the dermis, defined by fraction remaining in dermis with time $(F_{\text{dermis}} = \exp(-k_a t))$, and k_{el} is the elimination rate constant from the preparation. It can be shown, that after some rearrangement, Eq. (15.16) in Williams et al. [91] for the perfused porcine skin flap model simplifies to Eq. (15.80).

Reddy et al. [92] have described a one-compartment skin pharmacokinetic model to describe *in vivo* absorption. The model is identical to Model III in Wallace and Barnett [48] when the shunt transport in the latter is assumed to be negligible. They found that the general time lag model derived by McCarley and Bunge [51], and discussed earlier in Section 15.3, predicted best diffusion in a membrane for most situations.

Each of the above models may lead to erroneous estimates for the absorption rate constant if significant tissue distribution occurs in the body (as represented by the dotted lines in Fig. 15.12a). We have recently reported that estimates for k_a based on a single exponential (one compartmental) disposition are often twice those for the more correct biexponential (two compartmental) disposition model [93]. Surprisingly, to date, most analyses of *in vivo* percutaneous absorption kinetics have assumed mono exponential disposition kinetics and have not considered this potential error.

2. Delivery at a constant rate J_s, for a time period T

$$C_b = \begin{cases} \dfrac{J_{s,\text{in vitro}}F}{Cl_{\text{body}}}\left(1-\exp\left[-k_{el}(t-\text{lag})\right]\right), & t < \text{lag}+T \\ \dfrac{J_{s,\text{in vitro}}F}{Cl_{\text{body}}}\left(\left[1-\exp -k_{el}(t-\text{lag})\right]\right)\exp\left[-k_{el}(t-T-\text{lag})\right], & t \geq lag+T \end{cases} \qquad (15.82)$$

Singh et al. [94] used Eq. (15.81) to describe the *in vivo* absorption kinetics of a number of solutes iontophoresed transdermally *in vivo* at a presumed constant flux for a time T. Good fits were obtained in the nonlinear regression of each of the data sets.

Imanidis et al. [95] used a similar approach to describe a constant total flux of acyclovir from a patch $J_T\left(=C_s/\left[1/k_{pm}+1/k_{pp}\right]\right)$ into the blood stream when acyclovir disposition is described by a biexponential elimination after iv administration $(C_b = A\exp(-\alpha t)+B\exp(-\beta t))$. The resulting blood concentration (C_b)-time (t) profile is:

$$C_b = \frac{J_T}{V_c k_{el}}\left[1 + \frac{\beta - k_{el}}{\alpha - \beta}\exp(-\alpha t) + \frac{k_{el} - \alpha}{\alpha - \beta}\exp(-\beta t)\right] \tag{15.83}$$

where C_s is the acyclovir concentration in the patch, k_{pm} is the permeability coefficient of acyclovir in the skin, k_{pp} is the permeability coefficient of acyclovir in the rate controlling patch of the membrane, V_c is the apparent volume of distribution of the central compartment and A, B, α and β are constants describing the disposition process. Tegeder et al. [96] described muscle microdialysate after topical application by a first order absorption process after a lag time (t_{lag}) with a two-compartment disposition model and a fraction of drug unbound to tissue (fu):

$$C_{t,\text{free}}(t) = \frac{fu\,k_{21}\text{Dose}\,k_a}{V_c(\alpha - \beta)}\left[\frac{1}{\beta - k_a}\left(e^{-k_a(t-t_{\text{lag}})} - e^{-\beta(t-t_{\text{lag}})}\right) - \frac{1}{\alpha - k_a}\left(e^{-k_a(t-t_{\text{lag}})} - e^{-\alpha(t-t_{\text{lag}})}\right)\right] \tag{1584}$$

Hadgraft and Wolf [69] have examined the prediction of *in vivo* plasma data after topical application. Their modelling appeared to adequately describe the *in vivo* percutaneous absorption kinetics for a range of drugs.

3. Steady state conditions

$$C_{bss} = \frac{FJ_{s,\text{in vitro}}}{Cl_{\text{body}}} = \frac{Fk_pC_vA}{Cl_{\text{body}}} \tag{15.85}$$

This equation is a reduced form of Eq. (15.81) for $t \to \infty$ and $t < \text{lag} + T$. Eq. (15.84) has been used by Roberts and Walters [2] to define desired patch release rate for a number of drugs *in vivo* from a knowledge of the drug's clearance and desired plasma concentration.

4. A time-dependent transdermal flux best analysed assuming a model deduced from *in vivo* absorption kinetics (Section 15.1.7) or deconvolution analysis (Section 15.5.4).

15.6.2 Diffusion Pharmacokinetic Models

In vivo absorption models usually represent the body as one or more compartments with input into the body via percutaneous absorption. Cooper [97] derived an expression for the total amount of solute excreted into the urine after topical absorption. Other models of Guy, Hadgraft, Kubota, and Chandresekaran (described earlier) have adopted a similar approach in describing either plasma concentrations or urinary excretion rates from one- or two-compartment models. Cooper assumed diffusion through the skin according to Eq. (15.22) into the body, represented as a single compartment. When his model is modified to include a SC-vehicle parti-

tion coefficient K_m, the plasma concentration, $C_p(t)$, and the amount excreted into urine, $M(t)$, are defined by the equations:

$$C_p(t) = \frac{Ak_pC}{V_c}\left(\frac{1-\exp(-k_{el}t)}{k_a} + 2\sum_{n=1}^{\infty}\frac{(-1)^n}{k_{el}-n^2\pi^2/t_d}\left[\exp\left(-\frac{t}{t_d}n^2\pi^2\right)-\exp(-k_{el}t)\right]\right) \tag{1586}$$

$$M(t) = Ak_pC_vk_u\left(\frac{k_{el}t-1+\exp(-k_at)}{k_{el}^2} + 2\sum_{n=1}^{\infty}\frac{(-1)^n}{k_{el}-n^2\pi^2/t_d}\left[\frac{t_d}{n^2\pi^2}\left(1-\exp\left(-\frac{t}{t_d}n^2\pi^2\right)\right)-\frac{1}{k_{el}}\left(1-\exp(-k_{el}t)\right)\right]\right) \tag{15.87}$$

where k_{el} is the total effective elimination rate constant, k_u is the rate constant for excretion in the urine and V_c is the total effective volume of the compartment.

The steady state portion of the $M(t)$ versus t plot from Eq. (15.86) yields a slope of $k_uk_pA/k_{el} = f_ek_pA$ where k_p is the permeability coefficient $= K_mD_m/h_m$, A is the area of application, k_{el} is the elimination rate constant of the solute from the body and f_e is the fraction of the solute excreted in the urine. This plot is associated with a lag time t_L of:

$$t_L = \frac{1}{k_{el}} + \frac{t_d}{6}, \tag{15.88}$$

where again $t_d = h_m^2/D$, D is the diffusivity of the solute in a SC and h_m is the distance of the pathway of diffusion. Even for multicompartmental disposition kinetics, the total lag time for elimination of a solute is uncoupled and is the sum of epidermal diffusion and pharmacokinetic lag times [98]. When only a finite dose of solute is applied to the skin, the urinary excretion rate is also a function of the vehicle thickness [25].

In practice, the direct application of Eq. (15.22) to *in vivo* absorption may be limited. Equation (15.36), which takes into account the effectiveness of blood flow in removal of solute from the epidermis and the accumulation of solute in the epidermis *in vivo*, may be more appropriate. Accordingly, the actual steady state flux is less than k_pAC_v due to this limitation in blood flow clearance as defined by Eq. (15.38).

15.6.3 Physiologically-Based Pharmacokinetic and Pharmacodynamic (PBPK/PD) Models

A number of authors have advocated the use of physiological rather than compartmental representations of the body. McDougal [99] has summarised the modelling

in this area. These models utilise the numerical integration of a series of differential equations representing each organ, to solve for blood concentration-time profiles after topical application. Individual organs or types of tissues are represented as the compartments with blood flow into and out of the organs defining the transport in the body system. Input into the skin, as a perfused organ, is assumed to follow Fick's first law and may allow for evaporation. Jepson and McDougal [100] have used this model to estimate the permeability constants for halogenated methanes from an aqueous solution after topical application in a whole animal study.Timchalk et al. [101] described an integrated PBPK/PD model for the organophosphate insecticide chlorpyrifos using the McDougal model. The percutaneous absorption of perchlorethylene from a soil matrix has been described using modification of this model in which solute could evaporate from soil, reversibly partition into SC and subsequently reversibly partition into dermis [102]. Poet et al. [103, 104] and Thrall et al. [105] used exhaled breath data with the McDougal model to assess the percutaneous absorption of methyl chloroform, trichloroethylene and toluene. The dermal absorption, evaporation, distribution, metabolism, and excretion of a range of potential toxic solutes has been described using a multi-compartment "dermatatoxicokinetic" model based on skin surface, SC dosing device, plasma, tissue and urine pharmacokinetics after topical and intravenous administration [106]. This modelling has been used to suggest that urinary p-nitrophenol may be used as a marker for organophosphate insecticide exposure. The perfused skin flap enables a simpler model description as illustrated by its application in the study of jet fuel topical absorption [107].

15.6.4 Deconvolution Analysis in Pharmacokinetic Modelling

Deconvolution analysis is based on the principle that the observed plasma or blood concentrations-time profiles, $C_b(t)$, are defined by the percutaneous absorption flux, $J_s(t)$, and the disposition kinetics in the body after a unit intravenous bolus (impulse) injection, $C_{iv}(t)$:

$$\hat{C}_b(s) = \hat{J}_s(s)\hat{C}_{iv}(s). \tag{15.89}$$

Hence from the observed $C_b(t)$ and $C_{iv}(t)$ and inversion of the resulting Laplace domain expression for $\hat{J}_s(s)$ enables $J_s(t)$ to be defined. This technique is especially useful when the mathematical model for the percutaneous absorption process is not known. A comparison of the observed profile with theoretical profiles may define the underlying model for percutaneous absorption kinetics.

Examples of deconvolution analysis applied in this area include the evaluation of the absorption function from nicotine patches [108], the modelling of subcutaneous absorption kinetics [93] and modelling of a topically applied local anaesthetic agent [109].

15.6.5 Penetration into Tissues Underlying Topical Application Site

Recent reviews of models used for dermal transport are given in [110, 111]. An important model is the distributed elimination model which adds the elimination term to the diffusion equation:

$$\frac{\partial C_d}{\partial t} = D_d \frac{\partial^2 C_d}{\partial x^2} - k_e C_d \tag{15.90}$$

where C_d is concentration in the dermis, D_d is the molecular diffusion coefficient in the dermis and k_e is the elimination rate from the dermis due to blood clearance. Similar models have been used in a number of publications [112–114]. Anissimov and Roberts [115] recently analysed human biopsy data of Schaefer's group [116–120] with the distributed elimination model and established that for many solutes molecular diffusion alone cannot explain the data. Dancik et al. [121] used the distributed elimination model to analyse published human microdialysis data and also concluded that D_d in Eq. (15.89) must include the contribution to the transport processes from blood and/or lymphatics transport.

In a simpler modelling, epidermal concentrations *in vivo* after topical application, assuming D_e is sufficiently large to approximate well-stirred (i.e. compartmental representation), is defined by Eq. (15.40) and at long times ($t \to \infty$) by Eq. (15.33) via

$$C_{ss} = \frac{k_p A C_v}{Cl_r + k_p A / K_r} \tag{15.91}$$

where Cl_r is the *in vivo* epidermal clearance.

A similar expression can be defined for subsequent deeper tissues using a compartment in-series model in parallel with removal to the systemic circulation and recirculation to define deeper tissue concentrations after topical application [122]. Transport into deeper tissues could occur by either "convective" blood flow [123] or by diffusion. Nonlinear regressions of experimentally treated and contralateral tissue data with the model used simultaneous numerical integration of a series of differential equations [122, 124, 125]. The analysis showed that, whereas direct deep tissue penetration was apparent at early times, recirculation of drug from the systemic circulation accounted for tissue levels at longer times to define deep tissue penetration of dermally applied solutes. Roberts and Cross [126] have suggested that the half-life for elimination of a solute in such tissues is dependent not only on tissue blood flow (Q_p), but also on the fraction unbound of solute in the tissue (fu_T) and blood (fu_B) as well as the apparent unbound volume of distribution (V_T):

$$t_{1/2} \approx \frac{0.693 fu_B V_T}{fu_T Q_p}. \tag{15.92}$$

A further set of studies has been reported using stripped skin and an integrated application site—contralateral site model [127, 128]. This work extended earlier work [125, 126, 129–131] which showed significant direct penetration to deeper tissues underlining the topical application site in both rats and in humans. As discussed earlier, Tegeder et al. [96] have described muscle microdialysate pharmacokinetics (Eq. (15.83)) after topical application.

15.6.6 Pharmacodynamic Modelling

In principle, established pharmacodynamic models used in whole body pharmacokinetic modelling can be directly used when solutes are delivered by skin. Complexities can exist when the site of drug targeting is the skin itself. Imanidis et al [95] showed that the antiviral efficacy to HSV-1 skin infections of acyclovir was directly related to the logarithm of the flux from transdermal patches—consistent with classical log dose-response relationships. However, an equivalent systemic dose was relatively ineffective.

Beastall et al. [132] examined the onset of erythema (t_E) as a function of solute concentration (C_o). Applying Fick's law of diffusion they obtained the expression:

$$\log\frac{n_E}{h_m} = \log(C_v t_E^{3/2}) + \log\left(\frac{K_m}{1+K_m/p^{1/2}}\right) + \log\left(\frac{8D_{sc}^{3/2}}{\pi^{1/2}h_m^3}\right) - \frac{h_m^2}{9.2D_{sc}t_E}, \quad (15.93)$$

where D_{sc} is the diffusion coefficient of nicotinate in the SC, K_m is its partition coefficient between vehicle and skin, h_m is diffusion path length, p is the ratio of the diffusion coefficients of the nicotinate in the vehicle and the skin and n_E is the concentration of nicotinate required to trigger erythema. This expression showed a linear relationship should and did exist between $\log(C_v t_E^{2/3})$ and $1/t_E$. The gradient of the relationship D_{sc}/h_m^2 was greatly affected by the co-administration of the enhancer urea.

The human skin blanching assay for evaluating the bioequivalence of topical corticosteroid products should follow standardised guidelines as developed by the US Food and Drug Administration (FDA) in 1995. Demana et al. [133] evaluated the area under the effect curve (AUEC), also called the effect (E), for both visual and chromameter derived data. The visual data was best described by a sigmoidal $E_{\max}$ model (Eq. (15.93)) whilst the chromameter data was described by a simple $E_{\max}$ model (Eq. (15.94)):

$$E = E_0 \pm \frac{E_{\max} D}{D + ED_{50}}, \quad (15.94)$$

$$E = E_0 \pm \frac{E_{\max} D^{\gamma}}{D^{\gamma} + ED_{50}^{\gamma}} \quad (15.95)$$

where E_{max} is the maximal AUEC, D is the dose duration, ED_{50} is the dose duration for half maximal E and γ is a sigmoidicity factor related to the shape of the curve. The parameter E_o, not explicitly stated in the modelling by Demana et al. [133], should be included in the model fitting to correct for baseline readings [134]. Smith et al. [135] have pointed out that they had corrected for E_o using unmedicated site values in their earlier work [133]. A key aspect in this mathematical modelling is varying the dose administered by varying the duration of application. Varying dose duration is then used to relate the vasoconstrictor response to a range of corticosteroid amounts. Demana et al. [133] used a weighting of $1/AUEC$ and a number of goodness of fit criteria in their analyses.

More recently, Cordero et al. [136] developed an index to predict topical efficiency of a series of nonsteroidal anti-inflammatory drugs. This index took into account both, the biopharmaceutic aspect, based on the maximal flux, and the pharmacodynamic aspect, based on the ability to inhibit cyclooxygenase-2 *in vitro*.

15.7 Modelling-Facilitated Transdermal Delivery

15.7.1 Iontophoresis

There are a number of mathematical models used in iontophoresis. As described by Kasting [137], these are generally defined by the Nernst Planck and Poisson equations. Of particular practical usefulness is the iontophoretic flux of a solute through the epidermis. This flux can be incorporated into various pharmacokinetic models for the body to enable the description of plasma concentration and urinary excretion-time data. Singh et al. [94] examined *in vivo* plasma data after iontophoretic transport with simple pharmacokinetic models. *In vivo* blood concentrations for most solutes delivered by iontophoresis appear to be able to be described by zero order input into a one compartment model (Eqs. (15.81) and (15.84)) [94].

The iontophoretic flux depends on a number of factors, including: solute ionisation, interaction of solutes with pore walls, solute size, solute shape, solute charge, Debye layer thickness, solute concentration and presence of extraneous ions is accounted for. We have proposed an integrating expression for the flux of the *j*th solute [138]:

$$J_{j,iont} = C_j \left[\frac{2\mu_j fi_j Fz_j I_T \Omega PRT_j}{\left(k_{s,a} + k_{s,c}\right)\left[1 + fu_i \theta_{ju} + fi_j \theta_{ji}\right]} \pm \left(1 - \sigma_j\right) v_m \right], \qquad (15.96)$$

where C_j is concentration of the *j*th solute, μ_j is its mobility, fi_j and fu_j are ionised and unionised fractions of the solute, z_j is its charge, PRT_j is partial restriction term, σ_j is the reflection coefficient term, v_m is the velocity of water flow across the membrane due to electroosmosis, I_T is the total current across the mem-

brane, $|$ is the permselectivity for cations, $k_{s,a}$ and $k_{s,c}$ are conductivities of the anode and cathode solutions, θ_{ju} and θ_{ji} are parameters describing interaction of unionised and ionised fractions of the solute with the pore, and F is Faraday's constant.

Dermal and subcutaneous concentrations of solutes after *in vivo* iontophoretic application can also be determined in terms of clearance by blood supply to the tissue, clearance to deeper tissues and influx by iontophoresis [139].

15.7.2 Sonophoresis

The sonophoretic iontophoretic flux can also be included in pharmacokinetic models in a manner analogous to that described under Iontophoresis. Mitragotri et al. [140] have suggested that sonophoresis induces cavitation. They have suggested that the sonophoretic permeability $k_{p\,sono}$ can be defined in terms of the passive permeability coefficient k_p (units: cm/hr) and solute octanol-water partition coefficient (K_{ow}) as:

$$k_{p\,sono} = k_p + 2.5 \cdot 10^{-5} K_{ow}^{3/4} \tag{15.97}$$

Later work examined the threshold frequency dependency [141] and transport at low frequency [142].

15.8 Practical Issues in Applying Mathematical Models to Percutaneous Absorption Data

A major limitation in a number of reported percutaneous absorption studies, including those from our laboratories, has been the assumption of a given mathematical model. Whether that model is strictly the most appropriate one is often difficult to confirm. Most studies appear to have used the simplest model, as defined by Eq. (15.1), in which the steady state flux and lag time is defined by the steady state portion of the curve. There are a number of limitations in using such a model as discussed by Robinson [70] and other authors.

Robinson [70] pointed out that errors can be made if (a) the burst influx and lag containing through flux are represented by a steady state approximation at early times, (b) an infinite vehicle is assumed when the concentration is actually declining due to the finite volume used, (c) penetration of a solute by passive diffusion also involves modification of the skin barrier properties (solute-skin interactions), (d) vehicle effects on solute concentration, e.g. evaporation or skin permeability (vehicle-skin interactions) exist, (e) skin reservoir effects exist, as illustrated by the extensive uptake of sunscreens into, but not necessarily through, the skin [143], (f) discrepancies exist between *in vitro* and *in vivo* absorption due to the role of capillaries in absorption *in vivo* and (g) the resistance barrier of the skin is compromised.

The expressions for a number of the more complex models contain the necessary correction factors to overcome some of the inherent limitations in the simplest model (Section 15.1.1) representation of data. For instance, the steady state flux may be affected by the sampling rate from the receptor compartment as defined by Eq. (15.38). The lag time will be dependent on both this clearance and the volume ratios of the membrane and receptor phases, corrected for partitioning effects, as defined by Eq. (15.39). A different set of correction factors apply if an interfacial barrier or desorption rate constant exists (Eqs. (15.43) and (15.44)). As Kubota et al. [144] point out, although a simple compartmental model may describe percutaneous penetration kinetics, the parameters obtained may not necessarily represent the membrane diffusion and partition coefficient.

Relating data to a specific model using nonlinear regression techniques also requires an appropriate weighting of the data in accordance with the underlying errors associated with the data. In the absence of known error structures, a weighting of $1/y_{obs}$ may be appropriate. This weighting assumes that the coefficient of variation (standard deviation/mean) of the data is relatively constant.

Some of the dilemmas in the mathematical modelling of percutaneous absorption are enunciated in the letters to Pharmaceutical Research written by Singh et al. [134] and Smith et al. [135], especially in relation to pharmacodynamic modelling of skin blanching after topical application. Issues raised include (a) reliability of visual and chromameter methods (b) analysis of 'naive' pool data by nonlinear regression versus mixed effect modelling, (c) baseline correction, (d) consistency of parameter values, e.g. sigmoidicity with independent literature estimates, (e) precision of critical small and long dose duration data and (f) subject (skin) selection. Smith et al [135] suggested that the current methodology prepared by the FDA requires further evaluation.

Finally, there is probably a greater need for deconvolution techniques to be used with *in vivo* data. Such techniques do not make any assumption as to the underlying mathematical model of the absorption kinetics. Indeed, such an approach is a powerful way of determining whether assumed models are indeed applicable [93].

15.9 Conclusion

This chapter has attempted to overview some of the more important mathematical models used in percutaneous absorption. Given the substantive number of reported models and the complexity in many of the models, the overview is limited in its ability to give each of the models the credit they may deserve. However, it is hoped that the emphasis on the more practical models has enabled this fairly complex area to be presented in a manner useful for ready reference.

Our analysis has considered a number of boundary conditions associated with solute transport across a membrane, including clearance from the receptor solution, clearance from the membrane and diffusion in an underlying layer (eg epidermis below SC). Each situation is defined by a steady-state flux J_{ss} and lag time of the forms:

$$J_{ss} = \frac{k_p C_v}{1+M}, \tag{15.96}$$

$$\text{lag} = \frac{t_d}{6} N \tag{15.97}$$

where M and N are functions of the transport processes below the membrane. When the clearance of the solute is very high ($Cl >> k_p$), J_{ss} approaches the usual $k_p C_v$. Approximations for the lag time are less well-defined so that the use of lag time as an estimate for t_d is much less justified. Consequently, there are dangers of parameter misspecification with obvious consequences when extensions such as structure–transport relationships are based on the uncorrected parameters. Ultimately, therefore, mathematical modelling in this area is a balance between simplicity and an accurate representation of the underlying processes.

Acknowledgments We thank the NH&MRC of Australia PAH Research Foundation and the NSW and Qld Lions Medical Research Foundation for the support of this work.

References

1. Roberts MS, Anissimov YG. Mathematical models in percutaneous absorption. In: Bronaugh RL, Maibach HI, Editors. Percutaneous absorption drugs—cosmetics—mechanisms—methodology. New York: Marcel Dekker; 2005. pp. 1–44.
2. Roberts MS, Walters KA. The relationship between structure and barrier function of skin. In: Roberts MS, Walters KA, editors. Dermal absorption and toxicity assessment. New-York: Marcel Dekker; 1998. pp. 1–42.
3. Scheuplein RJ. A personal view of skin permeation. Skin Pharmacol Physiol. 2013;26:199–212.
4. Magnusson BM, et al. Molecular size as the main determinant of solute maximum flux across the skin. J Invest Dermatol. 2004;122(4):993–9.
5. Sloan KB, Wasdo SC, Rautio J. Design for optimized topical delivery: prodrugs and a paradigm change. Pharm Res. 2006;23(12):2729–47.
6. Roberts MS, Cross SE, Pellett MA. Skin transport. In: Walters KA, Editor. Dermatological and transdermal formulations. New York: Marcel Dekker; 2002. pp. 89–195.
7. Kuswahyuning R, Roberts MS. Concentration dependency in nicotine skin penetration flux from aqueous solutions reflects vehicle induced changes in nicotine stratum corneum retention. Pharm Res. 2014;31:1501–11.
8. Bunge AL, Persichetti JM, Payan JP. Explaining skin permeation of 2-butoxyethanol from neat and aqueous solutions. Int J Pharm. 2012;435(1):50–62.
9. Frasch HF, et al. Dermal permeation of 2-hydroxypropyl acrylate, a model water-miscible compound: effects of concentration, thermodynamic activity and skin hydration. Int J Pharm. 2014;460(1/2):240–7.
10. Crank J. The mathematics of diffusion. 2 ed. Oxford: Clarendon Press; 1975.
11. Yano Y, et al. Two-compartment dispersion model for analysis of organ perfusion system of drugs by fast inverse Laplace transform (FILT). J Pharmacokinet Biopharm. 1989;17(2):179–202.
12. Yano Y, Yamaoka K, Tanaka H. A nonlinear least squares program, MULTI(FILT), based on fast inverse Laplace transform for microcomputers. Chem Pharm Bull (Tokyo). 1989;37(4):1035–8.

13. Purves RD. Accuracy of numerical inversion of Laplace transforms for pharmacokinetic parameter estimation. J Pharm Sci. 1995;84(1):71–4.
14. Anissimov YG, Watkinson A. Modelling skin penetration using the Laplace transform technique. Skin Pharmacol Physiol. 2013;26(4/6):286–94.
15. Pirot F, et al. Characterization of the permeability barrier of human skin in vivo. Proc Natl Acad Sci. 1997;94(4):1562–7.
16. Frasch HF, Barbero AM. The transient dermal exposure: theory and experimental examples using skin and silicone membranes. J Pharm Sci. 2008;97(4):1578–92.
17. Siddiqui O, Roberts MS, Polack AE. Percutaneous absorption of steroids: relative contributions of epidermal penetration and dermal clearance. J Pharmacokinet Biopharm. 1989;17(4):405–24.
18. Roberts MS. Structure-permeability considerations in percutaneous absorption. In: Scott RC, et al., editors. Prediction of percutaneous penetration. London: IBC Technical Services; 1991. pp. 210–28.
19. Anissimov YG, Roberts MS. Diffusion modeling of percutaneous absorption kinetics: 1. Effects of flow rate, receptor sampling rate and viable epidermal resistance for a constant donor concentration. J Pharm Sci. 1999;88(11):1201–9.
20. Parry GE, et al. Percutaneous-absorption of benzoic-acid across human skin .1. invitro experiments and mathematical-modeling. Pharm Res. 1990;7(3):230–6.
21. Scheuplein RJ, Blank IH. Mechanism of percutaneous absorption IV. Penetration of non electrolytes (alcohols) from aqueous solutions and from pure liquids. J Invest Dermatol. 1973;60:286–96.
22. Roberts MS, et al. The percutaneous absorption of phenolic compounds: the mechanism of diffusion across the stratum corneum. J Pharm Pharmacol. 1978;30(8):486–90.
23. Guy RH, Hadgraft J. Physicochemical interpretation of the pharmacokinetics of percutaneous-absorption. J Pharmacokinetics Biopharmaceutics. 1983;11(2): pp. 189–203.
24. Kubota K, Ishizaki T. A theoretical consideration of percutaneous drug absorption. J Pharmacokinet Biopharm. 1985;13(1):55–72.
25. Cooper ER, Berner B. Finite dose pharmacokinetics of skin penetration. J Pharm Sci. Oct 1985.74:1100–2.
26. Anissimov YG, Roberts MS. Diffusion modeling of percutaneous absorption kinetics: 2. Finite vehicle volume and solvent deposited solids. J Pharm Sci. 2001;90(4):504–20.
27. Kasting GB. Kinetics of finite dose absorption through skin 1. Vanillylnonanamide. J Pharm Sci. 2001;90(2):202–12.
28. Scheuplein RJ, Ross LW. Mechanism of percutaneous absorption. V. Percutaneous absorption of solvent deposited solids. J Invest Dermatol. 1974;62(4):353–60.
29. Kakemi K, et al. Model studies on percutaneous absorption and transport in the ointment. I. Theoretical aspects. Chem Pharm Bull Tokyo. 1975;23(9):2109–13.
30. Guy RH, Hadgraft J. A theoretical description relating skin penetration to the thickness of the applied medicament. Int J Pharm. 1980;6(3/4):321–32.
31. Hadgraft J. The epidermal reservoir: a theoretical approach. Int J Pharm. 1979;2:265–74.
32. Cleek RL, Bunge AL. A new method for estimating dermal absorption from chemical exposure .1. General approach. Pharm Res. 1993;10(4):497–506.
33. Bunge AL, Cleek RL. A new method for estimating dermal absorption from chemical exposure: 2 Effect of molecular weight and octanol–water partitioning. Pharm Res. 1995;12(1):88–95.
34. Seko N, et al. Theoretical analysis of the effect of cutaneous metabolism on skin permeation of parabens based on a two-layer skin diffusion/metabolism model. Biol Pharm Bull. 1999;22(3):281–7.
35. Scheuplein RJ, Morgan LJ. “Bound water” in keratin membranes measured by a microbalance technique. Nature. 1967;214(87):456–8.
36. Roberts MS, Triggs EJ, Anderson RA. Permeability of solutes through biological membranes measured by a desorption technique. Nature. 1975;275:225–7.
37. Scheuplein RJ, Blank IH. Permeability of the skin. Physiol Rev. 1971;51(4):702–47.
38. Mitragotri S, et al. Mathematical models of skin permeability: an overview. Int J Pharm. 2011;418:115–29.

39. Anissimov YG, Roberts MS. Diffusion modeling of percutaneous absorption kinetics: 4. Effects of a slow equilibration process within stratum corneum on absorption and desorption kinetics. J Pharm Sci. 2009;98:772–81.
40. Watkinson AC, et al. Computer simulation of penetrant concentration-depth profiles in the stratum corneum. Int J Pharm. 1992;87:175–82.
41. Mueller B, Anissimov YG, Roberts MS. Unexpected clobetasol propionate profile in human stratum corneum after topical application in vitro. Pharm Res. 2003;20(11):1835–7.
42. Anissimov YG, Roberts MS. Diffusion modeling of percutaneous absorption kinetics: 3. Variable diffusion and partition coefficients, consequences for stratum corneum depth profiles and desorption kinetics. J Pharm Sci. 2004;93(2):470–87.
43. Hadgraft J. Calculations of drug release from controlled release devices: the slab. Int J Pharm. 1979;2:177–94.
44. Chandrasekaran SK, Bayne W, Shaw JE. Pharmacokinetics of drug permeation through human skin. J Pharm Sci, 1978;67:1370–4.
45. Iordanskii AL, et al. Modeling of the drug delivery from a hydrophilic transdermal therapeutic system across polymer membrane. Eur J Pharm Biopharm. 2000;49(3):287–93.
46. Higuchi WI. Diffusional models useful in biopharmaceutics. J Pharm Sci. 1967;56:315–24.
47. Riegelman S. Pharmacokinetics: pharmacokinetic factors affecting epidermal penetration and percutaneous adsorption. Clin Pharmacol Ther. 1974;16(5 Part 2):873–83.
48. Wallace SM, Barnett G. Pharmacokinetic analysis of percutaneous absorption: evidence of parallel penetration pathways for methotrexate. J Pharmacokinet Biopharm. 1978;6(4):315–25.
49. Roberts MS, Anissimov YG, Gonsalvez RA. Mathematical models in percutaneous absorption. In: Bronaugh RL Maibach HI, editors. Percutaneous absorption drugs—cosmetics—mechanisms—methodology. New York: Marcel Dekker; 1999. pp. 3–55.
50. McCarley KD, Bunge AL. Physiologically relevant two-compartment pharmacokinetic models for skin. J Pharm Sci. 2000;89(9):1212–35.
51. McCarley KD, Bunge AL. Physiologically relevant one-compartment pharmacokinetic models for skin. 1. Development of models. J Pharm Sci. 1998;87(4):470–81.
52. Zatz J. Simulation studies of skin permeation. J Soc Cosm Chem. 1992;43:37–48.
53. Anissimov YG. Mathematical models for different exposure conditions. In: Roberts MS, Walters KA, Editors. Dermal absorption and toxicity assessment. New York: Informa Healthcare; 2008, pp. 271–86.
54. Roberts MS, Anderson RA, Swarbrick J. Permeability of human epidermis to phenolic compounds. J Pharm Pharmacol. 1977;29(11):677–83.
55. Wu MS. Determination of concentration dependent water diffusivity in a keratinous membrane. J Pharm Sci. 1983;72:1421–3.
56. Gienger G, Knoch A, Merkle HP. Modeling and numerical computation of drug transport in laminates—model case evaluation of transdermal delivery system. J Pharm Sci. 1986;75(1):9–15.
57. Higuchi WI, Higuchi T. Theoretical analysis of diffusion movement through heterogeneous barriers. J Am Pharm Assoc Sci Ed. 1960;49:598–606.
58. Chandrasekaran SK, et al. Scopolamine permiation through human skin in vitro. AlChE J. 1976;22:828–32.
59. Kubota K, Koyama E, Twizell EH. Dual sorption model for the nonlinear percutaneous permeation kinetics of timolol. J Pharm Sci. 1993;82(12):1205–8.
60. Ando HY, Ho NFH, Higuchi WI. Skin as an active metabolizing barrier. 1. Theoretical analysis of topical bioavailability. J Pharm Sci. 1977;66:1525–8.
61. Yu CD, et al. Physical model evaluation of topical prodrug delivery-simultaneous transport and bioconversion of vidarabine-5′-valerate II: parameter determinations. J Pharm Sci. 1979;68(11):1347–57.
62. Yu CD, et al., Physical model evaluation of topical prodrug delivery-simultaneous transport and bioconversion of vidarabine 5′-valerate. Part 1. Physical model development. Part 2. Parameter determinations. J Pharm Sci. 1979;68:1341–6.

63. Yu CD, et al., Physical model evaluation of topical prodrug delivery-simultaneous transport and bioconversion of vidarabine 5'-valerate. Part 5. Mechanistic analysis of influence of non-homogeneous enzyme distributions in hairless mouse skin. J Pharm Sci. 1980;69:775–80.
64. Fox JL, et al. General physical model for simultaneous diffusion and metabolism in biological-membranes—computational approach for the steady-state case. Int J Pharm. 1979;2(1):41–57.
65. Hadgraft J. Theoretical aspects of metabolism in the epidermis. Int J Pharm. 1980;4:229–39.
66. Guy RH, Hadgraft J. Percutaneous metabolism with saturable enzyme-kinetics. Int J Pharm. 1982;11(3):187–97.
67. Kubota K, Ademola J, Maibach HI. Simultaneous diffusion and metabolism of betamethasone 17-valerate in the living skin equivalent. J Pharm Sci. 1995;84(12):1478–81.
68. Guy RH, Hadgraft J. Pharmacokinetics of percutaneous absorption with concurrent metabolism. Int J Pharm. 1984;20:43–51.
69. Hadgraft J, Wolff HM. In vitro/in vivo correlations in transdermal drug delivery. Dermal Absorption Toxicity Assessment. 1998;91:269–79.
70. Robinson PJ. Prediction: Simple risk models and overview of dermal risk assessment. In: Roberts MS, Walters KA, editors. Dermal absorption and toxicity assessment. New York: Marcel Dekker; 1998. pp. 203–29.
71. Yalkowsky SH, Valvani SC. Solubility and partitioning I: solubility of nonelectrolytes in water. J Pharm Sci. 1980;69(8):912–22.
72. Reifenrath WG, Robinson PB. In vitro skin evaporation and penetration characteristics of mosquito repellents. J Pharm Sci, 1982;71:1014–8.
73. Guy RH, Hadgraft J. Percutaneous absorption kinetics of topically applied agents liable to surface loss. J Soc Cosmet Chem. 1984;35:103–13.
74. Guy RH, Hadgraft J. A theoretical description of the effects of volatility and substantivity on percutaneous-absorption. Int J Pharm. 1984;18(1/2):139–47.
75. Saiyasombati P, Kasting GB. Disposition of benzyl alcohol after topical application to human skin in vitro. J Pharm Sci. 2003;92(10):2128–39.
76. Kasting GB, Miller MA. Kinetics of finite dose absorption through skin 2: volatile compounds. J Pharm Sci. 2006;95(2):268–80.
77. Miller MA, Bhatt V, Kasting GB. Dose and airflow dependence of benzyl alcohol disposition on skin. J Pharm Sci. 2006;95(2):281–91.
78. Scheuplein RJ. Mechanism of percutaneous absorption. II. transient diffusion and the relative importance of various routes of skin penetration. J Invest Dermatol. 1967;48(1):79–88.
79. Ghanem AH, et al. The effects of ethanol on the transport of beta-estradiol and other permeants in hairless mouse skin. II. a new quantitative approach. J Controlled Release. 1987;6:75–83.
80. Hatanaka T, et al. Prediction of skin permeability of drugs .2. development of composite membrane as a skin alternative. Int J Pharm. 1992;79(1):21–8.
81. Tojo K, Chiang CC, Chien YW. Drug permeation across the skin: effect of penetrant hydrophilicity. J Pharm Sci. 1987;76:123–6.
82. Yamashita F, et al. Analysis of skin penetration enhancement based on a 2-layer skin diffusion-model with polar and nonpolar routes in the stratum-corneum—dose-dependent effect of 1-geranylazacycloheptan-2-one on drugs with different lipophilicities. Biol Pharm Bull. 1993;16(7):690–7.
83. Edwards DA, Langer R. A linear-theory of transdermal transport phenomena. J Pharm Sci. 1994;83(9):1315–34.
84. Malkinson FD, Ferguson EH. Percutaneous absorption of hydrocortisone-4-C14 in two human subjects. J Invest Dermatol. 1955;25(5):281–3.
85. Vickers CF. Stratum corneum reservoir for drugs. Adv Biol Skin. 1972;12:177–89.
86. Reddy MB, Guy RH, Bunge AL. Does epidermal turnover reduce percutaneous penetration? Pharm Res. 2000;17(11):1414–9.
87. Roberts MS, Cross SE, Anissimov YG. Factors affecting the formation of a skin reservoir for topically applied solutes: skin pharmacology and applied skin. Physiology. 2004;17:3–16.

88. Rohatagi S, et al. Integrated pharmacokinetic and metabolic modeling of selegiline and metabolites after transdermal administration. Biopharm Drug Dispos. 1997;18(7):567–84.
89. Cross SE, Wu Z, Roberts MS. Effect of perfusion flow rate on the tissue uptake of solutes after dermal application using the rat isolated perfused hindlimb preparation. J Pharm Pharmacol. 1994;46(10):844–50.
90. Cross SE, Wu Z, Roberts MS. The effect of protein binding on the deep tissue penetration and efflux of dermally applied salicylic acid, lidocaine and diazepam in the perfused rat hindlimb. J Pharmacol Exp Ther. 1996;277(1):366–74.
91. Williams PL, Carver MP, Riviere JE. A physiologically relevant pharmacokinetic model of xenobiotic percutaneous-absorption utilizing the isolated perfused porcine skin flap. J Pharm Sci. 1990;79(4):305–11.
92. Reddy MB, McCarley KD, Bunge AL. Physiologically relevant one-compartment pharmacokinetic models for skin. 2. comparison of models when combined with a systemic pharmacokinetic model. J Pharm Sci. 1998;87(4):482–90.
93. Roberts MS, et al., Modeling of subcutaneous absorption kinetics of infusion solutions in the elderly using technetium. J Pharmacokinet Biopharm, 1997;25:1–21.
94. Singh P, Roberts MS, Maibach HI. Modeling of plasma-levels of drugs following transdermal iontophoresis. J Controlled Release. 1995;33(2):293–8.
95. Imanidis G, et al. Estimation of skin target site acyclovir concentrations following controlled (trans)dermal drug-delivery in topical and systemic treatment of cutaneous hsv-1 infections in hairless mice. Pharm Res. 1994;11(7):1035–41.
96. Tegeder I, et al. Application of microdialysis for the determination of muscle and subcutaneous tissue concentrations after oral and topical ibuprofen administration. Clin Pharmacol Ther. 1999;65(4):357–68.
97. Cooper ER. Pharmacokinetics of skin penetration. J Pharm Sci. 1976;65(9):1396–7.
98. Cooper ER, Effect of diffusional lag time on multicompartmental pharmacokinetics for transepidermal infusion. J Pharm Sci. 1979;68:1469–1470.
99. McDougal JN. Prediction—physiological models. In: Roberts MS, Walters KA, Editors. Dermal absorption and toxicity assessment. New York: Marcel Dekker; 1998. pp. 189–202.
100. Jepson GW, McDougal JN. Physiologically based modeling of nonsteady state dermal absorption of halogenated methanes from an aqueous solution. Toxicol Appl Pharmacol. 1997;144(2):315–24.
101. Timchalk C, et al. A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans. Toxicol Sci. 2002;66(1):34–53.
102. Poet TS, et al. PBPK modeling of the percutaneous absorption of perchloroethylene from a soil matrix in rats and humans. Toxicol Sci. 2002;67(1):17–31.
103. Poet TS, et al. Assessment of the percutaneous absorption of trichloroethylene in rats and humans using MS/MS real-time breath analysis and physiologically based pharmacokinetic modeling. Toxicol Sci. 2000;56(1):61–72.
104. Poet TS, et al. Utility of real time breath analysis and physiologically based pharmacokinetic modeling to determine the percutaneous absorption of methyl chloroform in rats and humans. Toxicol Sci. 2000;54(1):42–51.
105. Thrall KD, Weitz KK, Woodstock AD. Use of real-time breath analysis and physiologically based pharmacokinetic modeling to evaluate dermal absorption of aqueous toluene in human volunteers. Toxicol Sci. 2002;68(2):280–7.
106. Qiao GL, et al. Dermatoxicokinetic modeling of p-nitrophenol and its conjugation metabolite in swine following topical and intravenous administration. Toxicol Sci. 2000;54(2):284–94.
107. Riviere JE, et al. Dermal absorption and distribution of topically dosed jet fuels jet-A, JP-8, and JP-8(100). Toxicol Appl Pharmacol. 1999;160(1):60–75.
108. Benowitz NL, et al. Stable isotope method for studying transdermal drug absorption: the nicotine patch. Clin Pharmacol Ther. 1991;50(3):286–93.

109. Welin-Berger K, et al. In vitro-in vivo correlation in man of a topically applied local anesthetic agent using numerical convolution and deconvolution. J Pharm Sci. 2003;92(2):398–406.
110. Anissimov YG, et al. Mathematical and pharmacokinetic modelling of epidermal and dermal transport processes. Adv Drug Deliv Rev. 2013;65:169–90.
111. Jepps OG, et al. Modeling the human skin barrier—towards a better understanding of dermal absorption. Adv Drug Deliv Rev. 2013;65:152–68.
112. Gupta E, Wientjes MG, Au JL. Penetration kinetics of 2',3'-dideoxyinosine in dermis is described by the distributed model. Pharm Res. 1995;12(1):108–12.
113. Cross SE, et al. Self promotion of deep tissue penetration and distribution of methylsalicylate after topical application. Pharm Res. 1999;16(3):427–33.
114. Kretsos K, Kasting GB, Nitsche JM. Distributed diffusion-clearance model for transient drug distribution within the skin. J Pharm Sci. 2004;93(11):2820-35.
115. Anissimov YG, Roberts MS. Modelling dermal drug distribution after topical application in human. Pharm Res. 2011;28(9):2119–29.
116. Schaefer H, Stuttgen G. Absolute concentrations of an antimycotic agent, econazole, in the human skin after local application. Arzneimittelforschung. 1976;26(3):432–5.
117. Schaefer H, Zesch A. Penetration of vitamin A acid into human skin. Acta Derm Venereol Suppl (Stockh). 1975;74:50–5.
118. Schaefer H, Zesch A, Stuttgen G. Penetration, permeation, and absorption of triamcinolone acetonide in normal and psoriatic skin. Arch Dermatol Res. 1977;258(3):241–9.
119. Schaefer H, et al. Quantitative determination of percutaneous absorption of radiolabeled drugs in vitro and in vivo by human skin. Curr Probl Dermatol. 1978;7:80–94.
120. Zesch A, Schaefer H. [Penetration of radioactive hydrocortisone in human skin from various ointment bases. II. In vivo-experiments (author's transl)]. Arch Dermatol Forsch. 1975;252(4):245–56.
121. Dancik Y, et al. Convective transport of highly plasma protein bound drugs facilitates direct penetration into deep tissues after topical application. Brit J Clin Pharmacol. 2012;73(4):564–78.
122. Singh P, Maibach HI, Roberts MS. Site of effects. Dermal absorption toxicity assessment. 1998;91:353–70.
123. McNeill SC, Potts RO, Francoeur ML. Local enhanced topical delivery (LETD) of drugs: does it truly exist? Pharm Res. 1992;9(11):1422–7.
124. Singh P, Roberts MS. Blood-flow measurements in skin and underlying tissues by microsphere method—application to dermal pharmacokinetics of polar nonelectrolytes. J Pharm Sci. 1993;82(9):873–9.
125. Singh P, Roberts MS. Dermal and underlying tissue pharmacokinetics of salicylic acid after topical application. J Pharmacokinet Biopharm. 1993;21(4):337–73.
126. Roberts MS, Cross SE. A physiological pharmacokinetic model for solute disposition in tissues below a topical application site. Pharm Res. 1999;16(9):1392–8.
127. Nakayama K, et al. Estimation of intradermal disposition kinetics of drugs: I. analysis by compartment model with contralateral tissues. Pharm Res. 1999;16(2):302–8.
128. Higaki K, et al. Estimation of intradermal disposition kinetics of drugs: II. factors determining penetration of drugs from viable skin to muscular layer. Int J Pharm. 2002;239(1/2):129–41.
129. Singh P, Roberts MS. Effects of vasoconstriction on dermal pharmacokinetics and local tissue distribution of compounds. J Pharm Sci. 1994;83(6):783–91.
130. Cross SE, et al., Is there tissue penetration after application of topical salicylate formulations? Lancet, 1997;350:636.
131. Muller M, et al. Diclofenac concentrations in defined tissue layers after topical administration. Clin Pharmacol Ther. 1997;62(3):293–9.
132. Beastall J, et al. The influence of urea on percutaneous-absorption. Pharm Res. 1986;3(5):294–7.

133. Demana PH, et al., Evaluation of the proposed FDA pilot dose response methodology for topical corticosteroid bioequivalence testing. Pharm Res, 1997;14:303–8.
134. Singh GJ, et al. Evaluation of the proposed FDA pilot dose-response methodology for topical corticosteroid bioequivalence testing [letter]. Pharm Res, 1998;15(1):4–7 (Biol Sciences Journal holding: [v.1](1984)-12(1995);13(1996)).
135. Smith EW, Haigh JM, Walker RB. Analysis of chromameter results obtained from corticosteroid-induced skin blanching. Part 1. manipulation of data. Pharm Res. 1998;15:280–5.
136. Cordero JA, et al. In vitro based index of topical anti-inflammatory activity to compare a series of NSAIDs. Eur J Pharm Biopharm. 2001;51(2):135–42.
137. Kasting GB. Theoretical-models for iontophoretic delivery. Adv Drug Delivery Rev. 1992;9(2/3):177–99.
138. Roberts MS, Lai PM, Anissimov YG. Epidermal iontophoresis: I. development of the ionic mobility-pore model. Pharm Res. 1998;15(10):1569–78.
139. Cross SE, Roberts MS. Importance of dermal blood supply and epidermis on the transdermal iontophoretic delivery of monovalent cations. J Pharm Sci. 1995;84(5):584–92.
140. Mitragotri S, Blankschtein D, Langer R. An explanation for the variation of the sonophoretic transdermal transport enhancement from drug to drug. J Pharm Sci. 1997;86(10):1190–2.
141. Tezel A, et al. Frequency dependence of sonophoresis. Pharm Res. 2001;18(12):1694–700.
142. Tezel A, Sens A, Mitragotri S. Description of transdermal transport of hydrophilic solutes during low-frequency sonophoresis based on a modified porous pathway model. J Pharm Sci. 2003;92(2):381–93.
143. Jiang R, et al. Absorption of sunscreens across human skin: an evaluation of commercial products for children and adults. Br J Clin Pharmacol. 1999;48(4):635–7.
144. Kubota K, Sznitowska M, Maibach HI. Percutaneous-absorption—a single-layer model. J Pharm Sci. 1993;82(5):450–6.
145. Beckett AH, Taylor DC, Gorrod JW. Comparison of oral and percutaneous routes in man for systemic administration of ephedrines. J Pharm Pharmacol. 1972;24:65–70.

Chapter 16
Transdermal Patches: An Industrial Perspective on the Relevance of *In Vitro* Skin Permeation Studies and Approaches on Design of Manufacturing Processes

Björn Schurad and Marieke Gosau

16.1 Introduction

For the treatment of several diseases, the controlled delivery of active substances from the dosage form into the human body is of great importance. This especially holds true when highly potent drugs with a small therapeutic window are to be administered. Transdermal dosage forms are one option to allow for a constant drug release over a period of up to 7 days [1, 2] and may therefore represent a favorable formulation approach.

It needs to be considered that one of the skin's primary functions is to protect the human body from loss of endogenous substances on the one hand, and from entry of xenobiotics, including drugs, on the other. For this reason, the skin has evolved to become a highly efficient barrier [3], which must be overcome to deliver therapeutically relevant amounts of drugs into the body [4]. Once a suitable compound has been selected and successfully formulated within a transdermal patch, there are several advantages that make transdermal patches highly interesting drug delivery systems. First, with the ability to achieve constant plasma concentration levels, periods with noneffective plasma concentrations can be avoided as well as peak maxima which may be related to drug-induced side effects [5]. The dosing intervals may be reduced, especially when compared to conventional oral dosage forms, allowing the improvement of patient compliance. Also, the drug input can be easily interrupted by simply removing the patch from the skin and liver's first-pass effect can be circumvented. These advantages contribute to the subsequently growing transdermal market which comprises worldwide a volume of about 6 billion US dollars for 2012 [6].

Up to now, transdermal patches have been approved for a variety of indications, including nausea, smoking cessation, pain management, women's health (hormone replacement), contraception as well as cardiovascular and neurodegenerative

B. Schurad (✉) · M. Gosau
Acino AG, Am Windfeld 35, Miesbach 83714, Germany
e-mail: bjoern.schurad@acino-pharma.com

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_16

disorders. Major therapeutic advantages may especially be achieved, for example, in the dopaminergic therapy of Parkinson's disease. Here, the concept of continuous dopaminergic stimulation is considered to avoid, or at least to postpone, treatment-related motor fluctuations as they occur under treatment with L-dopa or other short-acting oral dopaminergic compounds bringing additional therapeutic benefit [7–11].

Generally, there are two different basic types of formulation designs established today: the reservoir-type patches and the matrix-type patches [12]. Reservoir patches usually consist of a backing foil to protect the patch from the environment, a liquid or semisolid drug reservoir, a membrane to govern the drug release from the transdermal system, and an adhesive layer facing the skin that is protected with a release liner until time of application. These systems have mainly been displaced from the commercial market since they are connected with serious potential for drug bursts and leakage once the system is damaged. This situation especially holds true for the first generation of fentanyl patches [13]. Moreover, the industrial production process is more complex and therefore results in higher costs compared with the production of the more modern matrix-type patches [12]. Within the simplest design of matrix patches, the drug is directly incorporated within the adhesive layer, which is situated between a backing foil and a release liner. However, there are also more complex designs, for example, multilayered matrix patches, where the drug containing polymeric matrix—offering a kind of reservoir function—is covered with an additional adhesive layer facing the skin. This allows more space during development to achieve the desired performance of the drug product. Different dosage strengths of matrix-type patches, which today dominate the commercially available transdermal products, may be easily die-cut from the same kind of intermediate laminate. This product type does not bear the risk of drug leakage since the active substances are fully entrapped within the respective matrix layers of the product.

As extensively discussed in the scientific literature, there are several approaches to increase the transdermal permeation of a compound through the skin tissue. These approaches include, but are not limited to, passive strategies as supersaturation [14, 15] and incorporation of chemical permeation enhancers [16, 17], or active strategies using an energy source as, for example, iontophoresis [18], sonophoresis [19, 20], electroporation [21], or shock waves [22]. Also, microneedles may be used to mechanically rupture the stratum corneum as the main barrier of the skin by puncturing the outer skin layers without inducing pain [23, 24].

16.2 Transdermal Drug Development: Skin Permeation Performance *In Vitro* and *In Vivo*

During drug development the scientists need appropriate *in vitro* tools to evaluate formulation approaches and to select promising formulations for first *in vivo* testing. Beside typical attributes that are considered to be critical to quality, as appearance of the patch, assay of active and functional ingredients, content uniformity, related substances (purity), drug release (dissolution), and microbial purity, there are some aspects that must be specifically focused on during development of transdermal

patches. These are the adhesive properties of the patch, which are described by adhesive strength and tack force. Moreover, the separation force to remove the patch from its release liner and cold flow characteristics, describing the tendency of the matrix to change its dimension, for example, by creeping under constant load, are considered as specific attributes for transdermal patches that should be kept in mind during product development as also requested by the recently published EMA Draft Guideline on Quality of Transdermal Patches [25]. As it also holds true for other controlled release dosage forms, the drug release should be paid significant attention. For transdermal dosage forms, the scientists should not only consider the liberation or release of the drug from the patch, but also the permeation of the drug through the skin tissue as a fundamental step to reaching the systemic circulation.

This can be investigated by *in vitro* skin permeation experiments. Here, it is the choice of the scientist, which skin model is to be used. Beside excised skin of rodents (e.g., hairless mice, rats), also porcine ear skin and human skin tissue are well described and accepted within the scientific literature [26–28]. The skin of rodents, especially excised hairless mouse skin, offers the advantage of easy access and availability. Moreover, the skin tissue can be "standardized" by limiting the sourcing to one single strain, animals of same sex and age, and raised under identical conditions. However, the skin's outermost layer, the stratum corneum, of hairless mice is with 4–10 µm significantly thinner than human skin, and is, thus, usually more permeable to drugs [29–31]. By contrast, human skin, in most cases derived from plastic surgery, is more complex to receive, limited in quantity and shows significant variability, especially from donor to donor. But after preparation (heat separation of epidermis as already described by Kligman and Christophers in 1963 [32] or dermatoming to a defined thickness, for example, 200 µm [33]), this tissue is closest to the *in vivo* barrier. In the last 15 years reconstructed skin tissues from human keratinocytes has gained more and more importance as model membrane for *in vitro* skin permeation experiments. These models already appear to be close to human skin regarding structure and composition [34, 35], but in most cases are still more permeable [36, 37]. Figure 16.1 illustrates the permeability of a model drug (buprenorphine) from a matrix patch formulation through different skin tissues *in vitro*. Information on the experimental set up for all skin permeation experiments described in this chapter is summarized in Table 16.1.

As might be expected, the hairless mouse skin is more permeable than dermatomised porcine ear skin (300 µm) and heat separated epidermis (HSE) derived from human skin. The reconstructed skin tissue (EpiDerm™ model, Mattek Corporation) was the most permeable, with the high-barrier EpiDerm-FT™ model allowing less drug to permeate—but still being somewhat more permeable than hairless mouse skin.

The question concerning correlation of *in vitro* skin permeation data with *in vivo* results is one of the key questions the scientists are faced with throughout the development. It is pointed out that during early development, when different formulations are screened for their skin permeation performance, the main focus is to establish a ranking between the different test formulations and the formulation approaches they are based on. For that purpose, these experiments should be carried out under well-controlled conditions eliminating test variability as far as possible to allow comparison between the various formulations tested in different sets of

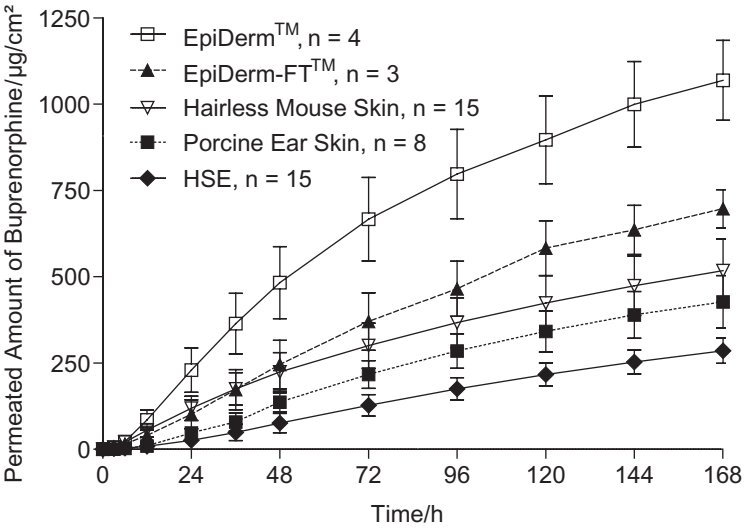


Fig. 16.1 *In vitro* skin permeation profiles of buprenorphine from a buprenorphine patch through different skin. A static diffusion cell system has been used and a validated analytical method to determine the amount of buprenorphine in the receptor fluid. Shown are the mean curves (mean ± standard deviation (SD)); number of replicates is given in the graphic

Table 16.1 Experimental set up for skin permeation experiments

Experimental set-up	Buprenorphine	Rivastigmine
Type of diffusion cells	Static, vertical	Static, vertical
Receptor volume	15 mL	15 mL
Receptor medium	Ammonium acetate buffer, pH 5.2	HEPES buffer, pH 7.2
Sample media replacement	Yes	Yes
Diffusion area	1.05 cm^2	1.05 cm^2
Skin tissue	As referenced in figures	As referenced in figures
Sample	Transdermal patch, diameter 1.2 cm applied to stratum corneum of skin sample	Transdermal patch, diameter 1.2 cm applied to stratum corneum of skin sample
Drug quantification in samples	Validated reversed phase HPLC with UV detection	Validated reversed phase HPLC with UV detection

HPLC high performance liquid chromatography, *HEPES* 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

experiments. Therefore, excised rodent's skin may be considered a suitable tool for this stage of development to identify the most promising formulations. In further confirmatory *in vitro* studies, it is often of benefit to compare the favorable formulations also in *in vitro* permeation studies using human skin preparations. In many cases, the permeated amounts in those experiments are lower than through rodent's skin and are, therefore, closer to what can be expected from the *in vivo* situation.

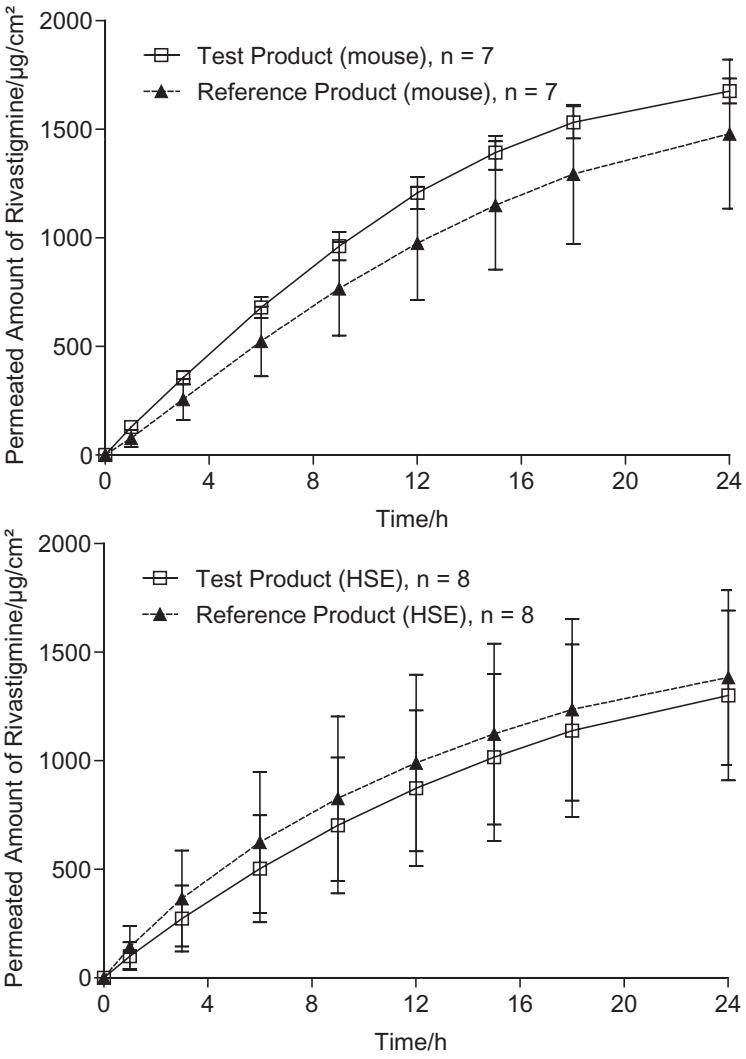


Fig. 16.2 *In vitro* skin permeation profiles of reference and test formulation through excised hairless mouse skin (*top*) and heat separated human epidermis (*bottom*). A static diffusion cell system has been used and a validated analytical method to determine the amount of rivastigmine in the receptor fluid. Shown are mean curves (mean ± SD) of at least $n = 7$ replicates

In what follows, a scenario is presented with rivastigmine as model drug, an acetyl choline esterase inhibitor to treat Alzheimer's disease. On both, excised hairless mouse skin and heat separated human epidermis obtained from plastic surgery, the drug permeation profiles and fluxes in steady state calculated as slope from the linear part of the permeation profiles are similar for the reference drug product and the drug product under investigation (no statistically significant difference, unpaired t-Test, $P > 0.05$, Fig. 16.2). Also, the onset of transdermal permeation, described by the lag time, is comparable for both formulations. Based on these results, a reasonable chance for bioequivalence could be concluded.

Table 16.2 Clinical trial information of bioavailability study carried out with two rivastigmine transdermal patch formulations

Study phase	Phase I
Formulations	2 patch formulations with nominal release rates of 9.5 mg rivastigmine per 24 h
Dosing	Single dosing
Design	Open label, twofold crossover
Number of volunteers	36
Drop outs	5
Included in PK analysis	31

Table 16.3 Summary of pharmacokinetic data derived from bioavailability study with two rivastigmine transdermal patch formulations; shown are geometric mean values with coefficient of variance given in parentheses

Parameter	Test	Reference
c_{max} (pg/mL)	6938.9 (43 %)	7415.7 (41 %)
$AUC_{0\text{-}t}$ (pg·h/mL)	121672.8 (38 %)	125833.2 (37 %)

These two drug products have also been administered to human volunteers in the framework of a bioavailability study conducted under GCP requirements as per ICH E6 [38] (Table 16.2).

Within this study both drug products behaved very similarly regarding rate and extent of exposure (Fig. 16.3) so that formal bioequivalence criteria for c_{max} and AUC were fulfilled. Key pharmacokinetic data are presented in Table 16.3.

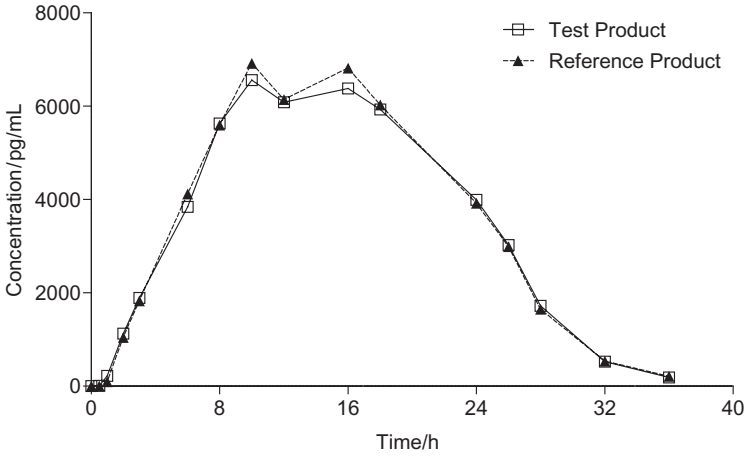


Fig. 16.3 Plasma concentration time profiles of two rivastigmine patch formulations in human volunteers. Shown are the mean curves (geometric mean) of $n = 31$ subjects

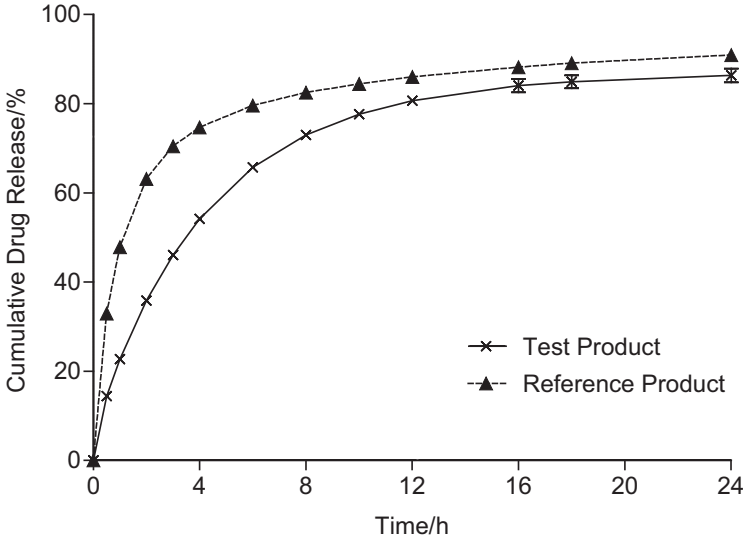


Fig. 16.4 Drug dissolution profile from reference and test formulation derived from a validated method using the compendial paddle over disk apparatus. Shown are the mean curves (Mean ± SD) of $n = 12$ replicates

This demonstrates that the qualitative results from the *in vitro* studies carried out on rodent's skin and human skin could be well transferred to the *in vivo* situation and underlines the relevance and suitability of *in vitro* skin permeation experiments for successful drug development.

By contrast, if the focus is set only on drug release as determined in the framework of dissolution testing, the situation may be different. When applying a validated paddle over disk dissolution test as described in USP <724> (apparatus 5), or Ph. Eur. 2.9.4-1 (disk assembly), both formulations can be distinguished in their drug release profile—whereas they perform equivalently *in vivo* [39, 40]. Especially during the initial hours of the drug release process, the rates are different until later on when the transdermal patches are (nearly) fully depleted (Fig. 16.4). This is also confirmed by a similarity factor f_2 of 35 calculated according to relevant Guidances [41–44].

Therefore, simply relying on drug dissolution during product development is not sufficient to formulate transdermal dosage forms with the required *in vivo* performance. For both formulations drug release in the dissolution test is rather quick and thus the permeation through the skin tissue appears to be the rate limiting step of absorption. These data also clearly highlight that *in vitro* dissolution shall be considered rather more as a quality control tool to demonstrate consistent drug release from the product during storage or from batch to batch [45]. As a formulation development tool for transdermal patches without considering the following step of skin tissue permeation it is limited in value.

Whereas in the present case the data from *in vitro* permeation studies qualitatively mirror what can be seen in man, this does not hold true for all active ingredients and/or all formulation approaches making transdermal drug product formulation a complex and difficult field. This can be seen from another comparable study based on the model drug buprenorphine, an opiod analgesic to treat severe pain.

In vitro and *in vivo* skin permeation data are compared from two buprenorphine transdermal patches for targeted dosing intervals of 7 days, a reference drug and a drug under investigation. Again, comparable skin permeation data were recorded using excised hairless mouse skin and heat separated human epidermis in a static diffusion cell system (Fig. 16.5).

The permeation data derived from excised mouse skin show a slightly more pronounced lag-phase for the test product, but after the initial phase the transdermal fluxes in steady state are concurrent during the distinctive stage of steady state for both formulations (no statistically significant difference, unpaired t-Test, $P > 0.05$). When heat separated human epidermis is used as the model membrane, the permeation of buprenorphine is more retarded due to the higher number of cell layers within the stratum corneum. However, the qualitative picture is comparable to the situation with excised hairless mouse skin. The lag time is somewhat more pronounced for the test formulation and the transdermal fluxes in steady state are comparable for the reference drug and the drug under investigation (no statistically significant difference, unpaired t-Test, $P > 0.05$). Also, the steady-state phases are maintained throughout the complete duration of the experiment. Therefore, the *in vitro* skin permeation data consistently indicate comparable drug permeation rates of the test and reference product over a prolonged steady-state phase, albeit the test product exhibits a somewhat longer lag phase.

These two drug products have also been introduced into a phase 1 pharmacokinetic study to characterize the plasma concentration time profiles. This study was designed as a crossover trial in 24 subjects (Table 16.4).

As can be seen from Fig. 16.6 and Table 16.5 depicting the pharmacokinetic results, a discrepancy between the plasma concentration time profiles of the two formulations were recorded.

The onset of action of the two patch formulations was comparable with reaching steady-state levels between 48 and 72 h. The prolonged constant drug release rate over the application period of 1 week can be observed for both formulations as predicted by the *in vitro* skin permeation data. The steady-state concentrations, by contrast, were strikingly different between reference and test formulation yielding also significantly lower AUC and c_{max} values for the test product. This, obviously, does not mimic the situation from the *in vitro* models, where steady-state permeation was found to be similar. The two patches do not show equivalent cumulative extent of buprenorphine exposure (AUC). In this case, the predictive power of the *in vitro* models seems to be limited.

To achieve a constant and sufficient permeation of buprenorphine through the skin into the blood stream over such a long application period, both formulations comprise complex excipient systems including different kind of chemical

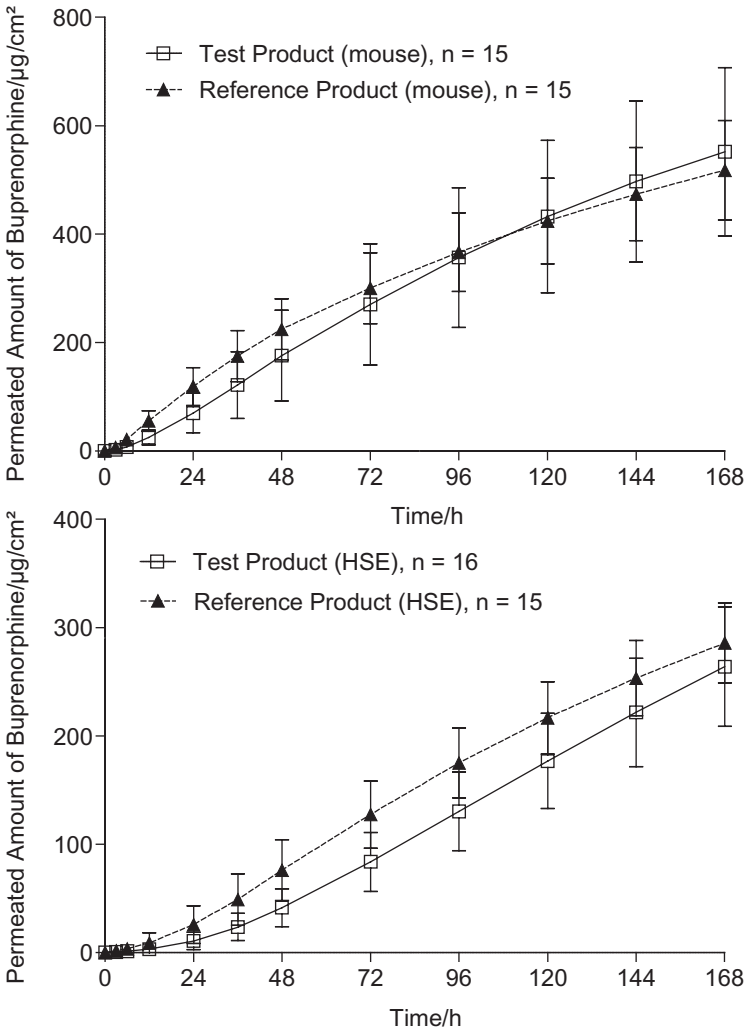


Fig. 16.5 *In vitro* skin permeation profiles of reference and test formulation through excised hairless mouse skin (*top*) and heat separated human epidermis (*bottom*). A static diffusion cell system has been used and a validated analytical method to determine the amount of buprenorphine in the receptor fluid. Shown are the mean curves (mean ± SD) of at least $n = 15$ replicates

permeation enhancers which directly act within the skin tissue. These enhancers migrate likewise to the active ingredient into the skin and may interact among themselves, with the active substance and predominantly also with components of the skin altering the skin barrier properties.

Table 16.4 Clinical trial information of bioavailability study carried out with two buprenorphine transdermal patch formulations

Study phase	Phase I
Formulations	2 patch formulations with nominal release rates of 20 µg buprenorphine per hour
Dosing	Single dosing
Design	Open label, twofold crossover
Number of volunteers	24
Drop outs	0
Included in PK analysis	24

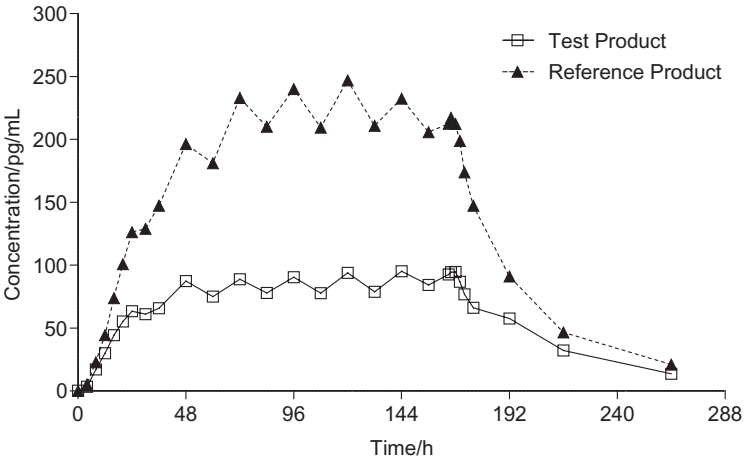


Fig. 16.6 Plasma concentration time profiles of two buprenorphine patch formulations in human volunteers. Shown are the mean curves (geometric mean) of $n = 24$ subjects

Table 16.5 Summary of pharmacokinetic data derived from bioavailability study with two buprenorphine transdermal patch formulations; shown are geometric mean values with coefficient of variance given in parentheses

Parameter	Test	Reference
c_{max} (pg/mL)	100.3 (46%)	261.2 (37%)
$AUC_{0\text{-}t}$ (pg·h/mL)	14985 (47%)	35350 (37%)

Returning to the *in vitro* data and taking these considerations into account, there appears a lack of the *in vitro* models to properly illustrate those effects. Regarding excised hairless mouse skin, there are differences in skin histology compared to human skin, which probably do not fully reflect the interactions with the functional enhancers. Furthermore, in addition to the stratum corneum, which is the main diffusion barrier to drugs, there are further layers of the skin with influence on drug

permeation. Lipophilic drugs are additionally controlled by deeper layers of the skin as the viable epidermis and dermis since these layers are essentially hydrophilic and may offer a relevant diffusion barrier to lipophilic drugs [46, 47]. Therefore, *in vitro* skin permeation experiments performed with HSE of human skin might not completely describe the complex permeation process and interactions of the enhancers, the drug, and the skin components as occurring *in vivo*. Although these explanations are somewhat speculative, this example for transdermal buprenorphine displays the limitation of *in vitro* models to properly predict what really happens *in vivo*. The *in vitro* data suggested comparable steady-state permeation of both formulations, but probably due to the complexity of the interactions especially with the various excipients *in vivo* and the lipophilic nature of the model drug, the *in vivo* situation could not be adequately predicted. This also underlines the need for *in vivo* data already during formulation development, when experience is not available from comparable formulation approaches with the respective drug.

However, *in vitro* permeation studies remain an important tool in patch development, for example, to screen different formulations, to characterize different formulation approaches, to establish a ranking regarding permeation behavior and to select first candidates for an *in vivo* testing. It may also be a beneficial tool to investigate different batches of a drug product for consistent permeation performance—as long as the skin tissue is limited in variability. The skin tissue should be selected by the scientist considering the development stage of the drug product and with focus on the respective scope of the experiment. Meanwhile, different guidance documents have been issued by competent authorities that address that the drug loading, when developing generic transdermal patches should be challenged during product development [25, 48]. Based on the principal release mechanism of diffusion certain amounts of the drug remain within the patch at the end of the application period. The scientists, therefore, should seek to minimize the drug loading of the patch, resulting in reduced residual drug content upon patch removal.

16.3 Transdermal Drug Development: Manufacturing Process Development

During development of transdermal drug products, batches on different scales are to be produced. Usually, starting within the galenical development laboratory, small-sized batches of various formulations are prepared on scales in gram ranges referring to liquid coating masses. Subsequently, the manufacturing process needs to be transferred into a GMP environment for up scaling to deliver clinical trial material and finally, after formal process validation is completed, to manufacture commercial supplies.

After the formulation of the transdermal patch is defined and the pilot meets its target product profile regarding any quality aspects, a thoroughly designed process development phase is mandatory to ensure that the final process is robust, stable, and efficient.

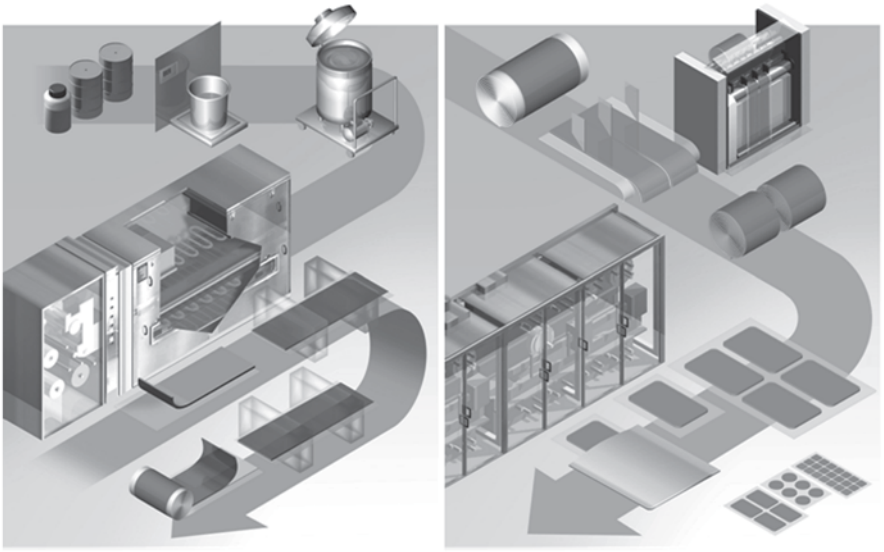


Fig. 16.7 Schematic drawing of production process using the solvent evaporation technique for single-layer matrix systems. *Left* (beginning *top left*): Weigh in, mixing, coating, drying, and lamination. *Right* (beginning *top left*): slitting of mother rolls to daughter rolls and die-cutting and pouch packaging

A typical production process of transdermal matrix patches—depending on the final design of the drug product, as, for example, the number of matrix layers—can generally be divided into the following production steps:

- Preparation of the (liquid) coating mass containing the active pharmaceutical ingredient, the matrix forming polymer, excipients, if any, and the process solvents
- Coating of the coating mass onto a substrate (e.g., release liner) and drying the wet film to remove the process solvents yielding a thin matrix layer
- Lamination of the matrix layer with an additional substrate (e.g., backing foil) to yield laminate mother rolls
- Slitting of laminate mother rolls to smaller daughter rolls that can be further processed
- Die-cutting, converting, and pouch packaging to individually packed units.

Obviously, in the case of transdermal patches with a more complex design including several matrix layers, a multitude of coating masses may be prepared and subsequent production steps such as coating, drying, and lamination are carried out more than once. In Fig. 16.7, the manufacturing steps of the most basic type of transdermal patches—the single-layer matrix system—are illustrated which are also described in more detail. Additionally, potential complications which may occur and issues which should be considered during the process development are outlined.

Early development work and especially initial process development work on production-scale equipment with a small batch size can be considered as a part of

the first stage—process design—of process validation according to the FDA Guidance for Industry "Process Validation" [49]. Process development shall provide detailed knowledge of the new drug product, shall reveal critical production steps, and shall deliver data regarding critical process parameters. This knowledge gained in line with the first process development batches shall finally lead to the exact definition of the manufacturing process.

For a standard manufacturing process of transdermal matrix patches, the preparation of the coating mass can be considered as the only discontinuous and therefore real scale-dependent step. It comprises the weighing, adding, and mixing of all components, including the active ingredient, matrix forming polymers, excipients as, for example, chemical permeation enhancers, crystallization inhibitors, tackifier, plasticizers, cross-linkers for the polymer or preservatives and the process solvents. Typically, the components including the active are separately weighed in and subsequently added to the adhesive polymer mixture. Care should be taken when the order of excipient addition is defined to avoid temporary incompatibilities, as, for example, phase separation or cluster formation within the coating mass. In some cases, the preparation of a premixture of some components may be helpful, that is then added to the polymer mixture. The nature and amount of process solvents needs to be thoroughly determined during formulation development. First, process solvents have to be removed during further production; therefore, all process solvents must be volatile enough, compatible with each other if more than one solvent is used, and toxicologically acceptable; class I solvents with (potential) carcinogenic properties must, therefore, be avoided. Also, in most cases, the drug substance is dissolved within the patch matrix. As a consequence, care should be taken that the process solvents offer sufficient solubility to completely dissolve the required amount of drug. The amount of process solvents also governs the viscosity of the coating mass, which directly influences the coatability of the wet mass. Extremes, too-low or too-high viscosity ranges may significantly complicate the subsequent coating process and may require a change of the coating technique or an adjustment of the solvent system. The viscosity can be altered by reducing or increasing the solids content of the coating mass or by adding viscosity affecting excipients like povidone. However, the addition of excipients to influence the viscosity should have been considered at an earlier development stage as they are still present in the dried matrix where it may contribute to the quality characteristics of the drug product. If applicable, the mixing process should be carried out under adequate temperature control.

The critical parameters of coating mass preparation typically are the mixing temperature, mixing time, and the applied shear forces of the mixing system. Since these parameters are scale dependent, they have to be evaluated accurately for different batch scales in different equipment systems in order to obtain a homogenous coating mass, which is one of the most important aspects for the quality of the drug product.

The stirring system within the mixing vessel also has to be scaled up, as the shear forces in a lab-scale mixing system usually differ from the mixing system of production scale. The mixing time and agitation speed need to be thoroughly investigated

depending on the respective equipment, including mixing tank and stirring device as well as batch size to safeguard that the individual components of the coating mass are uniformly distributed. The critical properties of the coating mass, which have an impact on the product quality, are appearance, viscosity, drug content, and especially the homogeneity. For systems designed with completely dissolved drug, it is highly recommended to microscopically check the coating mass for the absence of undissolved drug particles. Drug that is not fully dissolved may remain in an undissolved state within the matrix and trigger further drug crystallization, depending on the degree of matrix saturation. As a consequence, if significant amounts of drug crystals are present in a drug product that intentionally should contain the drug in a dissolved state, the product performance cannot be assured. Furthermore, if the drug is not homogenously distributed within the coating mass, the laminate which is subsequently produced will also lack homogenous drug distribution, which may lead to failure in uniformity of dosage units test as generally requested for transdermal patches by pharmacopoeias (e.g., European Pharmacopeia, United States Pharmacopeia, and Japanese Pharmacopeia).

Subsequently, the coating mass is typically cast onto the siliconized or fluorpolymer-coated side of a release liner made of polyethylene therephthalate or other plastic material, but also a coating onto the backing foil is possible and in some cases even advantageous based on formulation or the further manufacturing steps. Care must be taken that the coating mass is compatible with the substrate to ensure a homogeneous picture of the final laminate. There are different techniques of coating onto the carrier and the coating method is preferably selected with regards to, for example, the viscosity of the coating mass and/or the required dried matrix weight.

After the coating mass is spread evenly onto the web, the laminate is guided through a series of drying ovens, where the solvents are evaporated by heated air and the dried matrix is directly laminated with a further substrate, for example, the backing foil or a release liner foil. The complete process is conducted in a continuous manner and can therefore be considered as independent from the batch scale. The amount of coating mass that is to be coated impacts only on the process time. During the coating and drying process, a homogeneous laminate should be produced. Once the homogeneity of the coating mass has been confirmed, the thickness or the area weight of the matrix layer is directly correlated with the assay of the drug and should therefore be homogeneous in the cross machine direction as well as in the machine direction, that is, throughout the complete coating process.

The drying parameters have a major impact on relevant critical quality attributes and primarily define the quality of the drug product. Therefore, high attention should be paid to this process step. A transfer from a development laboratory to a production line requires proper planning and evaluation of the experiments. The drying performance of a continuous coating line is mainly governed by the drying time (dwell time), which is a result of the length of the drying compartments of the machine and the speed with which the web is driven through the ovens, as well as by the drying temperatures and the air circulation. Furthermore, the thickness of the coating is relevant. For a specified thickness of coating the drying performance (with respect to amount of evaporated solvent) is increased, when the web speed is

Table 16.6 Example of residual solvents present in dried matrix of an active laminate and the corresponding placebo laminate

Formulation	Placebo	Verum
Web speed (m/min)	1.5	1.2
Drying temperatures of four ovens (°C)	40/50/60/60	40/50/65/65
Residual 2-propanol (ppm)	536	4453

Placebo: Formulation not containing the active pharmaceutical ingredient
Verum: Formulation containing the active pharmaceutical ingredient

reduced and the temperatures and the air circulation are increased [50]. Although first process trials can be carried out with a placebo mass, additional trials with a coating mass containing the active are mandatory. Table 16.6 presents an example where two coating masses only differing by the presence of the active drug were coated in the same coating line by applying comparable drying conditions. Here, the extent of residual 2-propanol in the dried matrix was strikingly increased when the active drug was present—even when the drying conditions can be considered as somewhat more powerful.

The concentration of the drug within the formulation may influence the extent of those effects.

A high web speed connected with high temperatures may enable a quick and economic drying process, however, the process solvents need to be properly removed and complications such as bubble formation in the laminate should be avoided. Thus, the drying parameters are often a compromise of preferably somewhat lower temperatures to avoid inhomogeneities within the laminate, degradation of components or evaporation of the active or excipients and a high web speed to minimize the process time. An almost complete drying of the laminate is particularly important, if not only class III solvents (solvents with low toxic potential) but also solvents of class II (solvents that need to be limited) according to the ICH guideline Q3 C "Impurities: Guideline for Residual Solvents" [51] are employed. The use of class II solvents should ideally be avoided or if not possible at least be limited with narrow specification ranges to ensure the patient safety.

A preferred effect is that also potential residual monomers derived from polymeric material may be quantitatively evaporated. However, also other excipients—or the drug itself—that should be kept within the formulation may be partly evaporated during this process step. Substances with high volatility can evaporate to significant extent during the drying step (>20 %), thus altering the ratios of the components and in case of the evaporation of the active or permeation enhancing agents even the permeation characteristics of the drug product are disturbed. Therefore, it is essential to analyze the key components within the formulation such as the drug itself and relevant functional excipients as, for example, permeation enhancers as far as those can be considered as at least partly volatile. Consequently, analytical methods for quantification of the functional excipients have to be developed at an early formulation development stage and the final formulation has to be characterized

adequately. If components evaporate in a significant extent and it is not possible to adapt the drying parameters to quantitatively keep them within the formulation, it might become necessary to introduce a production overage to meet the targeted concentration in the drug product afterwards. A proper justification should be available for any overage. It is not advisable to assume that the components evaporate in the same extent in lab scale as in production scale manufacturing, since the process performance is usually not transferable on a 1:1 basis. Furthermore, in the framework of upcoming process optimization the drying parameters may be altered or the process is to be transferred to another coating and drying line. Then, those data may become of utmost importance to allow for proper comparison of the product manufactured under the different conditions.

As already mentioned, the drying process has a major impact on the quality of the drug product. The following attributes of the final transdermal patch that are usually considered as critical to quality may be influenced: appearance, assay, content uniformity, drug release, related substances, residual solvents and monomers, as well as potentially the adhesive properties.

In the context of the evaluation and establishment of drying parameters, the implementation of Design of Experiments (DoE) may be beneficial to assess the relationship of the critical parameters and to gain comprehensive knowledge of the process to establish ranges for the various process parameters. A deep understanding is an essential objective of the process development to define the critical process parameters and an appropriate design space within which the process operates in a robust manner to manufacture a drug product meeting its specification.

Having passed the drying ovens, the dried drug-containing matrix is covered with a backing film or release liner, respectively, wound up to a roll and stored for further processing. If the drug product tends to show cold flow, it is advisable to roll up the laminate with as little tension as possible. Once the homogeneity of the coating mass has been demonstrated, the area weight of the dried laminate can be considered to correlate with assay of the drug. It can be accepted that intermediate controls focus on appearance of the laminate and area weight, as far as other critical quality attributes are controlled on the final drug product.

The next process step usually comprises a downsizing of the mother roll to daughter rolls often dependent of size of the final drug product that is to be manufactured to optimize the yield. Different dosage strengths are usually produced from the same intermediate laminate; the dosage strength is then only defined by the surface area of the patch. This process step is connected with rather low risks.

The final process step of the manufacturing of the patch itself are the die-cutting, converting and pouch packaging steps which can often be performed within one single piece of equipment. Depending on the final patch design, for example, peel-off aids are slit into the release liner to allow convenient handling and an imprint for identification purposes of the drug product is printed onto the patch backing. Other features may be introduced to the drug product in parallel.

To a certain extent and depending on the composition almost all transdermal patch formulations exhibit cold flow. Depending on the degree the formulation tends to cold flow, sophisticated packaging designs may become necessary. Cold

flow describes a change of dimension without applying relevant stress to the formulation resulting in creeping of the polymeric matrix. This, in the worst case, may result in the patch sticking to the primary container making it inconvenient to use. Therefore, different approaches have been introduced into marketed formulations.

One option is the use of segregation sheets, basically impregnated (e.g., siliconized) foils put on top of the patch and which are removed prior to application. Also, features have been developed to increase the distance between the inner sachet layer and the patch so that potential cold flow of the matrix cannot get into contact with the packaging foil. For example, patches situated on an oversized release liner may be encircled with dimples that act as a mechanical spacer for the packaging foil. Alternatively, the packaging foil itself may offer an embossment positioned over the backing of the patch so that the packaging material is hindered to contact the patch border, where cold flow of the matrix may be present.

Summing up, a manufacturing process for transdermal matrix patches comprises multiple steps. Some of them are scale dependent and some of them are scale independent. Depending on the specific design of each single process step, critical process parameters can be defined whose variability have potential impact on critical quality attributes and therefore needs appropriate monitoring or control. Table 16.7 gives an exemplary overview in how far critical process parameters may be connected with critical quality attributes of the drug product. It is pointed out that these correlations need to be established individually for each drug product manufactured with its specific production process.

16.4 Conclusion

In conclusion, the development of effective and attractive transdermal dosage forms remains challenging. *In vitro* skin permeation studies are a useful and essential development tool for screening of various formulations to establish a ranking and add relevant information to select those formulations with satisfactorily performance. Moreover, for comparative analysis of, for example, a formulation under development and a reference listed drug or after implementation of changes to the formulation, skin permeation studies are of high value. However, during data evaluation it should be kept in mind that these data are derived from *in vitro* experiments that may not completely reflect the conditions of the *in vivo* situation. These data should, therefore, be interpreted with certain caution. *In vitro* dissolution should be considered solely as a quality control tool to demonstrate consistent quality of the transdermal patch during shelf life as well as from batch to batch. Most likely, the *in vitro* dissolution cannot be correlated with the performance of the product *in vivo*.

Already during early development, efforts should be made to establish a suitable manufacturing process. The process itself, its critical process parameters and its impact on critical quality attributes need to be well understood to allow the design of a robust and stable process and to assess changes to the process that may become necessary during scale up or life cycle management.

Table 16.7 Summary of critical process parameters and potentially affected critical quality attributes of the drug product

Process step	Critical process parameter	Scale dependency	Critical quality attribute of the drug product
Preparation of API containing coating mass	Mixing time Mixing speed Mixing temperature (Mixing equipment)	Yes	Appearance Content uniformity (Assay of API and functional excipients)
Manufacturing of laminate	Web speed Temperatures Fan speed	No	Appearance Assay of API and functional excipients Content uniformity Related substances Drug release Residual solvents (Adhesive properties as adhesive strength, separation force, tack) (Residual monomers)
Slitting	None	No	None
Punching and pouching	Sealing temperature Sealing pressure Sealing time	No	Tightness of pouch—and in consequence stability of product such as: Appearance Assay Related substances Drug release Microbiological purity Adhesive properties of the product (reflected by adhesive strength, separation force and tack).

API active pharmaceutical ingredient

References

1. Yum SI. Transdermal therapeutic systems and rate controlled drug delivery. Med Prog Technol. 1989;15(1/2):47–52.
2. Cleary GW (1993) The first two decades of transdermal delivery systems and a peek into the 1990's. In: Gurny R, Teubner A, editors. Dermal and transdermal drug delivery. APV Paperback, vol 31. Wissenschaftliche Vertragsgesellschaft Stuttgart, pp 13–32
3. Bos JD, Das PK, Kapsenberg ML. The skin immune system (SIS). In: Bos JD, editor. Skin immune system (SIS): cutaneous immunology and clinical immunodermatology. 2nd ed. Boca Raton: CRC Press; 1997. pp. 9–16.
4. Naik A, Kalia YN, Guy RH. Transdermal drug delivery: overcoming the skin's barrier function. Pharm Sci Technol To. 2000;3(9):318–26.

5. Pfister WR Transdermal and dermal therapeutic systems: current status. In: Gosh TK, Pfister WR, Yum SI, editors. Transdermal and topical drug delivery systems. Buffalo Grove: Interpharm Press Inc; 1997. pp 33–112 (Pharmacopoea Europaea 7.8, Chapter 2.9.4–1)
6. IMS Health; MIDAS Database. Pfeiffer RF (2002) potential of transdermal drug delivery in Parkinson's disease. Drug Aging. 2013;19(8):561–70.
7. Pfeiffer RF. Potential of transdermal drug delivery in Parkinson's disease. Drug Aging. 2002;19(8):561–70.
8. Stocchi F. Prevention and treatment of motor fluctuations. Parkinsonism Relat D. 2003;9:73–81.
9. Metman LV, Gillespie M, Farmer C, et al. Continuous transdermal dopaminergic stimulation in Parkinson's disease. Clin Neuropharmacol. 2001;24(3):163–9.
10. Olanow CW. Present and future directions in the management of motor complications in patients with advanced PD. Neurology. 2003;61 (Suppl 3):24–33.
11. Chase T. Levodopa therapy: consequences of the nonphysiologic replacement of dopamine. Neurology. 1998;50(5):17–25.
12. Subedi RK, Oh SY, Chun MK, et al. Recent advances in transdermal drug delivery. Arch Pharm Res. 2010;33(3):339–51.
13. Yeoh T. Profiles of recently approved transdermal drug delivery systems (TDDS) Part II: matrix-type fentanyl transdermal systems—design variation and delivery attributes. Transdermal Mag. 2011;3(5):9–15.
14. Hadgraft J. Passive enhancement strategies in topical and transdermal drug delivery. Int J Pharm. 1999;184:1–6.
15. Pellett M, Raghavan SL, Hadgraft J. et al. The application of supersaturated systems to percutaneous drug delivery. In: Guy RH, Hadgraft J, editors. Transdermal drug delivery. 2nd ed. New York: Marcel Dekker Inc; 2003. pp. 305–26.
16. Barry BW Lipid-protein-partitioning theory of skin penetration enhancement. J Control Release. 1991;15:237–48.
17. Karande P, Jain A, Ergun K, et al. Design principles of chemical penetration enhancers for transdermal drug delivery. Proc Natl Acad Sci USA. 2005;102:4688–93.
18. Kalia YN, Naik A, Garrison J, et al. Iontophoretic drug delivery. Adv Drug Deliv Rev. 2003;56:619–58.
19. Mitragotri S, Blankschtein D, Langer R. Transdermal drug delivery using low-frequency sonophoresis. Pharm Res. 1996;13(3):411–20.
20. Mitragotri S, Kost J. Low-frequency sonophoresis. Adv Drug Deliver Rev. 2000;56:589–601.
21. Denet A, Vanbever R, Préat V. Skin electroporation for transdermal and topical delivery. Adv Drug Deliver Rev. 2004;56:659–74.
22. Doukas AG, Kollias N. Transdermal delivery with a pressure wave. Adv Drug Deliver Rev. 2004;56:559–79.
23. Henry S, McAllister DV, Allen MG. Microfabricated microneedles: a novel approach to transdermal drug delivery. J Pharm Sci. 1998;88(9):948.
24. Prausnitz MR. Microneedles for transdermal drug delivery. Adv Drug Deliv Rev. 2004;56:581–7.
25. EMA European Medicines Agency Draft guideline on quality of transdermal patches. London, EMA/CHMP/QWP/911254/2011. 2012.
26. Schreiber S, Mahmoud A, Vuia A, et al. Reconstructed epidermis versus human and animal skin in skin absorption studies. Toxicol in Vitro. 2005;19:813–22.
27. Van Ravenzwaay B, Leibold E. A comparison between in vitro rat and human and in vivo rat skin absorption studies. Hum Exp Toxicol. 2004;23:421–30.
28. Park CW, Son DD, Kim JY, et al. Investigation of formulation factors affecting in vitro and in vivo characteristics of galantamine transdermal system. Int J Pharm. 2012;436:32–40.
29. Lee G, Parlicharla P. An examination of excised skin tissues used for in vitro membrane permeation studies. Pharm Res. 1986;3(6):356–9.
30. El-Kattan A, Asbil CS, Haidar S. Transdermal testing: practical aspects and methods. Pham Sci Technol To. 2000;3(12):426–30.

31. Simon GA, Maibach HI. Relevance of hairless mouse as an experimental model of percutaneous penetration in man. Skin Pharmacol Appl. 1998;11:80–6.
32. Kligman AM, Christophers E. Preparation of isolated sheets of human stratum corneum. Arch Dermatol. 1963;88:702–5.
33. Bronaugh RL, Stewart RF. Method for in vitro percutaneous absorption studies VI: preparation of the barrier layer. J Pharm Sci. 1986;75(5):487–91.
34. Ponec M, Boelsma E, Weerheim A, et al. Lipid and ultrastructural characterization of reconstructed skin models. Int J Pharm. 2000;203(1/2):211–25.
35. Ponec M, Boelsma E, Gibbs S, et al. Characterization of reconstructed skin models. Skin Pharmacol Appl Skin Physiol. 2002;15(Suppl 1):4–17.
36. Schmook FP, Meingasser JG, Billich A. Comparison of human skin or epidermis models with human and animal skin in in vitro percutaneous absorption. Int J Pharm. 2001;215(1/2):51–6.
37. Schäfer-Korting M, Bock U, Diembeck W, et al. The use of reconstructed human epidermis for skin absorption testing: results of the validation study. Altern Lab Anim. 2008;36(2):161–87.
38. ICH International conference on harmonisation. ICH guideline harmonised tripartite guideline: Guideline for good clinical practice E6(R1). 1996.
39. USP United states pharmacopeia 36/NF31, General Chapter 724 Drug release.
40. European Pharmacopoeia 8.0, Chapter 2.9.4-1 Dissolution test for transdermal patches—disk assembly method.
41. FDA Food and drug administration. Guidance for industry: dissolution testing of intermediate release solid oral dosage forms. 1997.
42. FDA Food and drug administration. Guidance for industry: Waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system. 2000.
43. EMA European Medicines Agency. Guideline on the investigation of bioequivalence. London, CPMP/EWP/QWP/1401/98. 2010.
44. EMA European Medicines Agency. Note for Guidance on Quality of Modified Release Products B: Transdermal Dosage Forms Section I (Quality). London, CPMP/ QWP/604/96. 1999.
45. Buskirk GA, Arsulowicz D, Basu P, et al. Passive transdermal systems whitepaper incorporating current chemistry, manufacturing and controls (CMC) development principles. AAPS Pharm Sci Tech. 2012;13(1):218–30.
46. Kou JH, Roy SD, Du J, et al. Influence of receiver fluid pH on skin flux of weakly acidic and basic drugs. Pharm Res. 1993;7:986–90.
47. Roy SD, Roos E, Sharma K. Transdermal delivery of buprenorphine through cadaver skin. J Pharm Sci. 1994;83(2):126–30.
48. FDA Food and drug adminstration. Guidance for industry: residual drug in transdermal and related drug delivery systems. 2011.
49. FDA Food and drug adminstration. Guidance for industry: process validation: general principles and practices. 2011.
50. Lipp R, Heimann G. Statistical approach to optimization of drying conditions for a transdermal delivery system. Drug Dev Ind Pharm. 1996;22(4):343–8.
51. ICH International conference on harmonisation. ICH guideline harmonised tripartite guideline: impurities: guideline for resiudal solvents Q3C(R5). 1997.

Chapter 17
Transdermal Drug Delivery Systems

Regulatory Considerations: NDA and ANDA Requirements

Vinod P. Shah and Roger L. Williams

17.1 Introduction

The first transdermal drug delivery system (TDS) appeared in the USA in 1979. It contained scopolamine and was approved for treatment of motion sickness. Since that time, a small number of TDS drug products have reached the US market (Table 17.1). The primary clinical argument for a TDS is that it provides clinical advantages compared to other routes of administration. Overall, the TDS has been a step forward, with significance both for the pharmaceutical industry and for medical practice and with many opportunities based on current research efforts.

Although applied topically, drug molecules in a TDS undergo percutaneous penetration/absorption and are taken up by the microcirculation in the dermal papillary layer for transport to target tissue via the systemic circulation. As a practical matter, a TDS is thus no different from administration of a drug via oral, inhalation, buccal, nasal, or other routes that lead to entry of a drug substance into the systemic circulation. During development, the general objective for a TDS is to optimize therapeutic efficacy and minimize side effects. Applied to the skin, a TDS is relatively noninvasive and has significant advantages compared with other routes of administration. These include:

- Possible improved therapeutic outcomes with greater safety compared to drugs delivered by other routes
- Improved patient compliance and practitioner acceptance
- Ease of removal of the dosage form in the event of an adverse drug reaction
- Availability of sophisticated formulation technology to create a reliable, pharmaceutically elegant TDS
- Avoidance of loss of drug substance in the gastrointestinal tract, e.g., through acid degradation in the stomach and first-pass elimination in liver

V. P. Shah (✉)
111309 Dunleith Place, North Potomac, MD 20878, USA
e-mail: Dr.vpshah@comcast.net

R. L. Williams
United States Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852-1790, USA

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_17

Table 17.1 Transdermal systems (TDS, patches, films) marketed in the USA

Drug name	Dosage form	Year	Therapeutic activity
Buprenorphine	Film, TDS	2001	Analgesic, chronic pain
Clonidine	Transdermal	1984	Hypertension
Diclofenac epolamine	Topical patch	2007	Analgesic
Estradiol (0.014 mg/24 h)	Transdermal	1986	Menopausal symptoms
Estradiol + norethidrone	Transdermal	1988	Menopausal symptoms
Ethinyl estradiol + noreigestromin	Transdermal	2001	Contraception
Fentanyl	Transdermal	1990	Chronic pain
Granisetron	Transdermal	2008	Antiemetic
Lidocaine	Topical patch	1995	Analgesic
Menthol/methyl salicylate	Topical patch	2008	Analgesic[a]
Methylphenidate	Transdermal	2006	ADHD[b]
Nicotine	Transdermal	1991	Smoking cessation
Nitroglycerin	Transdermal	1981	Angina pectoris
Oxybutynin	Transdermal	2003	Overactive bladder
Rivastigmine	Transdermal	2007	Dementia
Rotigotine	Transdermal	2007	Parkinson's disease
Scopolamine	Transdermal	1979	Motion sickness
Selegiline	Transdermal	2006	Depression
Testosterone	Transdermal	1993	Testosterone deficiency

[a] Introduced in Japan as Salonpas in 1934
[b] Attention deficit hyperactivity disorder

- Zero rather than first-order drug delivery with approximation of steady state drug concentrations in the systemic circulation and potential reduction in variability in blood levels compared to orally administered drugs
- Extension of market protection for off-patent drugs or drugs that are losing patent or exclusivity protection

The greatest challenge for TDS development is that only a small number of drugs are amenable for consideration. To allow transdermal penetration, a TDS drug substance generally should be a molecule of low molecular weight with high lipophilicity. Research is underway to increase the number of drug molecules that can be administered transdermally. With the advent of new technologies and advancement of science, second- and third-generation TDS formulations are approaching the market place. While there are many aspects of TDS medicines that merit consideration, this chapter focuses on regulatory considerations for a TDS submitted either as a New Drug Application (NDA) or as an Abbreviated New Drug Application (ANDA), with emphasis on bioavailability (BA) and bioequivalence (BE) documentation. For purposes of discussion a TDS also includes films and patches.

17.2 New Drug Applications

A TDS intended for the treatment or prevention of a systemic disease is considered a new drug under the US Food, Drug and Cosmetic Act and as such requires a full NDA for review by the Food and Drug Administration (FDA) for consideration of marketing approval [1, 2]. Because a TDS delivers drug over an extended period of time, it therefore must demonstrate controlled/extended release characteristics to support drug labeling. For all currently available TDS in the US market, approval for administration via other routes has preceded approval of a TDS formulation. In this setting, the NDA submission requirements may be substantially reduced, given that both safety and efficacy of the drug substance have been previously established. Thus, the main challenge in developing a TDS may lie in demonstrating that a changed exposure pattern, as reflected in BA measures, may result in improved efficacy and/or safety compared to administration of the drug substance by another route of administration. Typically, the predicate route has been oral.

17.2.1 Biopharmaceutics: General Issues

Knowledge of the biopharmaceutic properties of the drug intended for a TDS formulation is important. This is especially true for the pharmacokinetic and pharmacodynamic relationships that support the therapeutic activity of the drug, as reflected in expected clinical outcomes. A transdermal formulation delivers drug at a relatively constant rate to a relatively small circumscribed area of the skin. While the patient-controlled application of a TDS may offer significant clinical and marketing advantages, such a system also brings substantial clinical and regulatory questions, which should be addressed during the development process. Many of these questions arise because of the fundamental differences in BA exposure patterns that arise following application of a TDS in comparison to other routes of delivery. Biopharmaceutical issues specific to a transdermal formulation relate to (1) influence of the site of application on bioavailability; (2) effect of repeated application of the transdermal formulation at the same application site, including change in drug absorption associated with local irritation, inflammatory, or hypersensitivity reactions; (3) appropriate contact of the TDS as applied by the patient to the skin; (4) barrier properties of the skin, including whether the skin contributes to the control of the rate of entry of the drug into the systemic circulation; and (5) effects of occlusion of the site of application relative to both BA and irritancy/hypersensitivity.

Intimate contact with skin is essential to ensure drug absorption from a transdermal formulation and is facilitated by use of adhesives. Thermodynamic considerations are important as well and allow the possibility of formulation optimization to maximize transfer to drug across a localized area of the epidermis. Addition of an absorption enhancer to a transdermal formulation is possible but often brings additional concerns about the systemic effects of the enhancer itself.

17.2.2 Pharmacokinetics: General Issues

A drug can enter the body typically through five main routes or administration: oral, parenteral, topical, mucosal, and through inhalation [3]. The oral route is typically considered to facilitate entry of a drug into the systemic circulation, while the skin, primarily because of the barrier properties of the stratum corneum, is generally thought to impede drug access to the body. Gastrointestinal and epidermal/dermal membranes, however, have intrinsic permeability characteristics that impede entry of a drug to varying degrees depending on physicochemical characteristics of the drug and barrier properties of the respective membranes. If sufficiently important, these characteristics can preclude administration of a drug via either the oral or the transdermal route. For either an orally or transdermally administered drug, other, and at times formidable, barriers may exist to prevent access to the systemic circulation. Drugs administered orally are exposed to numerous digestive processes, including extremes of pH, mucosal, circulatory, and hepatic metabolic enzymes, and mechanical and mucous barriers, all designed to degrade and render absorbable dietary constituents. For a drug administered transdermally, equally formidable but at times substantially different barriers can block drug entry. The first of these is the highly keratinized layer of the stratum corneum, followed by the viable epidermis and dermis, and finally by the microcirculatory vasculature of the skin. As with an orally administered drug, a transdermally administered drug may encounter metabolic processes in the epidermis, dermis, and vasculature, but these degrading systems are likely to be less extensive compared to those that exist in the gastrointestinal tract. For this reason, the primary barrier to systemic drug access for a transdermally administered drug appears to be the stratum corneum.

Pharmacokinetic/BA studies recommended by the FDA for a transdermal drug formulation are designed to assess rate and extent of absorption of drug in the body across epidermal/dermal barriers compared to a suitable reference standard, typically given orally. These studies should delineate important pharmacokinetic parameters (clearance, volume, half-life, protein binding, blood/plasma concentration ratio, nonlinearity in parameters of drug absorption and elimination), assuming these have not previously been defined. Drug metabolism and chiral effects should be defined and compared to those observed following delivery of the drug via other routes of administration. Because most drugs now developed for transdermal delivery were previously developed for other routes of administration, changes in drug metabolism and chiral effects that might occur with transition to transdermal delivery should be understood. The skin is a viable organ that is capable of metabolizing certain drugs, and this capability, when present, may pose special regulatory requirements. As has been noted for nitroglycerin, which possesses two potentially active metabolites (1,2- and 1,3-dinitroglycerin), metabolite-to-parent ratios change depending on whether the drug is administered intravenously, orally, or transdermally [4]. Under these circumstances, the pharmacodynamics of a drug may change depending on the route of administration. Similarly, change in enantiomer ratios might occur depending on the route of administration and saturation of metabolizing enantiomer enzymes. Change in enantiomer ratio depending on rate and route

of administration might also lead to a change in the pharmacodynamic profile of a drug. Additional pharmacokinetic and BA studies defining demographic (e.g., age, gender, and race) and special population (e.g., renal and hepatic) effects may be required. Finally, study of drug absorption from the transdermal system when applied to diseased, irritated, or sensitized skin may be necessary.

17.2.2.1 *In Vivo* Studies

Despite differences between the presystemic barriers that a drug encounters in the epidermis and dermis and those that it encounters in the gastrointestinal tract, general regulatory pharmacokinetic and BA studies required for a TDS should be comparable to those required for an extended release formulation. Although specific requirements should be reviewed withthe FDA, some general statements are possible. As noted in an FDA guidance entitled *Format and Content of the Human Pharmacokinetics and Bioavailability Section of an Application* [5], five general types of studies are recommended to define the pharmacokinetics and BA of a TDS: (i) pilot studies to assess drug absorption and elimination after transdermal delivery; (ii) BA studies; (iii) pharmacokinetic studies; (iv) other *in vivo* studies as necessary; and (v) *in vitro* dissolution/drug release studies.

In vivo BA and pharmacokinetic parameters of a TDS should be fully characterized in comparison to those for an intravenous dose. When intravenous administration is not possible, oral and/or intramuscular administration may be employed. An initial step in the development of a transdermal formulation will be the determination of likely sites of application and the intended size of the transdermal system. The site and area of application should be optimized to the extent possible. Because percutaneous penetration and absorption of a drug from a transdermal formulation is area-dependent and may be site-dependent, pivotal pharmacokinetic and BA studies should be carried out at the proposed sites of application, with evaluation of each site for pharmacokinetics/BA. Assessment of the active drug delivery surface area of a transdermal formulation relative to target blood or plasma concentrations is an important determination and is a point at which many drugs become defined as unsuitable for transdermal delivery. Associated with this primary determination should be dose proportionality studies (active surface area of the formulation versus attained blood/plasma concentrations) and an estimate as to whether the skin or the transdermal system is rate-limiting relative to drug absorption. Lag time in attaining systemic drug delivery and depot effects following removal of a transdermal formulation should also be investigated.

17.2.2.2 *In Vitro* Studies

Two types of *in vitro* tests, product quality tests, and product performance tests, should be performed for all TDS [6]. Product quality tests specific to a TDS formulation include adhesion test, peel adhesion test, peel test, release liner test, tack

test, and leak test. The product performance test may include a drug release test. An *in vitro* drug release method for a dosage form provides a quality control method to assure batch-to-batch uniformity in drug release. USP general chapter <724> describes three procedures for determining drug release of a TDS. Apparatus 5 procedure developed by FDA is the simplest system applicable to all types, sizes, and strengths of marketed TDS. Apparatus 6 and 7 are product specific. Correlations between *in vitro* release characteristics and *in vivo* performance for a transdermal product may be useful to the pharmaceutical scientist but, at this time, are unlikely to supplant *in vivo* requirements for assessment of BA and bioequivalence.

17.2.3 Clinical Pharmacology: Considerations

Clinical pharmacology requirements for an NDA were determined in the 1985 Investigational New Drug (IND) /NDA rewrite and have been described in the 1988 CDER guideline *Format and Content of the Clinical and Statistical Sections of an NDA*. Clinical pharmacological studies for an NDA can be divided into two general classes: pharmacokinetic and pharmacodynamic. Pharmacokinetic study designs should assess drug–drug, drug–disease, and drug–demographic interactions. Specific applications of these recommendations to a transdermal formulation might include, for example, study of the influence of coadministration of other dermatological products on the drug absorption from the transdermal system, evaluation of the effect of dermatological disease states on drug absorption, and assessment of transdermal absorption in different age groups, in males versus females, and in individuals with different degrees of skin pigmentation. Pharmacodynamic clinical pharmacological studies should be designed to characterize the primary dose–response relationship (starting dose, duration of activity, maximum dose, requirements for dose titration), effects of enzymatic induction, and tolerance. Additional studies of drug–disease, drug–demographic, drug–drug, and other systemic pharmacodynamic effects (cardiovascular, renal, gastrointestinal, neurological, hematological, endocrinological, of the immune system) may be required. Careful attention to clinical pharmacological study designs and review of these designs with the appropriate FDA office can substantially reduce drug development time and costs [7].

17.2.4 Efficacy and Safety Requirements

The safety, efficacy, and pharmacological/toxicological requirements for a TDS are generally the same as for drugs intended for other routes of administration. Scientific data from adequate, well-controlled clinical trials are required to support clinical safety and efficacy claims for a transdermal drug formulation. The efficacy requirements may be reduced if the active ingredient is already approved for the same use by another route or administration. Intended label claims of superior efficacy or other advantages that accrue through transdermal administration must be

substantiated with acceptable data. Drugs with a narrow therapeutic index may need additional specialized studies. If systemic toxicity data for the active ingredient are already established, animal toxicity testing may be reduced or eliminated. Safety data must be investigated in relation to local and systemic toxicity of the drug substance.

Intimate contact with skin is essential to ensure drug absorption from a transdermal formulation. This is facilitated by use of adhesives. These adhesives may cause contact dermatitis or hypersensitivity. Irritation and perhaps hypersensitivity from a TDS may also be a function of the occlusive nature of the preparation, leading to accumulation of sweat and increase in subpatch microbial growth [8]. Individual ingredients at times may not show local irritation/contact dermatitis but when applied as a transdermal patch for a longer duration may show significant irritation. For these reasons, safety studies specific to a transdermal drug formulation should investigate propensities for local irritation, contact dermatitis, and hypersensitivity. When the blood level profiles generated by a transdermal formulation are comparable to those generated by a previously established nontransdermal route of administration, systemic safety issues may be ruled out, provided there is no differential cutaneous metabolism.

17.2.5 Studies Required for Transdermal Drug Formulations Approved for Other Routes of Administration

If the toxicity and efficacy data for the drug entity are already well established, blood level comparisons between a transdermal drug formulation and the approved marketed formulation, together with efficacy and local dermatotoxicity study data, may be sufficient to result in approval of the transdermal drug formulation via an NDA. The types of studies required will be determined on a case-bycase basis depending on several factors, including (a) the clinical pharmacology of the drug substance, (b) availability of previously marketed dosage form(s) of the drug, (c) the medical and biopharmaceutics rationale for transdermal drug delivery, (d) presence or absence of skin-specific routes of metabolism and chiral effects, (e) literature data on the drug entity, and (f) prior experience with the drug and/or drug delivery system.

As an example, a marketed scopolamine transdermal formulation was evaluated in comparison with oral and parenteral routes of administration of the drug [9, 10]. After a lag of several hours, the transdermal dosage form delivered scopolamine at a rate comparable to that obtained with the drug administered intravenously over a three-day period. The rate of scopolamine drug delivery was designed to maintain plasma concentrations sufficiently low to achieve an antiemetic effect and minimize drowsiness and tachycardia. Similarly, currently available estrogen and clonidine transdermal systems were approved based on clinical trials and comparative equivalence in blood level profiles between the patches and oral administration. In contrast to these examples, special clinical safety/efficacy studies were required of a

testosterone transdermal patch despite acceptable comparative blood level data because of enhanced cutaneous metabolism, resulting in high hydrotestosterone levels. Similarly, the absence of acceptable clinical data for transdermal nitroglycerin led to efficacy studies. These studies documented the development of tolerance to nitroglycerin with continued transdermal dosing and led to the current label recommendation to remove the system for 12 out of a 24-hour dosing cycle [11].

17.3 Abbreviated New Drug Applications

According to the current FDA policy, pharmaceutical equivalence between a generic is defined in the 'Orange Book' [12]. Mechanism of release, transdermal formulation drug content, and active surface area of the TDS are not considered necessary to define pharmaceutical equivalence, although the pertinence of each factor for a specific transdermal drug formulation will be determined on a case-by-case basis. Transdermal formulations containing chemical penetration enhancement methods can be judged pharmaceutically equivalent to transdermal formulations without enhancers. At this time, the presence or absence of a chemical enhancer is not pertinent to a determination of pharmaceutical equivalence of a transdermal formulation, provided that clinical safety and efficacy data for a specific enhancer have been previously reviewed and approved in FDA's new drug approval process for a transdermal dosage form.

Documentation of BE between the generic and innovator-listed products is necessary to achieve an AB rating in FDA's Approved Drug Products with Therapeutic Equivalence Ratings (Orange Book) [12]. When previously unapproved adhesives, patch materials, and/or inactive ingredients are used in a generic transdermal formulation, skin irritation and other safety data may be required. *In vitro* documentation of reproducible drug release characteristics for a generic transdermal system will also be required. Specific requirements for a generic transdermal formulation should be reviewed with the FDA, which has provided several draft guidances on the topic [13]. The following types of studies are required:

- Pharmacokinetic
- Bioequivalence
- Irritation and sensitization
- Adhesion performance, including a pharmacokinetic or irritation/skin sensitization study

A TDS typically contains a large amount of drug compared to the amount delivered to the patient. This large amount is needed as a driving force for the drug. Due to several mishaps associated with a discarded TDS, the amount of residual drug substance at the end of the labeled use period should be minimized [14].

17.4 Looking into the Future

Scientific and technological advances have raised the level of transdermal drug delivery to a new level of impact in the field of medicine. Prausnitz and Langer have classified the advances into three generations: (1) the first generation produced many TDS formulations using relatively low molecular weight drug substances with high lipophilicity that allows small content of drug in the system; (2) the second generation added chemical enhancers and iontophoresis, expanding opportunities for other molecules; and (3) third generation patches that include novel chemical enhancers and physical enhancers such as ultrasound, thermal ablation, and microneedles, which increase stratum corneum permeability and might enable transdermal delivery of macromolecules and vaccines [15]. Most enhancement approaches in the third generation result in stronger disruption of the stratum corneum and increase skin permeability.

17.5 Conclusions

TDSs containing a previously approved drug or a previously unapproved, new chemical entity may offer numerous clinical and commercial advantages. They also require careful evaluation in order to meet current regulatory approval criteria. Over the years, the FDA has provided general instructions and recommendations via regulations, guidances, and other publications. Applicants intending to submit an NDA or ANDA for transdermal formulations are encouraged to work with the reviewing staff at FDA to develop the necessary database for their proposed transdermal product.

References

1. Shah VP, Skelly JP. Regulatory considerations in transdermal drug delivery systems in the United States. In: Chien YW, Editor. Transdermal controlled systemic medication. New York: Marcel Dekker; 1987. pp. 399–410.
2. Peck CC, Shah VP. FDA requirements for approval of transdermal drug delivery systems. Scott RC, Guy RH, Hadgraft J, Editors. Proceedings of "Prediction of Percutaneous Penetration-Methods, Measurements. Modeling." Oxford: IBC Technical Services; 1989. pp. 286–9.
3. Marshall K, Foster TS, Carlin HS, Williams RL. Development of a compendial taxonomy and glossary for pharmaceutical dosage forms. Pharmacop Forum. 2003;29(5):1742–52.
4. Williams RL, Thakker KM, John V, Lin ET, Liang-Gee W, Benet LZ. Nitroglycerin absorption from transdermal systems: formulation effects and metabolite concentrations. Pharm Res. 1991;8:744–9.
5. FDA/CDER Guideline for Format and content of the human pharmacokinetics and bioavailability section of an application. February 1987.
6. No Author 2013. USP 36/NF 31. General Chapters <3> and <724>. 2013

7. Peck CC, et al. Opportunities for integration of pharmacokinetics, pharmacodynamics and toxicokinetics in rational drug development. Clin Pharmacol Ther. 1992;51:465–73.
8. Hurkmans JFGM, Boddie HE, Van Oriel LMJ, Van Doome H, Junginger HE. Skin irritation caused by transdermal drug delivery systems during long term (5 days) application. Br J Dermatol. 1985;112:461–7.
9. Good WR. Conference Proceedings of Drug Delivery Systems. Pharmaceutical Technology. Salem: Advanstar Communications; 1986. pp 40.
10. Shaw JE, Cramer MP, Gale R. Rate controlled transdermal therapy utilizing polymeric membranes. In: Kydoniens AF, Berner B, Editors. Transdermal delivery of drugs, Vol. I. Boca Raton: CRC Press; 1987. pp. 101–16.
11. Steering Committee, Transdermal Nitroglycerin Cooperative Study. Acute and chronic antianginal efficacy of continuous twenty-four hour application of transdermal nitroglycerin. Am J Cardiol. 1991;68:1263–73.
12. DHHS/FDA/CDER. Orange Book: Approved Drug Products with Therapeutic Equivalence Evaluations. n.d.
13. FDA. FDA Bioequivalence guidelines for transdermal drug delivery systems.n.d. http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidance/ucm075207.htm.
14. FDA Guidance for Industry, Residual drug in transdermal and related drug delivery system. Draft Guidance, August 2010.
15. Prausnitz MR, Langer R. Nat Biotechnol. 2008;26:1261–8.

Part VI
Clinical Studies and Bioequivalence

Chapter 18
Dermal Estradiol and Testosterone Transfer in Man: Existence, Models, and Strategies for Prevention

Aaron Gandhi, K. L. Busse and Howard I. Maibach

18.1 Introduction

Transdermal hormone application allows delivery of a clinically relevant hormone dose with less systemic exposure as compared to oral administration, thus decreasing many of the side effects associated with first-pass metabolism, gastrointestinal absorption, and peak plasma drug concentrations. The positive clinical benefits of transdermal estradiol [1–10] and testosterone [11–15] have been validated. Additionally, transdermal delivery may be a good option for older individuals who are often polymedicated [16]. Transdermal dosing is often available through different vehicles including patch, gel, emulsion, and spray preparations. While hormone patches may provide a protective covering to the dosed chemical, other formulations are applied directly to the skin and may increase the risk of transfer to another person via interpersonal contact.

Topical chemical transfer between a dosed individual and his or her interpersonal contacts including family members, children, partners, and friends is documented [17–20]. While transdermal delivery of estradiol and testosterone may be clinically relevant for the treated individual, unintentional transfer to a close contact can cause hormone imbalance and adverse effects. These imbalances may increase the risk of adverse cardiac events in men; cause hyperandrogenism, hirsutism, acne, coarsening of the voice, clitoris hypertrophy, and male-pattern baldness in women; and can lead to early puberty changes, virilization, and premature epiphyseal closure of the bones in children [21–28]. Cases of skin-to-skin transfer of transdermal testosterone products from father to son have caused precocious puberty and/or pronounced virilization [29–35]. In most instances, clothing barriers have shown to block transfer of testosterone by way of direct skin contact. Washing of the testosterone gel

A. Gandhi (✉) · K. L. Busse · H. I. Maibach
Department of Dermatology, School of Medicine, University of California, San Francisco, CA, USA
e-mail: aarongandhi@gmail.com

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_18

application site after use has been shown to significantly reduce the risk of transdermal transfer in direct skin-to-skin contact [36]. It is important to consider the potential for estradiol or testosterone transfer prior to utilizing transdermal hormone products.

18.2 Human Experiment Transfer Data: *In Vivo*

In vivo human transdermal transfer studies of topical estradiol products have been performed (Table 18.1). Wester et al. [37] conducted an *in vivo* bioavailability study to determine the transfer between six healthy, postmenopausal women dosed with a 0.16-g [14C]-estradiol gel formulation (5-mg [14C]-estradiol radioactivity per 8.5 g of gel) and a group of six healthy men or women. Attempted transfer was by ventral forearm contact by ten rubbing strokes and 15 min of direct skin-to-skin contact occurring 1 h after transdermal application. A protective sleeve was then placed over the dosed site. A ^{14}C-assay was performed on skin washings recovered 24 h after dosing to determine the amount of estradiol transfer. Transfer occurred between dosed subjects and naïve recipients as indicated by a 2.3 ± 2.0% (of applied estradiol dose) wash recovery and a 4.1 ± 3.6% sleeve recovery from the nondosed participants [37]. Additionally, the majority of the topically applied dose remained on the skin surface during the full 24 h dosing period and was, therefore, available for transfer. This observation has previously been attributed to a *mass balance* effect of the topically applied dose, allowing excess chemical to remain on the skin during a typical application period of 24 h [38].

In another gel study, ZumBrunnen et al. [39] conducted a single-center, randomized, open-label, crossover, multiple-dose trial to determine the amount of transfer between 48 healthy, postmenopausal women dosed with a 1.25 g estradiol gel 0.06% formulation (EstroGel©) and a group of nontreated postmenopausal women. Attempted transfer was by posterior arm contact by 3 min of rubbing in an up-down and side-to-side motion and an additional 12 min of direct skin-to-skin contact, occurring 1 h after transdermal application. Transfer was measured by the difference from baseline in the area under the serum concentration–time curve from time 0 through 24 h (AUC0–24), observed maximum serum concentration (C-max), and time averaged concentration over the dose administration interval (C_{avg}) of estradiol, estrone, and estrone sulfate for the nontreated group. The AUC0–24 data indicated that postcontact serum concentrations (estradiol 127.52 ± 66.60, estrone 529.00 ± 225.27, and estrone sulfate 11.44 ± 4.29) did not significantly increase from baseline hormone concentrations (estradiol 126.57 ± 61.79, $p = 0.898$; estrone 510.46 ± 262.69, $p = 0.541$; and estrone sulfate 11.45 ± 4.01, $p = 0.996$), thus indicating that significant interpersonal transfer did not occur [38]. The same conclusion was observed for C-max [39].

With estradiol emulsions, Taylor and Gutierrez [40] performed an open-label, nonrandomized clinical trial to investigate the amount of transfer between 14 postmenopausal women dosed with 1.74 g transdermal estradiol emulsion (Estra-

Table 18.1 *In vivo* human studies of transdermal transfer of topical estradiol and testosterone products [37, 39, 40, 42, 43]

	Wester et al. [37]	ZumBrunnen et al. [39]	Taylor and Gutierrez [40]	Schumacher et al. [42]	Rolf et al. [43]
Participants, *n*	6	48	14	20	12
Dosed individual	Postmenopausal, healthy women	Postmenopausal, healthy women	Postmenopausal, healthy women	Postmenopausal, healthy women	Healthy men: left forearm
Transdermal product hormone	Estradiol	Estradiol	Estradiol	Estradiol	Testosterone
Formulation	Gel (estrogel)	Gel 0.06% (EstroGel)	Topical emulsion (Estrasorb)	Transdermal spray (Evamist)	Gel 2.5%
Active ingredient[a]	[14C]-estradiol, [4-14C]-NEC-127 estradiol (specific activity 54.1 mCi/mmol)	Estradiol	Estradiol	Estradiol	Testosterone
Inactive ingredients[a]	Gel formulation	Purified water, alcohol, carbomer 934P, triethanolamine	Water, ethanol, polysorbate 80, soybean oil	Alcohol, octisalate	Purified water, carbomer 980, ethanol 67.0%, isopropyl, myristate, sodium hydroxide
Transdermal application site location	Forearm	Posterior arm	Leg	Forearm	Left inner forearm
Daily dose (total)	0.16 g	1.25 g	3.48 g	4.59 mg (3!90-μl sprays)	11.7 ± 2.2 mg
Applied area	100 cm^2 (5.0 × 20 cm^2)	"Maximal area" of posterior arm	Each leg	"Three nonoverlapping sites" on each inner forearm	80 cm^2
Amount applied/area	0.0016 g/cm^2	NA	1.74 g to each leg	1.74 g to each leg	0.15 mg/cm^2
	Wester et al. [37]	ZumBrunnen et al. [39]	Taylor and Gutierrez [40]	Schumacher et al. [42]	Rolf et al. [43]

Table 18.1 (continued)

Daily exposures, *n*	1	1	2	1	1
Total days, *n*	1	14 (× 2 treatment periods)	2	18	1
Skin-to-skin contact time after application, h	1	1	2, 8	4, 8, 12, 16, 20, 24	0.5
Transfer area/site	Ventral forearm	Posterior arm	Forearm	Inner forearm	Right forearm
Vigorous/rubbing contact	10 strokes	3 min	2 min	0 min	5 min
Constant contact/no movement	15 min	12 min	0 min	5 min	0 min
Outcomes measured	2.3 ± 2.0% of applied estradiol dose recovered from skin washing 4.1 ± 3.6% estradiol recovered from protective sleeve after 24 h	Postcontact serum estradiol (127.52 ± 66.60), estrone (529.00 ± 225.27), and estrone sulfate (11.44 ± 4.29) did not increase significantly from baseline serum estradiol (126.67 ± 61.79), estrone (510.46 ± 262.69), and estrone sulfate (11.45 ± 4.01)	In men, the average serum estradiol increased from 17.0 ± 4.3 pg/ml before contact to 21.0 ± 4.4 pg/ml after contact; 25% increase from baseline	90% CI for ratio of postcontact to precontact mean AUC0–24 of 1.00–1.07; entirely contained within a predetermined 90% CI equivalence range of 0.80–1.25	3.1±1.8% of applied testosterone dose was recovered from the non dosed site
Conclusion	Transfer to naive recipients occurred	Significant transfer did not occur	Significant transfer occurred	Significant transfer did not occur	Transfer occurred

NA data not available

[a] Ingredients from manufacturer product information

sorb© containing 2.5 mg of estradiol/g) to each leg and a group of 14 male partners. Attempted transfer was by vigorous skin-to-skin rubbing of 2 min, occurring 2 and 8 h after transdermal application, and was measured by serum concentration changes of estradiol, estrone, and estrone sulfate in female participants and their male partners. The average serum estradiol concentration in adult men increased from 17.0 ± 4.3 pg/ml before contact to 21.0 ± 4.4 pg/ml on day 2 after attempted transfer; signifying a 25% increase from baseline. The mean AUC0–24 from day 2 to baseline was 1.25 ($p< 0.0001$) for estradiol, 1.35 ($p< 0.018$) for estrone, and 1.16 ($p< 0.021$) for estrone sulfate, indicating that significant transfer did occur by means of vigorous skin-to-skin contact at the application site [40, 41].

While clinical *in vivo* human trials of a transdermal estradiol gel and emulsion formulation have reported measurable transfer of the applied hormone dose, a spray formulation study yielded opposite results. Schumacher et al. [42] conducted a single-center, open-label study to evaluate the amount of transfer between 20 postmenopausal women dosed with a 4.59 mg transdermal estradiol spray (Evamist) and a group of 20 untreated, healthy men. Attempted transfer was by forearm contact of 5 min without movement, occurring 1 h after estradiol application, and was measured by precontact and postcontact serum estradiol levels in the healthy men. The AUC0–24 was calculated to evaluate serum estradiol concentration of the men at 4, 8, 12, 16, 20, and 24 h after the attempted transfer. In this trial, significant transfer did not occur as determined by a 90% CI for the ratio of postcontact mean AUC0–24 (556.5) to precontact mean AUC0–24 (538.0) of 1.00–1.07, which was entirely contained within a predetermined 90% CI equivalence range of 0.80–1.25 [42].

Other trials focused on identifying the amount of hormone that remains on the skin after a transdermal application, and have, thus, considered the amount of hormone available for transfer. Rolf et al. [43] conducted a single-center, open, randomized study involving 12 healthy male volunteers (aged 22–27 years) to determine what percentage of a mean 11.0 ± 1.7 mg dose of a newly developed 2.5% testosterone gel applied to the abdomen and ventral forearm would remain on the skin after 10 and 30 min and after 1, 2, 4, and 8 h. After 10 min, approximately 60.2% of the applied testosterone dose could be recovered from the skin and 8 h later, approximately 50%. Notably, washing the skin with water reduced the amount of testosterone recovered. The study also evaluated the potential for transdermal hormone transfer to a nondosed site by rubbing the dosed (11.7 ± 2.2 mg) left forearm with the nondosed right forearm for 5 min after 30 min of application. Transfer did occur as 3.1 ± 1.8% of the applied testosterone dose was recovered from the nondosed forearm [43].

Manufacturers have studied the potential for transdermal hormone application to increase estradiol and testosterone levels in untreated close contacts. Although not all results have been published in peer-reviewed journals, excerpts of selected *in vivo* transfer studies (i.e., EstroGel, Estrasorb, Evamist, AndroGel©, and Testim©) are on the FDA website [44–48].

FDA product-specific studies have been conducted to determine the potential for transdermal estradiol transfer (Table 18.2) [44–48]. In a 14-day EstroGel [44]

Table 18.2 FDA data reflecting *in vivo* human studies of transdermal transfer of topical estradiol and testosterone products [44–48]

	EstroGel [44]	Evamist [45]	AndroGel [47, 48]	Testim (AUX-TG-206) [46]	Testim (AUX-TG-209) [46]
Participants, *n*	24	20	36	30	24
Dosed individual	Postmenopausal, healthy women	Postmenopausal, healthy women	Healthy men	Healthy men	Healthy men
	Naïve transfer recipients postmenopausal, healthy women	male partners	Female partners	Female partners	Female partners
Transdermal product					
Hormone	Estradiol	Estradiol	Testosterone	Testosterone	Testosterone
Formulation	Gel (EstroGel)	Transdermal spray (Evamist)	Gel (AndroGel)	(Testim)	(Testim)
Active ingredient[a]	Estradiol	Estradiol	Testosterone	Testosterone	Testosterone
Inactive ingredients[a]	Purified water, alcohol, carbomer 934P, Triethanolamine	Alcohol, octisalate	Purified water, carbomer 980, ethanol 67.0%, isopropyl myristate, sodium hydroxide	Purified water, acrylates, carbopol, ethanol (74%), glycerin, pentadecalactone, polyethylene glycol, propylene glycol, tromethamine	Purified water, acrylates, carbopol, ethanol (74%), glycerin, pentadecalactone, polyethylene glycol, propylene glycol, tromethamine
Transdermal application site					
Location	Posterior arm	Forearm	Abdomen, shoulders, upper arms	Abdomen	Arms, shoulders
Daily dose, total	1.25 g	3 × 90 μl sprays	10 g	100 mg	100 mg
Applied area	NA	NA	NA	NA	NA
Amount applied/area	NA	NA	NA	NA	NA
Daily exposures, *n*	1	1	1	1	1

Table 18.2 (continued)

	EstroGel [44]	Evamist [45]	AndroGel [47, 48]	Testim (AUX-TG-206) [46]	Testim (AUX-TG-209) [46]
Total days, *n*	14	NA	7	1	1
Skin-to-skin contact					
Time after application, *h*	1	1	2, 6, 12	1, 4, 8, 12	4
Vigorous/rubbing contact	0	0	15 min	15 min	15 min
Constant contact/no movement	15 min	5 min	0	0	0
Outcomes measured	No changes in endogenous mean serum estradiol concentration in non-dosed females	NA	Day 1: in females, threefold increase in C_{avg} Day 7: fourfold to sevenfold increase in C_{avg} 2 exposed women had max. testosterone levels of 250 and 350 ng/dl	Serum testosterone concentration in female partners increased 4-fold from baseline	Serum testosterone concentration in showered female partners increased 4-fold from baseline
Conclusion	Transfer did not occur	No significant transfer occurred	Transfer occurred	Transfer occurred	Transfer occurred

NA Data not available.

[a] Ingredients from manufacturer product information.

study, 24 healthy, postmenopausal women were dosed with 1.25 g of estradiol gel once daily on the posterior arm and permitted to engage in 15 min of direct skin-to-skin contact with a cohort of 24 nondosed healthy, postmenopausal females. No changes in endogenous mean serum estradiol concentration in nondosed female cohorts occurred after direct contact [44]. In another study on Evamist [45] transdermal spray, 20 healthy postmenopausal women were dosed with a 90 µl spray once daily on the ventral forearm and were permitted to engage in 5 min of direct continual contact with nondosed male partners. No significant transfer of estradiol to nondosed contacts occurred [45]. While initial transdermal estradiol experiments concluded that transfer did not occur, transdermal testosterone experiments yielded opposite results.

Two-brand-name testosterone gels, one for glandous skin and one for axilla, are available in the USA: AndroGel and Testim. Studies have been conducted with each to determine the potential for transdermal testosterone transfer. In a 7-day AndroGel [47] study, three groups of healthy men were dosed with 10 g of testosterone gel and permitted to engage in 15 min of daily vigorous skin-to-skin contact with their female partners starting at 2, 6, or 12 h after application. On day 1, the mean C_{avg} of testosterone in female partners revealed a threefold increase (18 ng/dl) and by day 7, C_{avg} increased by fourfold to sevenfold and exceeded the normal female range of 10–55 ng/dl. Additionally, two women who were exposed to male partners after 2 h of testosterone application had maximum testosterone levels of 250 and 350 ng/dl [47]. In a study of Testim [46] (AUX-TG-206), 30 healthy men applied 100 mg of Testim to the abdomen and were permitted to engage in 15 min of skin rubbing with female partners occurring 1, 4, 8, or 12 h after application. Serum testosterone concentrations in female partners increased fourfold from baseline. In a second trial [45] (AUX-TG-209), 24 healthy men applied 100 mg of Testim to the arms and shoulders and were permitted to engage in 15 min of skin rubbing with female partners occurring 4 h after application. In this study, female participants showered immediately after exposure. However, serum testosterone concentrations in female partners still increased fourfold from baseline.

Transdermal hormone transfer from dosed individual to naïve interpersonal contact can occur [37, 40, 43, 46–48] and may cause clinically significant hormone imbalance and adverse events [21–28]. Transfer from parent to young child, as has been previously described [29–35], is concerning. Wester et al. [49] reported that same-strength topical testosterone application to both an adult and newborn rhesus monkey increased systemic availability in the newborn by 2.7 times that of the adult. Not only do transdermal hormones demonstrate adequate penetration, but also, *in vivo* studies have indicated that interpersonal transfer via direct and vigorous skin-to-skin contact does occur. Comparison among such studies can highlight the propensity for different transdermal hormone formulations to transfer to naïve contacts and can be used to optimize testing and develop an ideal transdermal delivery system.

Although FDA data may indicate increased potential for transdermal hormone transfer from dosed individuals to nondosed personal contacts for some formulations, the studies are difficult to compare due to variations in contact time and

Table 18.3 Group I, 2.5 g gel applied to abdominal area of male, data from [36]

Parameter	Site of application	Gel amount	Site washed	T-shirt	Baseline least-squares mean	After application least-squares mean	After application/ baseline
AUC 0-24 (ng · h/dl)	Abdomen	2.5 g	No	No	485	615	127%
	Abdomen	2.5 g	No	Yes	485	469	97%
C_{av} (ng/dL)	Abdomen	2.5 g	No	No	20.2	25.6	127%
	Abdomen	2.5 g	No	Yes	20.2	19.5	97%
C_{max} (ng/dL)	Abdomen	2.5 g	No	No	31.6	39.1	124%
	Abdomen	2.5 g	No	Yes	31.6	23.2	73%

methods as well as different outcomes measured. Nonetheless, it is important to investigate methods to prevent unwanted transdermal hormonal transfer.

Stalhmen et al. used 24 healthy male and female couples in an open-label, randomized, parallel group, crossover study to assess transdermal hormonal transfer with respect to application site on the body and clothing barrier. A 1.62% testosterone gel was applied to three regions in male subjects: upper arms, shoulders, or abdomen. Group I received 2.5 g and group II 5.0 g of testosterone gel. After 2 h from initial gel application, male and female subjects had skin contact. Tests were done either with or without a cotton T-shirt. Contact was maintained for 15 min. In addition, subjects in group II were also tested based on washing or not washing the site on contact. Group III assessed the effects of 5 g of transdermal transfer without washing or T-shirt barrier [36].

Each examination was separated by a 1-week washout period. Testosterone exposure (C_{av} and C_{max}) in females increased up to 27% for the 2.5 g gel and up to 280% for the 5 g gel compared to baseline, 2 h after initial gel application. In both cases, the C_{av} remained within the normal female eugonadal range [36].

There is a significant risk of testosterone transfer from male patients using a 1.62% testosterone gel to their female partners with direct to skin contact (Table 18.3) [36]. After administration of the gel to the male's abdomen, females in both the groups I and II, demonstrated significantly higher exposure relative to baseline ($p < 0.01$ for AUC, C_{av}, and C_{max} for group I and $p < 0.002$ in group II) after direct contact with their male partner. When the test was performed again with the male partner wearing a t-shirt, there were no statistical differences found in any parameter compared to baseline. In group II, washing of the gel application site in there was a reduced overall exposure which was comparable to baseline, with C_{max} only 14% higher than baseline (Table 18.4).

With no barrier or washing of the gel (group III), there were significant increases when testosterone gel was applied to upper arms/shoulders of male participants ($P < 0.005$ for AUC, C_{av}, and C_{max}). Skin contact with upper arms/shoulders area

Table 18.4 Group II, 5 g gel applied to abdominal area of male, data from [36]

Parameter	Site of application	Gel amount	Site washed	T-shirt	Baseline least-squares mean	After application least-squares mean	After application/ baseline
AUC 0-24 (ng * h/dL)	Abdomen	5 g	No	No	380	575	152%
	Abdomen	5 g	Yes	No	380	413	109%
C_{av} (ng/dL)	Abdomen	5 g	No	No	15.8	24	152%
	Abdomen	5 g	Yes	No	15.8	17.2	109%
C_{max} (ng/dL)	Abdomen	5 g	No	No	19.8	33.7	170%
	Abdomen	5 g	Yes	No	19.8	22.6	114%

resulted in higher testosterone exposure for female participants when compared to that of the abdomen, as shown in Table 18.3.

Either washing the application site before skin-to-skin contact, or wearing a t-shirt, can prevent transfer of the testosterone gel. However, it should be noted that a t-shirt did not prevent total transmission of hormone, as seen with the 5 g testosterone gel and t-shirt barrier in Table 18.4 [36].

There are a number of limitations in this study that should be acknowledged. It should be noted that the time spent with direct skin-to-skin contact was exaggerated, and most likely does not reflect average real-world conditions. Participants were primarily of Hispanic descent and it is not clear how this affected the findings. An

Table 18.5 Group III, no barrier or washing. 5 g of testosterone gel, data from [36]

Parameter	Site of application	Gel amount	Site washed	T-shirt	Baseline least-squares mean	After application least-squares mean	After application/ baseline
AUC 0-24 (ng * h/dl)	Upper arms/ shoulders	5 g	No	No	369	1400	379%
	Abdomen	5 g	No	No	391	816	209%
C_{av} (ng/dL)	Upper Arms/ shoulder	5 g	No	No	15.4	58.4	380%
	Abdomen	5 g	No	No	16.3	34.0	209%
C_{max} (ng/dL)	Upper Arms/ Shoulders	5 g	No	No	30.2	111	366%
	Abdomen	5 g	No	No	32.1	53.9	168%

acknowledged limitation of the study was that menstrual status was not accounted for in the testosterone levels of premenopausal women in the data set. This could be a potential source of error in the data, as endogenous testosterone levels can fluctuate over the course of a menstrual cycle [36].

Emphasizing the risks of unwanted transdermal testosterone transfer to male patients being treated with gels is of high priority [36]. Physicians should suggest methods of transfer reduction, such as washing, along with limiting application sites to areas that are covered by clothing. In addition, a standardization of transfer methods would reduce risk of unwanted transfer.

18.3 Standardization of Transfer Methods

Clinical trials [37, 39, 40, 42, 43] and FDA data [41, 44–48] have examined the possibility of transdermal hormone transfer. However, the type of exposure, amount of transdermal hormone applied (i.e., total dose and active dose per applied area), number of exposures, applied area and transfer area, timing and frequency of contact, and specific transfer methods differ between studies. Standardization and quantification of such variables would permit better data comparison.

Accordingly, too little collective data exists to refine our knowledge about transdermal hormone formulations that may offer less transfer. Future experiments should consider standardization of skin-to-skin contact methods and duration of exposures based on realistic and exaggerated endpoints. It is difficult to compare clinical trial outcomes because some studies attempt transfer by vigorous contact and rubbing while others employ direct contact without movement. Taylor and Gutierrez [40] and Rolf et al. [43] attempted transfer by 2 min of vigorous skin rubbing and by 5 min of rubbing, respectively, and concluded that transfer did occur. Schumacher et al. [42] attempted transfer by 5 min of forearm contact without movement and concluded that transfer did not occur. In the real world, however, interpersonal contact may not be so straightforward.

In previously reported cases of transdermal hormone transfer from adult-to-adult or parent-to-child, it is unlikely that clinical effects were caused by one instance of 2–5 min of rubbing or 5 min of direct contact without movement. Transfer more likely occurred through everyday interactions and contact required for hugging, playing, cuddling, or even sleeping next to each other. For this reason, transfer studies based on exaggerated contact times may be more capable of reflecting real-life exposures.

While these *in vivo* human trials have started to evaluate the potential for transdermal hormone transfer, *in vivo* animal models as well as *in vitro* human and animal models are less studied.

18.4 Human Experiment Transfer Data: *In Vitro*

To our knowledge, no *in vitro* human experiments have specifically determined transfer potential of transdermal estradiol or testosterone.

18.5 *In Vitro* Transfer Methodologies

Several of the 15 steps in percutaneous penetrations have *in vitro* model [66]. We believe based on the Ngo et al. data that an *in vitro* method for transfer can be developed.

18.6 Animal Experiment Transfer Data: *In Vivo*

To our knowledge, no *in vivo* animal experiments have specifically determined transfer potential of transdermal estradiol or testosterone.

18.7 Animal Experiment Transfer Data: *In Vitro*

To our knowledge, no *in vitro* animal experiments have specifically determined transfer potential of transdermal estradiol or testosterone.

18.8 Possible Excipient Effect and Quantification of Transdermal Transfer

The controlled *in vivo* experiments in humans may, however, require further quantification. While overall conclusions indicate whether transfer "did" or "did not" occur, actual data comparisons are less straightforward. Different hormone formulations likely affect absorption to different degrees based on excipient properties of the vehicle (i.e., gels, spray, or emulsions). However, two clinical trials enabling an estradiol gel formulation yielded different conclusions with similar attempted transfer methods. Despite different outcome measures, Wester et al. [37] observed a positive estradiol gel transfer to nondosed subjects via measurement of a ^{14}C assay, while ZumBrunnen et al. [39], via measuring of changes in estradiol, estrone, and estrone sulfate concentrations from baseline, did not. We suspect that excipients will be crucial in determining transfer. Unfortunately, inadequate data exist to develop a quantitative structure-activity relationship (QSAR) model.

The chemical endpoints that appear most accurate would enable quantification of hormone change after attempted transfer through measurement of a radioisotope (^{14}C) or stable isotope as would be measured by mass spectrometry. Transdermal transfer is difficult to measure by changes in serum hormone concentrations due to interference from endogenous hormones. This concept is in agreement with Wester and Maibach [50], suggesting that serum concentrations of an applied compound *in vivo* are extremely low following topical application and may not be detectable unless by tracer methodology. Accordingly, the most accurate detection assay would be one that does not interfere with endogenous hormone concentrations. Any changes from baseline may be too small to measure and may explain the negative studies. Furthermore, diurnal variations in serum testosterone [51–58] and estradiol [55–59] levels are well documented. Accordingly, it may be difficult to observe a transfer effect by measuring changes in endogenous hormones before and after transdermal hormone application. The methods by which transdermal hormones are applied (i.e., vehicle formulations) may also affect percutaneous penetration.

18.9 Intrinsic Properties of Transdermal Hormones

Chemical properties of applied hormones and frictional properties of skin may affect transdermal transfer between individuals. For example, the friction coefficient can interpret skin differences on various anatomical locations and between individuals and can also monitor skin changes resulting from topical chemical applications [60, 61]. The friction coefficient of the skin may be increased by water or moisturizers that hydrate the skin; it may be decreased by alcohol or other drying agents [60]. By understanding the mechanical state of the skin, it may be possible to devise better transdermal application methods that focus on site of application, character of the vehicle, and differences in age, gender, race, and anatomical sites of the dosed individual. Understanding to what degree the friction coefficient affects transdermal hormone transfer between individuals may help formulate better application vehicles.

18.10 Anatomic Effect

It is necessary to explore how different anatomic sites affect transdermal hormone penetration, and thus potential for interpersonal transfer. Regional variation of percutaneous absorption is described with the genitals as the anatomic site with greatest absorption, followed by the face, trunk, arms, and legs in decreasing order [62, 63]. The potential for transfer is complicated by *application* site of the dosed individual and *contact* site of the naïve contact. For example, to what degree would transfer occur if a father applied topical testosterone to his trunk and held his son's face against his skin? Would transfer occur if his dosed arms contacted his son's legs? Furthermore, forearm percutaneous penetration data may underestimate

absorption at all body sites [62] which is significant because many clinical trials focus on attempted transfer via forearm contact or rubbing. Frictional studies have also reported differences based on anatomical site. Cua et al. [64] described the frictional coefficient difference from 0.12 on the abdomen to 0.34 on the forehead and postauricular skin, and Elsner et al. [65] described a difference from 0.66 on the vulvar surface to 0.48 on the forearm. As new technology is able to explore potential for percutaneous absorption based on intrinsic properties of the skin, studies on transfer of topically applied hormones may become more valuable.

18.11 Absorption Properties of the Skin

Percutaneous absorption depends on many variables. In theory, transdermal hormone that is not absorbed may become available for interpersonal transfer. Therefore, it is important to understand the details of percutaneous absorption and expand our knowledge regarding transfer potential. Wester and Maibach [50] defined the ten steps to percutaneous absorption in 1983 as functions of the following absorption parameters:

1. Vehicle release of applied hormone which may depend on the partition of the hormone between vehicle and skin, the solubility of the hormone in the vehicle, hormone concentration, and pH
2. Absorption kinetics of the hormone with attention to application site, individual variation, skin condition, occlusion, drug concentration, surface area, and multiple dose application, which may affect penetration and thus potential for transfer
3. Excretion kinetics and bioavailability of the hormone from percutaneous administration
4. Effective cellular and tissue distribution allowing part of the dosed hormone to remain on the skin surface
5. Substantivity, or the part of the applied dose which binds to the skin surface and is lost by exfoliation
6. Wash-and-rub resistance, referring to the ability to remove the hormone by washing or rubbing
7. Volatility, as the partition of the hormone between its vehicle on the skin surface and the surrounding air
8. Binding of the hormone in the skin
9. Anatomic pathways at different body sites and in the cellular structure of the skin
10. Cutaneous metabolism of the viable epidermis and the ability of a transdermally applied hormone to penetrate through the stratum corneum. Examination of “other” steps of percutaneous absorption may be necessary to develop a transcutaneous product with low probability of interpersonal transfer including absorption and transfer from clothing and transfer from daily-use objects such as tables, desks, and even handlebars

Addition details on the 15 steps to percutaneous penetration in man are detailed by Ngo [66].

18.12 Analytic Chemistry and Human Pharmocokinetics

Appropriate FDA package labeling is greatly aided by data from refined analytic chemistry. The radioisotope method utilizing urine and liquid scintillation counting permits greater analytic sensing compared to cold chemistry and blood sampling [67]. The latter may provide false-negative transfer results. The principles outlined here related to transfer of chemicals, other than dry, a field requiring elucidation [68].

18.13 Development and Optimization of Transfer Models

While *in vivo* human model experiments reflect transdermal transfer, less is known about transfer potential among *in vivo* animal models and *in vitro* human and animal models. Although not classically an *in vitro* model in the sense that it does not involve applying a hormone to one side of excised skin in a diffusion chamber and performing a radioactivity assay in the collection vessel on the opposite side [69], clothing transfer studies may serve as artificial membranes to detect the amount of topical hormone removed from the skin through direct contact. Initial clinical trials have reported that transfer from dosed individual to clothing can occur [37, 70, 71].

The development and validation of *in vivo* animal models and *in vitro* human and animal models to depict transdermal hormone transfer may be necessary. When developing animal models for transdermal transfer studies, attempts should be made to abide by the 3Rs (i.e., replacement, reduction, and refinement) principle for humane testing as originally described by Russell and Burch [71]. Animal models should additionally take into consideration the differences in percutaneous absorption of hormones from humans. Wester and Maibach [72] and Andersen et al. [73] examined the percutaneous absorption of a 4 μg/cm^2 testosterone dose among animal models. The percutaneous absorption of testosterone was highest in the rabbit (69.6% of dose absorbed), followed by the rat (47.4%), guinea pig (34.9%), pig (29.4%), and rhesus monkey (18.4%), which was closest to absorption observed in man (13.2%; ratio rhesus monkey to man: 1.4) [50, 73, 74]. The Organization for Economic Co-Operation and Development (OCED) adopted guidelines for assessing absorption by using human and animal skin *ex vivo* in order to standardize and regulate testing of chemical compounds [75–77]. New *in vitro* human skin models are in development based on OCED guidelines [77, 78]. Yet, the ideal animal model for determination of transdermal transfer potential has not yet been identified. Optimization of such models would permit further understanding of the potential for transdermal hormone transfer among interpersonal contacts and its relevance in clinical practice.

18.14 Conclusion

In the age of rapidly developing medical advances, different delivery routes for medications are becoming a reality. Transdermal hormone application has proven clinically significant, often with fewer side effects for the patient as compared to oral alternatives. Despite benefit to the dosed individual, transdermal hormone transfer to a naïve interpersonal contact can occur and may cause hormone imbalance and adverse effects ranging from cardiovascular events and masculinization in adults to precocious puberty and virilization in children. Clinical trials have focused on *in vivo* transfer potential of topical estradiol and testosterone formulations in humans and have determined that transfer can occur. In order to more fully appreciate the potential for transdermal hormone transfer, developments are necessary that focus on the possible excipient effect of transdermal transfer, appropriate quantification of hormone changes after attempted transfer (e.g., tracer methodology), standardization of skin-to-skin contact methods and duration of exposure based on real and exaggerated endpoints, a greater understanding of chemical properties of the applied hormone and frictional properties of the skin that affect percutaneous absorption, determination of which anatomical site offers the greatest clinical benefit to the dosed individual and least potential for transfer, and development and optimization of *in vivo* animal models and *in vitro* human and animal models to enhance our understanding of percutaneous absorption in daily life.

References

1. Place VA, Powers M, Darley PE, Schenkel L, Good WR. A double-blind comparative study of Estraderm and Premarin in the amelioration of postmenopausal symptoms. Am J Obstet Gynecol. 1985;152:1092–9.
2. Scott RT Jr, Ross B, Anderson C, Archer DF. Pharmacokinetics of percutaneous estradiol: a crossover study using a gel and a transdermal system in comparison with oral micronized estradiol. Obstet Gynecol. 1991;77:758–64.
3. Sivanandy MS, Masimasi N, Thacker HL. Newer hormonal therapies: lower doses, oral, transdermal, and vaginal formulations. Cleve Clin J Med. 2007;74:369–75.
4. Yasui T, Uemura H, Takikawa M, Irahara M. Hormone replacement therapy in postmenopausal women. J Med Invest. 2003;50:136–45.
5. Pratapkumar AV. A comparison of transdermal and oral HRT for menopausal symptom control. Int J Fertil Womens Med. 2006;51:64–9.
6. Sumino H, Ichikawa S, Kasama S, et al. Different effects of oral conjugated estrogen and transdermal estradiol on arterial stiffness and vascular inflammatory markers in postmenopausal women. Atherosclerosis. 2006;189:436–42.
7. Shaw JE, Prevo ME, Amkraut AA. Testing of controlled-release transdermal dosage forms: product development and clinical trials. Arch Dermatol. 1987;123:1548–56.
8. Powers MS, Schenkel L, Darley PE, Good WR, Balestra JC, Place VA. Pharmacokinetics and pharmacodynamics of transdermal dosage forms of 17 beta-estradiol: comparison with conventional oral estrogens used for hormone replacement. Am J Obstet Gynecol. 1985;152:1099–106.

9. Vrablik M, Fait T, Kovar J, Poledne R, Ceska R. Oral but not transdermal estrogen replacement therapy changes the composition of plasma lipoproteins. Metabolism. 2008;57:1088–92.
10. Shifren JL, Desindes S, McIlwain M, Doros G, Mazer NA. A randomized, open-label, crossover study comparing the effects of oral versus transdermal estrogen therapy on serum androgens, thyroid hormones, and adrenal hormones in naturally menopausal women. Menopause. 2007;14:985–94.
11. Swerdloff RS, Wang C, Cunningham G, et al. Long-term pharmacokinetics of transdermal testosterone gel in hypogonadal men. J Clin Endocrinol Metab. 2000;85:4500–10.
12. Wang C, Berman N, Longstreth JA, et al. Pharmacokinetics of transdermal testosterone gel in hypogonadal men: application of gel at one site versus four sites: a General Clinical Research Center Study. J Clin Endocrinol Metab. 2000;85:964–9.
13. McNicholas T, Ong T. Review of Testim gel. Expert Opin Pharmacother. 2006;7:477–84.
14. de Ronde W. Testosterone gel for the treatment of male hypogonadism. Expert Opin Biol Ther. 2009;9:249–53.
15. Bouloux P. Testim 1% testosterone gel for the treatment of male hypogonadism. Clin Ther. 2005;27:286–98.
16. Kaestli LZ, Wasilewski-Rasca AF, Bonnabry P, Vogt-Ferrier N. Use of transdermal drug formulations in the elderly. Drugs Aging. 2008;25:269–80.
17. Johnson R, Nusbaum BP, Horwitz SN, Frost P. Transfer of topically applied tetracycline in various vehicles. Arch Dermatol. 1983;119:660–3.
18. Yerasi AB, Butts JD, Butts JD. Disposal of used fentanyl patches. Am J Health Syst Pharm. 1997;54:85–6.
19. Lu C, Fenske RA. Dermal transfer of chlorpyrifos residues from residential surfaces: comparison of hand press, hand drag, wipe, and polyurethane foam roller measurements after broadcast and aerosol pesticide applications. Environ Health Perspect. 1999;107:463–7.
20. Wolf A, Burkhart K, Caraccio T, Litovitz T. Childhood poisoning involving transdermal nicotine patches. Pediatrics. 1997;99:1–5.
21. Rolf C, Kemper S, Lemmnitz G, Eickenberg U, Nieschlag E. Pharmacokinetics of a new transdermal testosterone gel in gonadotrophin-suppressed normal men. Eur J Endocrinol. 2002;146:673–9.
22. Choi BG, McLaughlin MA. Why men's hearts break: cardiovascular effects of sex steroids. Endocrinol Metabl Clin North Am. 2007;36:365–77.
23. Aksglaede L, Juul A, Leffers H, Skakkebaek NE, Andersson AM. The sensitivity of the child to sex steroids: possible impact of exogenous estrogens. Hum Reprod Update. 2006;12:341–9.
24. Rolf C, Nieschlag E. Potential adverse effects of long-term testosterone therapy. Baillieres Clin Endocrinol Metab. 1998;12:521–34.
25. Merhi ZO, Santoro N. Postmenopausal virilization after spousal use of topical androgens. Fertil Steril. 2007;87:975–6.
26. de Ronde W. Hyperandrogenism after transfer of topical testosterone gel: case report and review of published and unpublished studies. Hum Reprod. 2009;24:425–8.
27. Delanoe D, Fougeyrollas B, Meyer L, Thonneau P. Androgenisation of female partners of men on medroxyprogesterone acetate/percutaneous testosterone contraception. Lancet. 1984;1:276.
28. Moore N, Paux G, Noblet C, Andrejak M. Spouse-related drug side-effects. Lancet. 1988;1:468.
29. Brachet C, Vermeulen J, Heinrichs C. Children's virilization and the use of a testosterone gel by their fathers. Eur J Pediatr. 2005;164:646–7.
30. Yu YM, Punyasavatsu N, Elder D, D'Ercole AJ. Sexual development in a two-year-old boy induced by topical exposure to testosterone. Pediatrics. 1999;104:e23.
31. Kunz GJ, Klein KO, Clemons RD, Gottschalk ME, Jones KL. Virilization of young children after topical androgen use by their parents. Pediatrics. 2004;114:282–4.

32. Bhowmick SK, Ricke T, Rettig KR. Sexual precocity in a 16-month-old boy induced by indirect topical exposure to testosterone. Clin Pediatr (Phila). 2007;46:540–3.
33. Stephen MD, Jehaimi CT, Brosnan PG, Yafi M. Sexual precocity in a 2-year-old boy caused by indirect exposure to testosterone cream. Endocr Pract. 2008;14:1027–30.
34. Franklin SL, Geffner ME. Precocious puberty secondary to topical testosterone exposure. J Pediatr Endocrinol Metab. 2003;16:107–10.
35. Svoren BM, Wolfsdorf JI. Sexual development in a 21 month-old boy caused by testosterone contamination of a topical hydrocortisone cream. J Pediatr Endocrinol Metab. 2005;18:507–10.
36. Stahlman J, Britto M, Fitzpatrick S, McWhirter C, Testino SA Jr, Brennan JJ, ZumBrunnen TL. Effect of application site, clothing barrier, and application site washing on testosterone transfer with a 1.62% testosterone gel. Curr Med Res Opin. 2012;28:281–90.
37. Wester R, Hui X, Maibach H. In vivo human transfer of topical bioactive drug between individuals: estradiol. J Invest Dermatol. 2006;126:2190–3.
38. Wester RC, Maibach HI. Percutaneous absorption of drugs. Clin Pharmacokinet. 1992;23:253–66.
39. ZumBrunnen TL, Meuwsen I, de Vries M, Brennan JJ. The effect of washing and the absence of interindividual transfer of estradiol gel. Am J Drug Deliv. 2006;4:89–95.
40. Taylor MB, Gutierrez MJ. Absorption, bioavailability, and partner transfer of estradiol from a topical emulsion. Pharmacotherapy. 2008;28:712–8.
41. Novavax Inc. Estrasorb (estradiol topical emulsion) prescribing information. NDA No. 021371. www.accessdata.fda.gov/drug-satfda_docs/label/2003/21371_estrasorb_lbl.pdf. Accessed 8 Oct 2010.
42. Schumacher RJ, Gattermeir DJ, Peterson CA, Wisdom C, Day WW. The effects of skin-to-skin contact, application site washing, and sunscreen use on the pharmacokinetics of estradiol from a metered-dose transdermal spray. Menopause. 2009;16:177–83.
43. Rolf C, Knie U, Lemmnitz G, Nieschlag E. Interpersonal testosterone transfer after topical application of a newly developed testosterone gel preparation. Clin Endocrinol (Oxf). 2002;56:637–41.
44. Ascend Therapeutics: EstroGel (estradiol gel). NDA No. 021166. www.accessdata.fda.gov/drugsatfda_docs/label/2008/021166s007 lbl.pdf. Accessed 8 Oct 2010.
45. DPT: Evamist (estradiol transdermal spray). NDA No. 022014. www.accessdata.fda.gov/drugsatfda_docs/label/2008/022014s001lbl. pdf. 2010 Accessed 8 Oct 2010.
46. Auxilium Pharmaceuticals: Testim 1% (testosterone gel). NDA No. 021454. www.accessdata.fda.gov/drugsatfda_docs/label/2009/021454s008lbl.pdf. 2010. Accessed 8 Oct 2010.
47. Summary basis of approval, application 21-015. Medical review. www.fda.gov/cder/foi/nda/2000/21-015_AndroGel_Medr.pdf. Accessed 20 Feb 2010.
48. Clinical pharmacology and biopharmaceutic reviews www.fda.gov/cder/foi/nda/2000/21-015_AndroGel_BioPharmr/pdf. Accessed 20 Feb 2010.
49. Wester RC, Noonan PK, Cole MP, Maibach HI. Percutaneous absorption of testosterone in the newborn rhesus monkey: comparison to the adult. Pediatr Res. 1977;11:737–9.
50. Wester R, Maibach H. Cutaneous pharmacokinetics: 10 steps to percutaneous absorption. Drug Metabol Rev. 1983;14:169–205.
51. Diver MJ. Analytical and physiological factors affecting the interpretation of serum testosterone concentration in men. Ann Clin Biochem. 2006;43:3–12.
52. Bremner WJ, Vitiello MV, Prinz PN. Loss of circadian rhythmicity in blood testosterone levels with aging in normal men. J Clin Endocrinol Metab. 1983;56:1278–81.
53. Cooke RR, McIntosh JE, McIntosh RP. Circadian variation in serum free and non-SH-BG-bound testosterone in normal men: measurements, and simulation using a mass action model. Clin Endocrinol (Oxf). 1993;39:163–71.
54. Diver MJ, Imtiaz KE, Ahmad AM, Vora JP, Fraser WD. Diurnal rhythms of serum total, free and bioavailable testosterone and of SHBG in middle-aged men compared with those in young men. Clin Endocrinol (Oxf). 2003;58:710–7.

55. Andersson AM, Carlsen E, Petersen JH, Skakkebaek NE. Variation in levels of serum inhibin B, testosterone, estradiol, luteinizing hormone, follicle-stimulating hormone, and sex hormone-binding globulin in monthly samples from healthy men during a 17-month period: possible effects of seasons. J Clin Endocrinol Metab. 2003;88:932–7.
56. Brambilla DJ, Matsumoto AM, Araujo AB, McKinlay JB. The effect of diurnal variation on clinical measurement of serum testosterone and other sex hormone levels in men. J Clin Endocrinol Metab. 2009;94:907–13.
57. Nicolau GY, Lakatua D, Sackett-Lundeen L, Haus E. Circadian and circannual rhythms of hormonal variables in elderly men and women. Chronobiol Int. 1984;1:301–19.
58. Nicolau GY, Haus E, Lakatua DJ, et al. Circadian and circannual variations of FSH, LH, testosterone, dehydroepiandrosterone-sulfate (DHEA-S) and 17-hydroxy progesterone (17 OH-Prog) in elderly men and women. Endocrinologie. 1985;23:223–46.
59. Clark MK, Sowers M, Levy BT, Tenhundfeld P. Magnitude and variability of sequential estradiol and progesterone concentrations in women using depot medroxyprogesterone acetate for contraception. Fertil Steril. 2001;75:871–7.
60. Sivamani RK, Goodman J, Gitis NV, Maibach HI. Coefficient of friction: tribological studies in man-an overview. Skin Res Technol. 2003;9:227–34.
61. Sivamani R, Maibach H. Tribology of skin. Proc IMechE. 2006;220:729.
62. Guy R, Maibach H. Calculations of body exposure from percutaneous absorption data. In: Bronaugh RL, Maibach HI, editors. Topical absorption of dermatological products. New York: Marcel Dekker; 2002.
63. Wester R, Maibach H Regional variation in percutaneous absorption. In: Bronaugh R, Maibach H, editors. Percutaneous absorption drugs-cosmetics-mechanisms-methodology. 3rd Ed. New York: Marcel Dekker; 1991. pp. 107–16.
64. Cua AB, Wilhelm KP, Maibach HI. Frictional properties of human skin: relation to age, sex and anatomical region, stratum corneum hydration and transepidermal water loss. Br J Dermatol. 1990;123:473–9.
65. Elsner P, Wilhelm D, Maibach HI. Frictional properties of human forearm and vulvar skin: influence of age and correlation with transepidermal water loss and capacitance. Dermatologica. 1990;181:88–91.
66. Ngo MA, Maibach HI. Historical perspective and advances. Toxicol Appl Pharmacol. 2010;243:225–38.
67. Gilman SD, Gee SJ, Hammock BD, Vogel JS, Haack K, Buchholz BA, Freeman SP, Wester RC, Hui X, Maibach HI. Analytical performance of accelerator mass spectrometry and liquid scintillation counting for detection of 14 C-labeled atrazine metabolites in human urine. Anal Chem. 1998;70:3463–9.
68. Hui X, Lamel S, Qiao P, Maibach HI. Isolated human/animal stratum corneum as a partial model for 15 steps in percutaneous absorption: emphasizing decontamination, Part I. J Appl Toxicol. 2013;33(3):152–72
69. Tregear R. Physical properties of skin. New York: Academic; 1966.
70. Mazer N, Bell D, Wu J, Fischer J, Cosgrove M, Eilers B. Comparison of the steady-state pharmacokinetics, metabolism, and variability of a transdermal testosterone patch versus a transdermal testosterone gel in hypogonadal men. J Sex Med. 2005;2:213–26.
71. Mazer N, Fisher D, Fischer J, Cosgrove M, Bell D, Eilers B. Transfer of transdermally applied testosterone to clothing: a comparison of a testosterone patch versus a testosterone gel. J Sex Med. 2005;2:227–34.
72. Russell WMS, Burch RL. The principles of humane experimental technique. London: Methuen; 1959.
73. Wester R, Maibach H. Percutaneous absorption in man and animal: a perspective. In: Drill V, Lazar P, editors. Cutaneous toxicity. New York: Academic; 1977. pp. 111–26.
74. Andersen KE, Maibach HI, Anjo MD. The guinea-pig: an animal model for human skin absorption of hydrocortisone, testosterone and benzoic acid? Br J Dermatol. 1980;102:447–53.
75. OECD. Skin absorption: in vitro method. Guideline 428. 2004.

76. OCD. Guidance document No. 28 for the conduct of skin absorption studies. 35th Joint Meeting, August 2003.
77. Ackermann K, Lombardi Borgia S, Korting HC, Mewes KR, Schafer-Korting M. The Phenion® full-thickness skin model for percutaneous absorption testing. Skin Pharmacol Physiol. 2010;23:105–12.
78. Schreiber S, Mahmoud A, Vuia A, et al. Reconstructed epidermis versus human and animal skin in skin absorption studies. Toxicol In Vitro. 2005;19:813–22.

Chapter 19
Effects of Occlusion on Dermal Drug Delivery: Implications for Bioequivalence Measurement

Nilab Osman and Howard I. Maibach

19.1 Introduction

Occlusion of the skin can occur from everyday use of gloves, stockings, and clothing. Research has shown that it can affect various physiological parameters such as pH balance, carbon dioxide (CO_2) levels, transepidermal water loss (TEWL), and produces histological and microbiological changes in the skin. Recently, there has been a greater understanding of the properties of occlusion as an active agent, which can potentially be used to enhance epidermal drug delivery in healthy skin and to facilitate healing in diseased skin. A comprehensive overview of the current published research is provided in this chapter.

19.2 Effects of Occlusion on Skin Physiology and Chemistry

19.2.1 pH Changes

In experimental models of occlusion, dressings such as bandages or vinylidene polymer plastic film (Saran Wrap®) were used to trap moisture within the skin, thus preventing the evaporation of water from the skin surface. Many researchers have noted an increase in pH postocclusion. Aly et al. [1] performed an experiment using Saran Wrap® on human forearm skin. A statistically significant rise in pH was noted from a preocclusion value of 4.3 to a postocclusion value of 7.0 [1]. In

N. Osman (✉)
Department of Dermatology, UCSF, San Francisco, CA 94143-0989, USA
e-mail: Nilyosman@yahoo.com

H. I. Maibach
Department of Dermatology, School of Medicine, University of California, San Francisco, CA, USA

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_19

a similar study conducted by Hartmann [2] using impermeable and nonadhesive polyethylene foil, there was an increase in pH from an average baseline of 4.9–7.2. Interestingly, 24 h after removal of the occlusion, the pH fell to 5.2 [2]. Faergemann et al. [3] conducted a study using the same method of occlusion with Saran Wrap® for 8 days and noted that throughout the entire period of occlusion the pH remained elevated [3] (Table 19.1).

19.2.2 Carbon Dioxide Emission (pCO_2)

Aly et al. found a statistically significant increase in the pCO_2 emission levels from a preocclusion value of 25 mg/cm^2/h to a postocclusion level of 118 mg/cm^2/h on human forearm skin [1]. The degree of occlusion seems to also play a role in the rate of pCO_2 emission. Studies conducted using occlusive Blenderm™ plastic tape vs. Micropore™ semiocclusive paper tape demonstrated that complete occlusion resulted in a statistically significant increase in the rate of pCO_2 emissions, while semiocclusive paper tape did not induce a statistically significant change [4]. Faergemann et al. [3] used statistical analysis to confirm the increase in pCO2, and noted that a statistical significant increase was noted only after the first 24 h of occlusion [3].

19.2.3 Transepidermal Water Loss (TEWL)

TEWL measures the quantity of water that passes through the epidermal layer to the surrounding atmosphere using the process of diffusion and/or evaporation. Various researchers have demonstrated that occlusion of the skin impacts TEWL greatly. Friebe et al [5] examined the effect of occlusion using sodium lauryl sulfate (SLS), water, or nothing within an aluminum test chamber, and specifically looked at the impact on TEWL. They concluded that occlusion with water increased TEWL for 180 min, while occlusion with an empty test chamber increased the TEWL for 120 min [5]. These findings were attributed to a disruption of the stratum corneum (SC), which ultimately increased its permeability allowing for a greater movement of water. When skin was completely occluded with Saran Wrap® for 5 days, there was an increase in the TEWL from 0.56 to 1.87 mg/cm^2/hr [1]. Faergemann et al. [3] occluded human skin with Saran Wrap® for 8 days and found that TEWL values do not stay constant throughout the entire period of occlusion; rather in this particular experiment there was a noted peak on day 3, followed by a decrease on day 8 [3].

19.2.4 Relative Skin Moisture, Hydration, and Skin Water Content (WC)

By utilizing an evaporimeter, relative skin moisture can be measured. In the study conducted by Hartmann [2] the forearm of human subjects were occluded continuously

Table 19.1 Overview of significant findings of past publications pertaining to the effects of occlusion on skin

Parameter	Study	Results	Reference
pH	5-day occlusion with Saran Wrap on forearm	-pH more basic from 4.3 preocclusion to 7.0 postocclusion	[1]
	3-day continuous occlusion of the forearm of 26 subjects with impermeable nonadhesive polyethylene foil	-pH increased after occlusion from 4.9 to 7.2 -pH fell to 5.2 , 24 h postocclusion	[2]
	8-day occlusion with Saran Wrap®	-pH remained elevated for the entire 8 days	[3]
CO_2	5-day occlusion with Saran Wrap® on the forearm	-CO_2 emissions increased from 25 to 118 nl/cm^2/min	[1]
	8-day occlusion with Saran Wrap®	-CO_2 remained elevated for the entire 8 days -A significant increase seen after 24 h	[3]
TEWL	Comparing the effect of occlusion with sodium lauryl sulfate, water, or nothing	-Occluding with water in the test chamber or with an empty test chamber, increased TEWL, and lasted 180 and 120 min after removal from test chamber -A simple occlusion without an irritant can disturb the stratum corneum permeability barrier	[5]
	5-day occlusion with Saran Wrap® on forearm	-TEWL increased from 0.56 to 1.87 mg/cm^2/h	[1]
	8-day occlusion with Saran Wrap®	-TEWL peaked at day 3 and then decreased -TEWL was significantly lower after 8 days of occlusion than the 3-day peak	[3]
Relative skin moisture	3-day continuous occlusion of the forearm of 26 subjects with impermeable nonadhesive polyethylene foil	-The moisture contents as measured with an evaporimeter increased from 20 to 75% -24-h postocclusion moisture content dropped to 5% (15% below baseline)	[2]
Skin water content (WC)	8-day occlusion with Saran Wrap®	-WC remained elevated for the entire 8 days -Significant increase seen after 24 h	[3]
Hydration	Effects of occlusion of forearm skin with wet occlusion patch and wet diaper for 3 and 8 h	-Skin hydration was significantly higher with a patch and diaper occlusion compared to nonoccluded skin	[6]

Table 19.1 (continued)

Histological	The effect of occlusive treatment on human skin: an electron microscopic study on epidermal morphology as affected by occlusion and dansyl chloride.	-With a greater period of occlusion there was widening of the stratum spinosum noted after 6 h and the formation of keratinocytes within the cytoplasm and perinuclear zone after 24 h	[7]
	Reactive changes in the Langerhans' cells of human skin caused by occlusion with water and sodium lauryl sulfate	-If skin is left nonoccluded for 24 h, the LC population has shown the capacity for reactive proliferation within the dermal layer	[9]
Microbiological	Occlusion, carbon dioxide, and fungal skin infections	-With a greater period of occlusion there was widening of the stratum spinosum noted after 6 h and the formation of keratinocytes within the cytoplasm and perinuclear zone after 24 h	[7]
	Reactive changes in the Langerhans' cells of human skin caused by occlusion with water and sodium lauryl sulfate	-If skin is left nonoccluded for 24 h, the LC population has shown the capacity for reactive proliferation within the dermal layer	[9]

for 3 days with impermeable nonadhesive polyethylene foil, and the moisture content increased from 20 to 75%. However, 24 h postocclusion the moisture content dropped significantly from a baseline of 15 to –5% [2]. Skin water content also appears to be affected by occlusion. Zhai et al. [6] occluded human forearm skin with a wet occlusion patch and a wet diaper for 3 and 8 h. They noted a statistically significant higher level of skin hydration in the wet patch and diaper-occluded skin compared to nonoccluded skin [6]. The skin WC has also been shown to increase after occlusion, with a statistically significant increase occurring after 24 h [3].

19.2.5 Histological Changes

With the use of an aluminum chamber and an electron microscope, morphological changes can be observed after occlusion of human skin. Linderberge et al. [8] noted changes to the intracellular space within the basal layer. The changes were time dependent and demonstrated an increase in the size of the intracellular space with an increase in the number of mononuclear cells 3 h postocclusion. With a greater period of occlusion there was widening of the stratum spinosum noted after 6 h and the formation of keratinocytes within the cytoplasm and perinuclear zone after 24 h [7].

Langerhans cells (LC) are antigen-presenting cells present within all layers of the skin but are most prominent within the stratum spinosum. Research has shown that post occlusion there is a decrease in the number of organelles, with a more electron dense cytoplasm and an increased concentration of filaments [8]. However, if skin is left nonoccluded for 24 h, the LC population has shown the capacity for reactive proliferation within the dermal layer [9]. These findings demonstrate that occlusion has the potential to activate the epidermal immune system. Such findings may indicate that skin could be hypersensitive to stimulation, particularly from irritants post occlusion.

Microscopic changes within the SC are also visible after occlusion of forearm skin using an occlusive chamber. Postocclusion shave biopsy of the skin has revealed water pools within the intercorneocyte lipid layers. There was also an increase in thickness of the SC as a result of the water retention and swelling of the corneocytes [10].

19.2.6 Microbiological Changes

Research has shown that the balance of skin flora will shift if human skin is occluded for a significant duration of time. As stated above, occlusion causes an increase in CO_2 levels, which has been linked to an activation of dermatophytes, forming infective arthrospores, leading to a potential for proliferation of microbiological organisms such as fungus [10].

Forty-eight hours post occlusion with sterile gauze moistened with sterile water and a Teflon® (Dupont™) sheet placed on top, Bible et al. [11] found a four-order increase in magnitude of the bacteria colonies. It was particularly interesting that *Klebisella pneumonia,* which was not present in significant numbers preocclusion, appeared in one of three subjects post occlusion [11].

Many researchers have observed the increase of bacterial colonies after a short period of occlusion. With as little as 24 h of occlusion, bacterial counts have been observed to increase from 1.8×10^2 organisms/cm^2 to 1.4×10^6 organisms/cm^2. A peak colony count was observed 4 days post occlusion to a value of 9.8×10^7 organisms/cm^2. Interestingly, after 5 days of occlusion the colony count was noted to decrease to 7.5×10^6 organisms/cm^2 [1]. No explanation was proposed for such findings.

Certain types of bacteria were observed to increase more so than others. *Staphylococci* were noted to increase five logs and *Cornynebacterium* increased by four logs [2]. Examining the effect of occlusion on the *Pityrosporum orbiculare* population, which is the main etiologic agent in tinea versicolor, it was noted that 10 days of occlusion increased the density of colonies by a factor of ten [3]. The effect of occlusion on Gram-negative bacteria has not been very conclusive. Aly et al. [1] observed an increase in Gram-negative rods. However, Hartman (1983) did not find such an increase in Gram-negative bacteria.

19.3 The Effect of Occlusion on Topical Drug Delivery

Using *in vivo* and *in vitro* models, many researchers have looked at the impact of occlusion on the topical absorption of pharmaceutical drugs such as opioid antagonists and steroidal and nonsteroidal anti-inflammatory agents (NSAIDs).

Moon-Kyoung et al. [12] used *in vivo* models with hairless mice to examine the cutaneous absorption of hydrocortisone-containing liposomes. They monitored the temporal profiles of drugs within occluded and nonoccluded skin. When examining the amount of drug found within the SC as a percentage of the initial dose, the authors observed an increase over time within occluded skin, while nonoccluded skin showed a maximum amount of drug at 1 h, then a rapid decrease afterwards, with the lowest concentration at 6 h. These findings were attributed in part to the gelation of liposomes, affecting the degree to which partitioning of the drug occurs in circumstances of dehydration, as is observed under nonoccluded conditions and in occluded skin, which has a higher degree of hydration [12].

The SC layer has been found to be hydrophobic, with the remainder of the epidermis having hydrophilic properties. Bucks et al. [13] used this relationship as a possible explanation for why there are varying degrees of penetration of corticosteroids in occluded and nonoccluded skin [13]. As mentioned above, the SC layer in occluded skin has a higher WC and thus is more hydrated, making it and the surrounding epidermis more similar in hydrophilicity, which allows for a greater movement of hydrophobic molecules such as steroids into deeper layers of the epidermis.

Pharmacologically, another area of great interest has been NSAID drug delivery via transcutaneous routes rather than per os, which carries a risk of gastric ulcer formation. Oral NSAIDs are one of the most prescribed drugs globally, and have been reported as being the cause of 25% of reported drug side effects. By delivering NSAIDs through the epidermis, unwanted side effects can be minimized [14]. Compared to oral NSAID delivery, topical applications penetrate through the skin slowly and in much smaller quantities before reaching the systemic circulation. The bioavailability is typically less than 5% while the maximum plasma NSAID concentration can reach 15% [14]. Occlusion can impact NSAID penetration into the epidermis as shown in *ex vivo* studies using human cadavers. Occlusion increased the penetration of NSAIDs twofolds. However, this finding should be cautiously interpreted since cadaver skin obviously lacks the necessary flow of blood and may be a poor model for living skin [14].

The product formulation and the vehicle used may have a dramatic impact on NSAID drug delivery, specifically absorption rates and the depth of penetration. Cevc et al. [15] have researched and developed formulation of Ketoprofen (propionic acid class of NSAIDs) based on Transfersome technology, which is artificially created vesicles meant to act in a similar fashion as cell vesicles. These vesicles are ultradeformable and possess an aqueous core, with an outer bilayer made of complex lipids. Their research has shown that using this type of vehicle resulted in

a more effective delivery of the active compound to the muscle when compared to standard formulations of ketoprofen [15].

The pH of a drug may also impact topical drug delivery. Opioid antagonists such as naltrexone are particularly sensitive to such shifts in pH. Naltrexone's active metabolite is naltrexol, with both possessing two ionizable groups. One that is an aliphatic nitrogen and the other a phenolic group. An ionized form of naltrexol at a pH of 4.5 was shown to be two orders of magnitude more soluble than the unionized donor solution at a pH of 8.5, with the topical solubility of naltrexone best at a low pH. Skin occlusion has shown to induce an increase in pH, and thus could hinder dermal penetration of opioid antagonists such as naltrexone [16].

19.4 Effect of Occlusion on Healthy and Diseased Skin

Occlusion has been found to be a risk factor for developing conditions such as irritant contact dermatitis [17]. However, occlusion has also been shown to benefit chronic dermatological conditions such as plaque psoriasis [18].

As described above, occlusion compromises the barrier function of skin, specifically altering the protein and lipid composition of the SC [19]. This change in composition seems to be related to an increase in the pH, thus deactivating many of the enzymes needed for lipid synthesis [20]. Jungerstead et al. [19] investigated the skin barrier response to occlusion in healthy skin, and in skin that had sustained damage from tape stripping or the application of SLS. They assessed skin barrier function by measuring the TEWL, observed for erythema, and examining the lipid composition. Their results showed that healthy skin barrier function was not significantly affected by occlusion. In skin that had undergone trauma with tape stripping, occlusion resulted in a lower TEWL and facilitated the healing process. SLS irritation caused the TEWL to be higher than in the control arm of the study, and prolonged healing time. However, lipid analysis showed no statistically significant deference in the ceramide subspecies or the ceramide/cholesterol ratio after various intervals of occlusion [19].

Using occlusion as a noninvasive treatment for such chronic dermatological conditions such as plaque psoriasis may have great value. Friedman [18] compared occlusion to steroid and Ultraviolet B (UVB) therapy. His findings were quite remarkable. Using fluocinolone cream daily and occluding the skin showed a statistically significant improvement of psoriatic plaque. Interestingly, after 10 weeks of treatment, occlusion was superior to steroid therapy alone. In plaque psoriasis that was treated solely with occlusion, there was complete remission in 40.9% of plaques treated. The UVB light treatment showed no statistically significant difference when compared to occlusion [18].

19.5 Conclusion

Occlusion has shown great promise, as clinicians increasingly look to noninvasive treatments for dermatological conditions such as mild psoriasis. The research also suggests that it may lead to an activation of the immune system and is a risk factor for developing irritant contact dermatitis. Occlusion alters various physiological parameters such as pH, CO_2 emissions, TEWL, and induces histological and microbiological changes in the skin. Occluding the skin has also shown great promise in improving the delivery of certain formulations of NSAIDs and corticosteroids topically. The practical and clinical use of occlusion as a therapy will have to undergo greater research. On bioequivalence evaluations much remains to be assessed regarding not only the effect of total but also partial occlusion, such as provided by certain vehicles.

References

1. Aly R, Shirley C, Cunico B, Maibach H. Effect of prolonged occlusion on the microbial flora, pH, carbon dioxide and transepidermal water loss on human skins. J Invest Dermatol. 1978;71(6):378–81.
2. Hartmann AA. Effect of occlusion on resident flora, skin moisture and skin-pH. Arch Dermatol Res. 1983;275:251–4.
3. Faergemann J, Aly R, Wilson DR, Maibach HI. Skin occlusion: effect on Pityrosporum orbiculare, skin PCO_2, pH, transepidermal water loss and water content. Arch Dermatol Res. 1983;275:383–7.
4. King RD, Cunico RL, Maibach HI, Breenberg JH, West ML, Jeppsen JC. The effect of occlusion on carbon dixide emission from human skin. Acta Derm Venereol. 1978;58(2):135–8.
5. Friebe K, Effendy I, Loffler H. Effect of skin occlusion in patch testing with sodium lauryl sulfate. Br J Dermatol. 2003;148:65–9.
6. Zhai H, Ebel JP, Chatterjee R, Stone KJ, Gartstein V, Juhlin KD, Pelosi A, Maibach HI. Hydration versus skin permeability to nicotinates in man. Skin Res Technol. 2002;8:13–8.
7. Lindberg M, Johannesson A, Forslind B. The effect of occlusive treatment on human skin: an electron microscopic study on epidermal morphology as affected by occlusion and dansyl chloride. Acta Derm Venerol. 1982;62:1–5.
8. Lindberg M, Johannesson A, Forslind B. The effect of occlusive treatment on human skin: an electron microscopic study on epidermal morphology as affected by occlusion and dansyl chloride. Acta Derm Venerol. 1982;62:1–5.
9. Mikulowska A. Reactive changes in the Langerhans' cells of human skin caused by occlusion with water and sodium lauryl sulphate. Acta Derm Venerol. 1990;70:468–73.
10. Allen AM, King RD. Occlusion, carbon dioxide and fungal skin infections. Lancet. 1978;1:360–2.
11. Bibel DJ, Lovell DJ, Smiljanic RJ. Effects of occlusion upon population dynamics of skin bacteria. Dr J Dermatol. 1976;95:607–12.
12. Moon-Kyoung Kim, Suk-Jae Chung, Min-Hwa Lee, Chang-Koo Shim. Delivery of hydrocortisone from liposomal suspensions to the hairless mouse skin following topical application under non-occlusive and occlusive conditions. J Microencapsul. 1998;15:21–9.
13. Bucks DA, McMaster JR, Maibach HI, Guy RH. Bioavailability of topically administered steroids: a 'mass balance' technique. J Invest Dermatol. 1988;91:29–33.
14. Heyeman CA, Lawless-Liday C, Wall GC. Oral versus topical NSAIDs in rheumatic diseases. Drugs. 2000;60(3):555–74.

15. Cevc G, Mazgareanu S, Rother M, Vierl U. Occlusion effect on transcutaneous NSAID delivery from conventional and carrier-based formulations. Int J Pharm. 2008;359:190–7.
16. Banks SL, Pinninti RR, Gill HS, Crooks PA, Prausnitz MR, Stinchcomb AL. Flux across [corrected] microneedle-treated skin is increased by increasing charge of naltrexone and naltrexol in vitro. Pharm Res. 2008;25(7):1677–85.
17. Ramsing DW, Agner T. Effect of glove occlusion on human skin (II) long term experimental exposure. Contact Dermat. 1996;34:258–62.
18. Friedman SJ. Management of psoriasis vulgaris with a hydrocolloid occlusive dressing. Arch Dermatol. 1987;123:1046–52
19. Jugersted JM, Hogh JK, Hellgren LI, Jemec GBE, Agner T. Skin barrier response to occlusion of healthy and irritated skin: differences in trans-epidermal water loss, erythema and stratum corneum lipids. Contact Dermat. 2008:313–9.
20. Rippke F, Schreiner V, Schwanitz HJ. The acidic milieu of the horny layer: new findings on the physiology and pathophysiology of skin pH. Am J Clin Dermatol. 2002;3(4):261–72.

Chapter 20
Challenges with Clinical Endpoints—Bioequivalence

A Brief Walk Through the History of Topical Bioequivalence

Garrett Coman, Nicholas Blickenstaff and Howard I. Maibach

20.1 Introduction

In 1962, Europe and Canada were reeling from the devastating teratogenic effects of thalidomide, a new sedative that had been used to treat morning sickness. While the FDA had previously refused to approve the drug, citing insufficient safety data, the company had already distributed the drug to over 1200 physicians around the USA. The American people were concerned over the FDA's inability to protect the public from dangerous drugs. Coincidentally, Senator Estes Kefauver was working on legislation to limit drug companies' misleading and extravagant claims and used this opportunity to pass the Kefauver–Harris Amendments. These expanded the FDA's authority and responsibility in ensuring not only drug safety but also its efficacy. For the first time, drug companies had to scientifically prove the safety and efficacy of their drugs to the FDA before selling in the USA [1].

Interest in clinical trials intensified in attempting to scientifically prove drugs' effectiveness and safety. At first, the FDA did not have specific requirements on clinical trial design. However, as the FDA passed the Drug Efficacy Study Implementation (DESI) Act that withdrew over a thousand ineffective drugs previously marketed, it published hundreds of critiques of clinical studies that had been previously submitted for drug approval before 1962. To be granted an administrative hearing to dispute the FDA's withdrawal of their drug, companies were required to have adequate and well-controlled clinical investigations and at least two clinical trials to prove safety and efficacy. Regulations stated non-controlled studies were not acceptable evidence to support claims of effectiveness. These helped shape the

G. Coman (✉) · N. Blickenstaff
Department of Dermatology, University of California-San Francisco, San Francisco, CA 94143, USA
e-mail: garrett.coman@hsc.utah.edu

School of Medicine, University of Utah, Salt Lake City, Utah, USA

H. I. Maibach
Department of Dermatology, School of Medicine, University of California, San Francisco, CA, USA

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_20

FDA's continued role into the 1970s as they developed drug class clinical guidelines outlining study designs and expected data required for approval of almost 30 drug classes. The FDA's increasing role in defining required data helped elevate the double blind placebo controlled trial's role in proving safety and efficacy. This, in addition to the organization of the Society for Clinical Trials in 1978, helped bring attention to clinical trial design and the analysis of clinical trials in government as well as industry sponsored clinical trial research [2].

Bioequivalence is the property wherein two drugs with identical active ingredients or two different dosage forms of the same drug possess similar bioavailability and produce the same effect at the site of physiological activity. When the drug acts on its target, the two drugs should deliver the same amount of active ingredient to the target site. After patents and FDA exclusivity expired, companies were free to make generic drugs. This has influenced the landscape of topical drug products, with the majority of topical drugs eventually having generic equivalents. When two drugs have been proven to be bioequivalent, the FDA then considers them therapeutically equivalent, and allows the new formulation to be sold based on previous safety and efficacy data from the therapeutically equivalent drug. Being able to measure bioequivalence is helpful for getting similar or slightly altered drugs approved through the FDA. The goal in creating a generic topical drug product is Q1: quantitative sameness (same components as the reference-listed drug) and Q2: qualitative sameness (same excipients as the reference-listed drug). Additionally, Q3: physical and chemical similarity in microstructure needs to be given careful thought. While oral bioequivalence has been more straightforward, many times just needing blood samples to check drug levels or *in vitro* dissolution testing, proving topical bioequivalence is more challenging. Since the target site is the skin, often very little of the active drug is delivered into systemic circulation, making systemic blood levels irrelevant to the drug levels in the skin. Finding a universally standard method of measuring topical bioequivalence has eluded dermatology to date [3].

The following are selected examples of advances and creative insights into clinical endpoints and attempts at topical bioequivalence, giving a historical understanding of the current state of bioequivalence with a view towards the future:

20.2 Corticosteroid Vasoconstriction

An early success in topical bioequivalence came from McKenzie and Stoughton in 1962, when a simple method for assessing the percutaneous absorption of corticosteroid preparations was suggested. Taking advantage of the vasoconstriction activity of steroids, the skin was inspected for signs of pallor, blanching, or vasoconstriction after topical application of a steroid. While varying the concentration of steroid, the presence or absence of pallor was used as an indication of activity. Their results suggested that the method could be of use in determining penetrability and activity of new compounds, as well as determining bioequivalence [4].

20.3 Photography—Acne

In dermatology, there was an effort to standardize visual observation to allow clinicians to compare results from different trials. An engineer, Cyril Cook, at P&G in 1979 published a standard for observational studies in dermatology with inter- and intratrial consistency, proposing a 0–8 grading scale of acne severity using a set of photographs as reference standards. Photos including both sides of the patient's face were captured on a single exposure by positioning the face parallel to a front-surface mirror with a strobe light source above the camera. When trained clinicians were asked to rate the severity of a patient's acne based on these reference photos, there was a higher rate of reproducibility among physicians than previous efforts at quantifying individual lesions and inflammation of the skin [5]. Since its introduction, the Cook method has been tested and expanded upon, including combining fluorescence, polarized photography, computer alignment, imaging segmentation, and three-dimensional analysis in an effort to improve accuracy of diagnosis. Chiang et al. provides a comprehensive review of the subject [6]. The method still stands as an objective and usable method to assess acne severity in clinical practice, and there remains strong evidence promoting the use of the photographic method in clinical trials for good inter- and intrasubject reliability while efficiently reducing the patients needed in a study [6].

20.4 Hair Weight—Hair Growth

Price et al. used hair weight to measure the change in hair growth after starting finasteride 1 mg daily for 48 and 96 weeks. An area was marked with a permanent tattoo on the chosen scalp site and all hair in the square template was clipped to 1 mm in length. Every 6 weeks the hair was clipped to 1 mm again, with the clipped hairs collected for weighing at the end of the 48 weeks. Not only did hair weight increase in those treated with finasteride versus placebo (an average of 20.4% increase in finasteride and a 5.2% decline in placebo), but hair weight increased to a larger extent than hair count did, implying that other factors such as increased thickness and length play a role in increasing hair growth in men with androgenic alopecia [7]. This insight could not have been made with hair counting alone.

20.5 Hair Photography

Another method for measuring finasteride's (1 mg daily) affect on hair growth is global photographic assessment. Olsen et al. described using 35 mm photographs of 4 areas of the scalp (vertex, anterior/mid, frontal, and temporal scalp) at months 6, 12, 18, and 24. These photographs were sent to three dermatologists at a central site who were blinded to the study center, patient, and treatment. These dermatologists rated each photo on a seven-point scale from greatly decreased to greatly increased. There was increased hair growth and decreased hair loss in all four regions of the scalp, with the greatest affect on the vertex and anterior-/mid-scalp regions [8].

20.6 Paired Comparison Assay

In 1945, Marion Sulzberger had concerns over the divergent opinions of clinicians and investigators in the treatment of fungal infections. He believed the difference in opinions could be attributed to the low incidence of active cases in studies of prophylaxis, dissimilar course of disease, lack of *in vivo* correlation with *in vitro* data, and the cultural demonstration of the presence or absence of fungus as not being a reliable criterion for relative effectiveness. He proposed a novel method to examine relative effectiveness of treatment for foot fungal infections. Since most fungal infections of the feet are symmetrical, using both feet of one patient in a paired comparison would constitute a rapid screening method for determining the relative effectiveness of two therapies [9]. This demonstrated the power of a paired comparison method.

20.7 Psoriasis Plaque Assay

Another application of the paired comparison was Katz et al.'s analysis of Dumas and Scholtz's unpublished psoriasis small plaque assay data of 1965–1983. Using psoriasis patients' stable psoriatic lesions, several compounds, concentrations, and formulations were tested simultaneously on the same subject with positive and negative controls. Small test sites and minute amounts of drug minimized systemic side effects. Suppression of acanthotic, hyperplastic epithelium and square test sites made "all or none" endpoints easily observable. Katz's analysis concluded small plaque bioassay was able to successfully differentiate between corticoids, concentrations, and vehicles; and suggested that it has been an underutilized therapeutic bioequivalent standard for topical dermatologic dosage forms [10].

20.8 Armitage Statistics

P. Armitage published a book in 1975 as a clinician's guide to sequential medical analysis. The method does not have a fixed study patient size (n) in advance of starting the trial. Using statistics, the researchers determine a "stopping rule" based on number of patients and outcomes. Patients are enrolled into the trial and data is collected and analyzed until significance is reached, then the trial is ended. In some instances, this allows a trial to be stopped earlier than with more classical methods of trial design, saving time and resources [11].

20.9 Placebo Response

The placebo response is an interesting aspect of the clinical trial, and still incompletely understood. It has long been known that patients within placebo arms of randomized controlled trials routinely endorse and exhibit considerable improvement [12]. This placebo response has extended in some studies to actual physiologic changes, such as increasing dopamine in Parkinson's patients [13]. Chiou investigated the percent contribution of the vehicle to drug effect in topical drugs. and found it large, in some cases up to 90%, with the average being 55% over eight commonly prescribed topical tetracyclines on reduction of inflammatory and noninflammatory lesions [14] (Fig. 20.1). This has caused concern and increased interest in the real efficacy of the active ingredients in some topical drugs.

However, there is hope, as psoriasis biologics have shown remarkably low placebo response rates. Lamel extensively reviewed randomized controlled trials for biologics' efficacy in psoriasis treatment. Rates of placebo responses were significantly lower (4.14%) than active drug responders (48.4%), with an overall odds ratio of 23.94 [15]. Factors associated with placebo response included treatment indication,

NAME	REDUCTION BY BRAND (%)	REDUCTION BY VEHICLE (%)
0.1 % TRETINOIN CREAM	39 (143)	20 (139)
0.1% ADAPALENE CREME	32 (294)	17 (293)
5% DAPSONE GEL	38 (1,506)	31 (1,504)
1% CLINDAMYCIN	38 (162)	27 (82)
0.1% ADAPALENE + 2.5% BP	50 (564)	27 (489)
1% CLINDAMYCIN + 5% BP	47 (215)	20 (168)
1% CLINDAMYCIN + 5% BP	54 (397)	19 (177)
0.0255 TRETINOIN GEL	41 (845)	23 ± 5.0
MEAN ± SD	42 ± 7.1	23 ± 5.0

Fig. 20.1 Mean percent reduction in total lesion counts from baseline at the end of study. (Compiled from data in [14])

Q1 Q2 Q3 + Option	>Biowaiver based on similarity/ formulation complexity
Amenable to VCA	>Accepted VCA method
Amenable to PK characterization	>PK-based bioequivalence, alone or in conjunction with other methods
Site of action, stratum corneum/epidermis	>Clinical endpoint study >Skin permeability >DPK >Confocal Raman Spectroscopy
Site of action, epidermis/dermis	>Clinical endpoint study >microdialysis >skin permeability >Confocal Raman Spectroscopy
Site of action, below dermis	>Clinical endpoint study >PK-based bioequivalence >microdualysis

Fig. 20.2 Draft decision tree for determination of topical bioequivalence. (Compiled from data in [3])

randomization fraction, a Psoriasis Area and Severity Index (PASI) score inclusion requirement, and the time period of outcomes measure documentation. This lends more confidence to the effectiveness and understanding of therapies.

The Product Quality and Research Institute organized a workshop in 2013 to evaluate challenges in bioequivalence, quality, and novel assessment technologies for topical drug products. They published an overview of the discussion, examining the current topical bioequivalence assessment studies including clinical endpoints, pharmacokinetic trials, pharmacodynamics, dermatopharmacokinetics, microdialysis, open flow microperfusion, confocal raman spectroscopy, *in vitro* skin permeation studies, *in vitro* release testing, and modeling/pharmacometric approaches. While there are merits to each method, it is clear there is still no ideal "one size fits all" model for topical bioequivalence. The paper includes a decision tree to assist in finding the best method to test for topical bioequivalence for each scenario (Fig. 20.2). The report concludes with a plea for creative new approaches to optimize the existing methods and explore alternative methodologies, while adding that the clinical trail remains the "standard" for establishing clinical bioequivalence for topical products [3].

20.10 Percutaneous Penetration

A thorough understanding of percutaneous penetration is a good starting point for creative methods to determine bioequivalence. Ngo and Maibach's 15 Factors of Percutaneous Penetration is a thorough review of the current understanding, identifying factors associated with penetration. Only one of these deals with the physiochemical

Steps	Factors determining percutaneous absorption
1	Physiochemical properties of penetrant
2	Vehicle effects
3	Dose, duration, surface area, and frequency
4	Sub-anatomical pathways
5	Regional variability
6	Population variability
7	Surface conditions
8	Health and integrity
9	Substantivity and binding
10	Distribution
11	Exfoliation
12	Wash effect
13	Metabolic and photochemical transformation
14	Excretion kinetics
15	Method of determination

Fig. 20.3 Factors determining percutaneous penetration. (Compiled from data in Ngo 2012)

properties of the penetrant; the others are: dose, duration, surface area, frequency, sub anatomical pathways, regional variability, population variability, surface conditions, health and integrity, substantivity and binding, distribution, exfoliation, wash effect, metabolic and photochemical transformation, excretion kinetics, and method of determination [16] (Fig. 20.3). With an increased understanding of the basic science behind penetration, the ability to compare topical bioequivalence increases.

Care must be taken when choosing a skin model for *in vitro* models, as research has shown significant differences. When comparing two skin models: synthetic hydrophobic membrane and neonatal porcine skin, significant differences in permeation of a wide range of small hydrophilic molecules released from dissolving microneedle arrays was found, with the porcine skin being closer to *in vivo* data [17]. Care should be taken when synthetic membranes are used, and further research is necessary to determine an ideal standard for testing.

20.11 Skin Variability

Most believe oral and topical absorption of drugs to be widely variable; however, recent research has shown the overall mean and median coefficient of variation for topical and oral absorption for ten commonly used drugs were comparable (within 10% of each other). While interindividual variation may not be as divergent as previously assumed, in a drug-to-drug comparison certain medications demonstrated considerably more variation when absorbed orally versus topically. These

differences were unable to be explained by molecular weight and octanol partition coefficient alone [18]. As topical drug delivery results in decreased dose-related side effects, more stable systemic circulation levels, and decreased dosing frequency for patients, further investigation into topical dosing is warranted.

20.12 Comparative Effectiveness

Comparative effectiveness research aims to produce actionable evidence on the safety and effectiveness of medical products and interventions as they are used outside of the controlled research setting. There are many problems with early comparative effectiveness data immediately following a new medication release—increased patient channeling due to patient-, physician-, and system-related factors, changes in the characteristics of patient population during early marketing, and the small number of users in the first few months of marketing [19]. Schneeweiss et al. proposed sequential cohort monitoring with secondary health care data and propensity score balancing, extended follow-up of phase III and phase IV trials, indirect comparisons of placebo-controlled trials, and modeling and simulation of virtual trials. While establishing comparative effectiveness for newly marketed drugs is challenging, being able to adjust and correct use patterns early in the drug's life will be easier before utilization patterns have been set.

Insights into questions that have puzzled dermatologists give hope for an promising future in topical bioequivalence. Shaw and Maibach [20] recently investigated the unanswered differences in patch testing and repeat open application testing (ROAT) rates with hydrocortisone creams versus ointments. While topical corticosteroid ointments generally induce more vasoconstriction than corticosteroid creams, *in vitro* assays of excised human skin have shown statistically significant greater hydrocortisone penetration from creams than ointments [21]. Shaw and Maibach found 15 of 20 patients (75%) had a positive ROAT test with hydrocortisone cream versus 6 of 16 (38%) with hydrocortisone ointment. Patch testing was positive with hydrocortisone cream in 23 of 27 patients (85%) versus 1 of 14 (7%) with hydrocortisone ointment, which the authors attribute to differences in transepidermal penetration with different vehicles. This suggests a lack of bioequivalence between hydrocortisone creams and ointments as well as different formulations, which is troublesome for our currently used vasoconstrictor assay "bioequivalence standard." Further *in vitro* and *in vivo* studies are needed to fully understand these clinically significant issues.

20.13 Conclusions

As the focus continues to be on evidence-based medicine, subsequent generations of physicians need training and experience in understanding the literature, easily accessible institutional memory, experimental design, grading, and designing creative

new ways to generate reliable data. Perhaps certification, as is more common in Europe, would provide more structure and standardized learning. The FDA's role will continue to evolve, reflecting the ever-globalization of medicine development and its role as a generator of public data. Emphasis on understanding bioequivalence through a combination of continued support from government, industry, and philanthropy will be a key contributor to providing evidence for topical drug effectiveness and the field of dermatology.

References

1. Commissioner O of the Consumer Updates—Kefauver-Harris Amendments Revolutionized Drug Development. http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm322856.htm. Accessed 16 Jan 2014.
2. Commissioner O of the Overviews on FDA history—FDA and clinical drug trials: a short history. http://www.fda.gov/AboutFDA/WhatWeDo/History/Overviews/ucm304485.htm. Accessed 17 Feb 2014.
3. Yacobi A, Shah VP, Bashaw ED, et al. Current challenges in bioequivalence, quality, and novel assessment technologies for topical products. Pharm Res. 2014. doi:10.1007/s11095-013-1259-1.
4. McKenzie AW. Percutaneous absorption of steroids. Arch Dermatol. 1962;86(5):611–14. doi:10.1001/archderm.1962.01590110047006.
5. Cook CH, Centner RL, Michaels SE. An acne grading method using photographic standards. Arch Dermatol. 1979;115(5):571–5.
6. Chiang A, Hafeez F, Maibach HI. Skin lesion metrics: role of photography in acne. J Dermatol Treat. 2014;25(2):100–5. doi:10.3109/09546634.2013.813010.
7. Price VH, Menefee E, Sanchez M, Ruane P, Kaufman KD. Changes in hair weight and hair count in men with androgenetic alopecia after treatment with finasteride, 1 mg, daily. J Am Acad Dermatol. 2002;46(4):517–23.
8. Olsen EA, Whiting DA, Savin R, et al. Global photographic assessment of men aged 18 to 60 years with male pattern hair loss receiving finasteride 1 mg or placebo. J Am Acad Dermatol. 2012;67(3):379–86. doi:10.1016/j.jaad.2011.10.027.
9. Sulzberger MB, Shaw HC, Kanof A. Evaluation of measures for use against common fungous infections of the skin: screening tests by means of paired comparisons on human subjects. US Nav M Bull. 1945;45:237–48.
10. Katz M, Shaikh Z, Maibac HI. Scholtz–Dumas psoriasis small plaque bioassay. J Dermatol Treat. 2000;11(1):15–9. doi:10.1080/095466300750163717.
11. Armitage P. Sequential medical trials. Wiley; 1975, 2nd edition, John Wiley & Sons Inc, New York City, NY.
12. Beecher HK. The powerful placebo. J Am Med Assoc. 1955;159(17):1602–6.
13. Strafella AP, Ko JH, Monchi O. Therapeutic application of transcranial magnetic stimulation in Parkinson's disease: the contribution of expectation. Neuroimage. 2006;31(4):1666–72. doi:10.1016/j.neuroimage.2006.02.005.
14. Chiou WL. Low intrinsic drug activity and dominant vehicle (placebo) effect in the topical treatment of acne vulgaris. Int J Clin Pharmacol Ther. 2012;50(6):434–37.
15. Lamel SA, Myer KA, Younes N, Zhou JA, Maibach H, Maibach HI. Placebo response in relation to clinical trial design: a systematic review and meta-analysis of randomized controlled trials for determining biologic efficacy in psoriasis treatment. Arch Dermatol Res. 2012;304(9):707–17. doi:10.1007/s00403-012-1266-9.
16. Knaak JP, Timchalk C, Tornero-Velez R, Ngo MA, Maibach HI. 15 factors of percutaneous penetration of pesticides. In: Parameters for pesticide QSAR and PBPK/PD models for human risk assessment, vol. 1099. ACS Symposium Series. American Chemical Society ,

Oxford Press, Oxford, Great Britain; 2012:67–86. http://dx.doi.org/10.1021/bk-2012–1099.ch006. Accessed 5 March 2014.
17. Garland MJ, Migalska K, Tuan-Mahmood TM, et al. Influence of skin model on in vitro performance of drug-loaded soluble microneedle arrays. Int J Pharm. 2012;434(1–2):80–9. doi:10.1016/j.ijpharm.2012.05.069.
18. Levin J, Maibach H. Interindividual variation in transdermal and oral drug deliveries. J Pharm Sci. 2012;101(11):4293–307. doi:10.1002/jps.23270.
19. Schneeweiss S, Gagne JJ, Glynn RJ, Ruhl M, Rassen JA. Assessing the comparative effectiveness of newly marketed medications: methodological challenges and implications for drug development. Clin Pharmacol Ther. 2011;90(6):777–90. doi:10.1038/clpt.2011.235.
20. Shaw DW, Maibach HI. Clinical relevance of tixocortol pivalate-positive patch tests and questionable bioequivalence of different hydrocortisone preparations. Contact Dermatitis. 2013;68(6):369–75. doi:10.1111/cod.12066.
21. Stoughton RB. The vasoconstrictor assay in bioequivalence testing: practical concerns and recent developments. Int J Dermatol. 1992;31(Suppl. 1):26–8.

Chapter 21
Clinical Considerations of Bioequivalence for Topical Dermatologic Drugs

Jessica Schweitzer and Howard I. Maibach

21.1 Introduction

Bioequivalence (BE) is used to compare the rate and extent to which different drugs are released from their vehicles and cross the stratum corneum to exert their local skin or systemic effect. A central concept of BE is that drugs with the same mechanism of action and equivalent efficacy will have equivalent effects if presented in the same concentration at the site of action. If two drugs can be shown to have the same efficacy and the same pharmacokinetics, or two formulations of the same drug can be shown to have the same pharmacokinetics, they can be considered bioequivalent.

Many well-tested methods of determining BE, such as *in vitro* dissolution testing, are limited to solid oral drugs, and cannot be applied to the vast array of semisolid topical products used in dermatology [22]. An alternative method for determining BE between topical drugs includes visual assessment, but this provides the challenge of defining reproducible clinical endpoints for these drugs. In this chapter, clinical endpoints that have been used successfully to determine BE are outlined. These endpoints may provide guidance for the development of a more generalized, cost-efficient, and effective method to determine topical BE across other drug classes.

J. Schweitzer (✉)
Largo Medical Center, Largo, FL 33770, USA
e-mail: jschweitzer09@gmail.com

H. I. Maibach
Department of Dermatology, School of Medicine, University of California, San Francisco, CA, USA

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_21

21.2 Suction Blister Technique, Dermal Microdialysis, Skin Stripping, and *In Vitro* Release to Determine Bioequivalence

Techniques have been developed to measure the effect or pharmacokinetics of transdermally applied drugs to demonstrate BE.

An example is the suction blister technique. A skin blister is created and aspirated fluid is collected for analysis. This technique has been used successfully to compare the efficacy of tanning products, citropten and bergapten, to deliver eight-methoxypsoralen in PUVA bath therapy, and also to compare the pharmacokinetics of bergapten in different vehicles [4]. In the latter case, blister fluid analysis showed that bergapten was more effectively delivered in an ethanolic solution than in an oil solution.

Dermal microdialysis (DMD) uses a probe, consisting of a semipermeable membrane, which is introduced into the dermis and the use of the technique is described in detail in chapters chapter 10. The probe is connected to a precision pump, which pumps a tissue-compatible fluid through the probe. Small molecules in the dermis, such as the nonprotein bound fraction of a drug, can diffuse across the membrane of the probe into the perfusate [3]. The drug concentration in the perfusate can be measured and is determined by the concentration in the interstitial fluid, the partition coefficient between the interstitial fluid and the perfusate, and the flow rate. Many studies have reported an accurate use of DMD to assess BE [3, 5, 7, 14, 16, 17]. As DMD can simultaneously monitor test and reference formulations on a single subject using separate probes, DMD reduces intersubject variability and reduces the number of subjects required for a statistically significant BE evaluation. In one study using DMD to assess for BE, 27 subjects were required when using two probes, whereas only 18 subjects were needed when using three probes [16]. With DMD, a subject number of 20 can be used to determine BE with 80 % power and within 80–125 % confidence limits [19]. DMD allows for continuous monitoring of drugs and is less invasive than skin biopsy.

Other methods to evaluate BE include skin stripping and *in vitro* release [21]. *In vitro* release is discussed in detail in chapter 4. In skin stripping, layers of the stratum corneum are removed via tape stripping and tested for drug concentrations [21]. Although there have been varying results from this technique, it is used to evaluate BE among topical products [11, 24]. Although the current methods to determine BE have been described, these methods can be tedious, time consuming, and painful. Reaching an endpoint to determine BE between two topical formulations via a cost-effective, timely, patient-conscientious method has yet to be created.

21.3 Reaching Clinical Endpoints in the Vasoconstrictor Assay with Chromametry, Digital Image Analysis, and Visual Inspection

The vasoconstrictor assay is one of the most widely studied and successful clinical methods to determine BE for steroids [4, 12, 13, 20, 26, 28]. This assay uses the dose-dependent local vasoconstriction and blanching effect of corticosteroids to compare potency between them. Three methods are used to measure skin blanching: chromametry, digital image analysis, and visual inspection alone. Although chromametry and digital image analysis have been somewhat successful, the third method of visual inspection can be carried out across other drug classes beyond steroids. In addition to the previously mentioned methods of determining BE, the concept of using visual inspection as seen in the vasoconstrictor assay could be utilized when developing a more standardized method of determining BE amongst other classes of drugs.

Chromameters have an added potential benefit of recording vasoconstriction results; however, questions of its efficacy have been raised [12]. The chromameter measures skin blanching by quantifying the reflectance of a xenon light pulse with three parameters: red-green, yellow-blue, and light-dark [4]. These three parameters are used to specify a point in three-dimensional space that records the color or extent of blanching [4]. Limitations of chromameter use include color changes that are not directly related to the steroid effect, such as blanching produced by the pressure exerted by the head of the chromameter, existing variables in skin color due to nevi and hair, and even the angle of application of the chromameter [4].

The second and most recent method to assess blanching in the vasoconstriction assay is the image analysis of digital photographs. The data is obtained in high resolution which allows a greater data size to analyze from the image [4]. Furthermore, this method is not that sensitive to visual irregularities due to blemishes or nevi, as seen in chromametry. In a preliminary study comparing this method with chromametry, the digital image analysis was shown to be superior to the chromameter method in detecting levels of vasoconstriction with predetermined concentrations of steroids [4]. The digital image analysis accurately detected various blanching effects depending on preidentified strength, whereas the chromameter detected no statistically significant difference between the treatments.

The third method to estimate blanching is visual inspection, which may be used with the most ease and efficacy. Moreover, visual inspection can be used to compare the efficacy of two steroids in the same class and/or composition, such as creams or gels [26]. Visual inspection requires less subjects and has shown to be as accurate, if not more, than chromametry [2, 19]. Thus, it may be the best method to explore when developing an accurate, efficient, and inexpensive standardized method to assess BE in topical drugs that goes beyond comparison of vasoconstriction in steroids. One limitation of this method is the subjective nature of clinical inspection. However, with proper training, visual inspection has proven to be a reliable method in determining BE [2, 26].

21.4 Other Trials Using Visual Inspection to Reach Clinical Endpoints

In addition to the vasoconstrictor assay, visual inspection has been utilized to determine clinical endpoints in other studies to compare tretinoin and adapalene, psoriasis severity, acne severity, and in the treatment of androgenetic alopecia with finasteride. When determining a method to estimate BE, these trials and their methods can be of use.

21.4.1 Tretinoin and Adapalene

Topical medications for acne have been successfully compared using methods largely relying on visual inspection. Visual inspection was used to record two of the three clinical endpoints to differentiate between tretinoin concentrations, different tretinoin vehicles including gel versus cream, and two different drugs: tretinoin and adapalene [18]. Tretinoin or adapalene were applied on the forearms of different subjects for 21 days to compare the pharmacologic activity of each drug, but not for acne treatment. Pharmacologic activity was analyzed using three clinical endpoints: erythema, exfoliation (scaling/peeling), and increased transepidermal water loss (TEWL). The extent of exfoliation was recorded using a number scheme (0–3) for severity [18]; TEWL was measured with an evaporimeter, and erythema was assessed between a severity range of 0–4. With two of the three endpoints determined via visual inspection (excluding TEWL), this pharmacodynamics model could serve as a template to assess BE between two different retinoids.

21.4.2 Psoriasis Small Plaque Bioassay

Visual assessment has also been successful when used with the psoriasis small plaque bioassay. Twelve test medications in the form of cream, ointment, or gel vehicles were applied to assigned psoriatic plaques on each subject [15]. A 4-point visual scale was applied for the scoring of improved plaques with each treatment. The data shows the accurate assay reflected dose-response curves and differentiates potency between varying corticosteroid concentrations and vehicles [15].

21.4.3 Assessing Acne Severity

Many scales for assessing acne severity incorporate visualization to reach clinical endpoints in their standards as well [6, 10, 27]. For example, the acne-grading method by Cook uses a severity scale ranging from 0 to 8, and incorporates photographic examples of patients at levels 0, 2, 4, 6, and 8 [10]. This method can also

be used to standardize graders by comparing these photographic examples with the current patients. Different acne grading systems use either lesion counting or photographic assessment, as in the Cook method, to assess acne severity [27]. Advancing technology in photographic methods continues to be a promising technique to assess clinical endpoints. This approach can be applied to determine BE using visual inspection amongst other drugs.

21.4.4 Androgenetic Alopecia and Finasteride

Similarly, visual inspection using photography is a well-documented endpoint in clinical trials when comparing finasteride to placebo for hair loss treatment [23]. In a multicenter, double-blind, randomized study of men with vertex hair loss, the global photographic assessment of clinical photographs was successfully used to contrast results between finasteride and the placebo. Furthermore, the results were consistent with those obtained from more objective measurements of hair weight [25]. Based on these previous studies, it may be possible to develop a method of BE based on visual observation. The success in these areas of research can be used to develop similar methods in determining BE.

21.5 Clinical Endpoints in BE

The challenges of using clinical endpoints to determine BE include the large number of sample subjects required and the subjective nature of evaluation. A possible solution to this problem is the Cochran–Armitage design, which can be used in clinical trials to assess a dose-response relationship by using a smaller sample size. One subset of this design, called the exact permutation Cochran–Armitage trend test, can be used for smaller sample sizes in dose-response studies [1, 8]. With this test, the experiment is complete once statistical significance has been found, so a larger sample size is not needed. Thus, a smaller number of subjects may be used in trials assessing BE. Furthermore, Corcoran et al. have also developed computational tools to estimate the power of tests with a smaller sample size [9]. Therefore, using this test could allow for a smaller sample size in future BE assays and lead to a more cost-effective method.

In addition to visual assessment, attempts to assess BE by percutaneous penetration have been investigated, largely by comparisons of total penetration into the reservoir, but, to a lesser extent, drugs in the skin layers. Percutaneous penetration of a compound consists of at least 15 steps, including absorption and excretion kinetics, characteristics of the vehicle, and tissue deposition [29]. It has been shown with percutaneous penetration that some compounds have similar diffusion coefficients in the stratum corneum [29]. With adequate replicates, and including some of the other

clinically relevant 15 steps of penetration, a viable BE algorithm could be developed [29]. These methods are further reviewed and discussed throughout this book.

21.6 Conclusion

Taken together, there is much room to improve the assessment of topical BE using clinical endpoints. Many current methods to determine BE at this point are costly, invasive, or inefficient. The vasoconstrictor assay in BE and other methods in other areas of research use visual inspection to reach clinical endpoints. Together, these methods can be further developed into future BE parameters that are cost effective. Activities endeavoring to refine BE for topicals is unevenly spread—with few efforts focused on a long-term approach. Hopefully, this overview provides a stimulus for additional funding and founding efforts to provide reliable topical generic formulations.

References

1. Armitage P. Tests for linear trends in proportions and frequencies. Biometrics. 1955;11:375–86.
2. Au WL, Skinner M, Kanfer I. Bioequivalence assessment of topical clobetasol propionate products using visual and chromametric assessment of skin blanching. J Pharm Pharm Sci. 2008;11(1):160–6.
3. Benfeldt E, Hansen SH, Volund A, Menne T, Shah VP. Bioequivalence of topical formulations in humans: evaluation by dermal microdialysis sampling and the dermatopharmacokinetic method. J Invest Dermatol. 2007;127:170–8.
4. Bronaugh R, Maibach H. Topical absorption of dermatological products. New York: Marcel Dekker; 2002. (Print).
5. Brunner M, Dehghanyar P, Seigfried B, Martin W, Menke G, Muller M. Favourable dermal penetration of diclofenac after administration to the skin using a novel spray gel formulation. Br J Clin Pharmacol.2005;60:573–7.
6. Chiang F, Hafeez F, et al. Skin lesion metrics: role of photography in acne. J Dermatolog Treat. 2013;25(2):100–5. Jul 1. [Epub ahead of print]
7. Chik Z. Letter to the editor: comparative pharmacokinetic assessments of topical drugs: evaluation by dermatopharmacokinetics, microdialysis, and systemic measurement. J Invest Dermatol.2010;130:2828–30.
8. Cochran WG. Some methods of strengthening the common x2 tests. Biometrics. 1954;10:417–51.
9. Cocoran CD, Mehta CR. Exact level and power of permutation, bootstrap and asymptotic tests of trend. J Mod Appl Stat Methods. 2002;1:42–51.
10. Cook C, Center R, et al. An acne grading method using photographic standards. Arch Dermatol. 1979;115(5):571–5.
11. Franz TJ. Study # 1, avita gel 0.025 % vs retin-a gel 0.025 %. http://www.fda.gov/ohrms/dockets/ac/01/slides/3804s2_03_Franz.pdf. Accessed 15 Sept 2014.
12. Guidance for Industry: Guidance: topical dermatologic corticosteroids: in vivo bioequivalence. Division of bioequivalence, Office of Generic Drugs, Food and Drug Administration. Rockville, MD. June 1995.

13. Haigh JM, Smith EW, Maibach HI. New developments in the methodology available for the assessment of topical corticosteroid-induced skin blanching. In: Bronaugh RL, Maibach HI, editors. Percutaneous absorption. Vol 3. New York: Marcel Dekker; 1998. pp. 487–98.
14. Hegemann L, Forstinger C, et al. Microdialysis in cutaneous pharmacology: kinetic analysis of transdermally derived nicotine. J Invest Dermatol. 1995;104(5):839–43.
15. Katz M, Shaikh Z, Maibach H. Scholtz-Dumas psoriasis small plaque bioassay. J Dermatol Treat. 2000;11:15–19.
16. Klimowicz A, Farfal S, Bielecka-Grzela S. Evaluation of skin penetration of topically applied drugs in humans by cutaneous microdialysis: acyclovir vs. salicylic acid. J Clin Pharm Ther. 2007;32:143–8.
17. Korinth G, Jakasa I, Wellner T, Kezic S, Kruse J, Schaller KH. Percutaneous absorption and metabolism of 2-butoxyethanol in human volunteers: a microdialysis study. Toxicol Lett. 2007;170:97–103.
18. Lehman PA, Franz TZ. Assessing the bioequivalence of topical retinoid products by pharmacodynamics assay. Skin Pharmacol Physiol. 2012;25:269–80.
19. McCleverty D, Lyons R, Henry B. Microdialysis sampling and the clinical determination of topical dermal bioequivalence. Int J Pharm. 2006;308:1–7.
20. McKenzie AW, Stoughton RB. Method for comparing percutaneous absorption of steroids. Arch Dermatol. 1962;86:608–10.
21. Narkar Y. Bioequivalence for topical products–an update. Pharm Res. 2010;27:2590–601.
22. National archives and records administration: Code of federal regulations (title 21). Food and drugs, in: revised 2001 April 1 p. url access.gpo.gov/cgi-bin/cfrassemble.cgi?title=200121. Accessed 15 Sept 2014.
23. Olsen EA, Whiting DA, et al. Global photographic assessment of men aged 18–60 years with male pattern hair loss receiving finasteride 1 mg or placebo. J Am Acad Dermatol, 2012;67(3):379–86.
24. Pershing LK. Bioequivalence assessment of three 0.025% tretinoin gel products: dermatopharmacokinetic vs. clinical trial methods. http://www.fda.gov/ohrms/dockets/ac/01/slides/3804s2_02_Pershing//index.htm. Accessed 15 Sept 2014.
25. Price VH, Menefee E, et al. Changes in hair weight in men with androgenetic alopecia after treatment with finasteride (1 mg daily): three- and 4-year results. J Am Acad Dermatol. 2006;55(1):71–4.
26. Ramirez E, Laosa O, et al. Acceptability and characteristics of 124 human bioequivalence studies with active substances classified according to the biopharmaceutic classification system. J Clin Pharmacol. 2010;70(5):694–702
27. Ramli R, Malik AS, et al. Acne analysis, grading and computational assessment methods: an overview. Skin Res Technol. 2012;18(1):1–14.
28. Smith EW, Meyer E, Haigh JM. Accuracy and reproducibility of the multiple reading skin blanching assay. In: Maibach HI, Surber C, editors. Topical corticosteroids. Basel: Karger; 1992. pp. 65–73.
29. Hui X, Lamel S, Qiao P, Maibach HI. Isolated human/animal stratum corneum as a partial model for 15 steps in percutaneous absorption: emphasizing decontamination, Part I. J Appl Toxicol. 2013;33:157–72.

Part VII
Guidelines and Workshop Report

Chapter 22
OECD Test Guideline 428—A Method for *In Vitro* Percutaneous Absorption Measurement?

John Jenner

22.1 Introduction

The goal of measuring percutaneous absorption is to predict the amount of chemical penetrant that will be absorbed over a period of time following application to, or contamination of the skin. It would seem intuitively obvious that the best way to do this would be to measure the percutaneous absorption of the penetrant in a group of humans, which is not always possible, particularly if the penetrant is toxic. Moreover, estimation of penetration rates *in vivo* can be difficult and sometimes less precise than *in vitro* measurement. The attraction of *in vitro* methods is that they measure only a limited number of the variables involved in a biological system under precisely controlled conditions. For instance, an *in vitro* system can estimate the flux of a chemical across the skin directly, whereas measurement of urinary or blood concentration can be done only if the influence of other processes such as redistribution to other body compartments, elimination and excretion can be excluded. Paradoxically, this advantage is also a disadvantage in that having removed a large number of the effects of redistribution, elimination and excretion on percutaneous absorption; these must then be accounted for when interpreting the results to predict bioavailability *in vivo*.

Our need to adopt similar or identical methods to carry out experimental research is driven by the requirement to show that the experiments can be reproduced by other scientists. It is only by the experiments of different scientists showing the same results that experimental research can become established as a scientific fact. In some areas of science, the accurate recording of the experimental method is sufficient to enable precise reproducibility of an experimental result. In biology and medicine, however, researchers must contend with the inherent variability of biological organisms that are the subjects of their experiments. Reproducibility is very important if the results of experimental investigations are to be used to support

J. Jenner (✉)
Biomedical Sciences Department, Dstl Porton Down, Salisbury, Wiltshire, SP1 3FZ, UK
e-mail: jjenner@dstl.gov.uk

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_22

conclusions about the safety of substances to be administered to humans or to which humans may become exposed in their everyday lives.

Although the principles of measuring the diffusion of molecules across the skin *in vitro* were published in the middle years of the twentieth century [1], some 20 years passed before the publication of a definitive description of the measurement method [2, 3] started a debate within the scientific community about how such experiments should be best performed. The method had been adopted by the pharmaceutical industry to determine if their products might be hazardous by percutaneous absorption and later to test formulations to deliver drugs by this route. This debate is described in the reports of several workshops [4, 5] where the various methods and limitations on those methods were discussed. The recommendation of this workshop—that standardisation of methods for measuring skin absorption were required—was instrumental in the publication of a series of guidance documents by various reviewing groups [6–8], as well as Test Guideline 428 "Skin absorption: *in vitro* method" by the Organisation for Economic Cooperation and Development (OECD) in 2004 [9]. These documents are being continually updated and, though in themselves not a thorough review of the scientific literature, they are an excellent starting point for scientists new to the area.

Taken together, the clear message from these documents is that *in vitro* dermal absorption studies should not be used in isolation but as a part of a hierarchical decision-making process in safety testing and with *in vivo* and clinical studies to define or predict percutaneous absorption in man.

22.2 The Contents of Guideline 428

An OECD Guideline is not intended to be a definitive method. Instead, it sets out the best method to use, leaving the option to adopt other methods if those can be justified. The justification usually comes from the reason for undertaking the test. For instance, if measurement of the rate of skin diffusion is being carried out as a part of toxicological risk assessment, the conditions may be set to maximise the measured diffusion rate, on the basis that this will build a safety factor into the test result. This approach retains flexibility in experimental design, enabling the widest range of scientists to follow the good practice described in the guideline. The following is a summary of the key methodological aspects covered by Guideline 428 with some comments on the implementation of each.

22.2.1 Diffusion Cell Design

Test Guideline 428 does not identify a specific horizontal diffusion cell design, simply describing the cell as two chambers separated by a skin membrane with a suitable facility for taking samples from the receptor chamber. The important characteristics of a diffusion cell are mandated as described in Chap. 5 (e.g. there

should be a good seal between the skin and the chambers). Occlusion or non-occlusion is permitted to enable the conditions of the test to be adapted to the user's requirements. Although not specified in the guideline, the material used to make the diffusion cells should be chosen such that the penetrants being tested are not absorbed into them. If this occurs continually across tests, cross contamination is possible and the penetration rates within an individual test can be compromised.

22.2.2 Choice of Receptor Fluid

The type of receptor fluid is not mandated, though a physiologically compatible one is preferred and it should not damage the membrane. Over the years, there has been much debate on which receptor fluid gives the most representative measure of an *in vivo* absorption rate. The most important attributes of the receptor fluid are that the solubility of the penetrant in the receptor fluid should not be rate limiting and that it should not damage the membrane so that a falsely high rate is measured. Many workers strongly support the use of a physiological buffer as a receptor fluid, but the limited solubility of some penetrants with established dermal absorption has led to the use of various additives such as bovine serum albumin to increase solubility or include some protein binding. There is also some concern that for highly lipophilic compounds, the solubility of the penetrant in the receptor does become rate limiting and a falsely low rate can be measured. Conversely, others recommend the use of a mixture of ethanol and water to maximise the penetration rates of the widest range of chemical possible, though concerns have been raised about the possible extraction of skin lipids artificially increasing penetration. This may work well for risk assessment, but may give a falsely high rate that would not work well for predicting release from transdermal delivery devices of topical drug formulations. The choice of receptor fluid must be carefully considered and justified on the basis of the purpose for which the penetration data is to be used. Definitive studies that link penetration rates measured *in vivo* with *in vivo* pharmacokinetics across a range of penetrant types remain to be performed and represent a considerable technical challenge.

22.2.3 Skin

The guideline makes recommendations about the types of skin that should be used whilst recognising the limitations on individual scientists by national legislation and ethical requirements. Although human skin is preferred for predicting human absorption, animal skin can be used when human skin is not available. Indeed, animal skin may be preferred if the aim of the work is to perform experimental work in animals. Skin can be viable or non-viable, but viable is preferred, and prepared by any suitable means such as enzymic digestion, heat, chemical separation and cutting with a dermatome to prepare membranes 200–400 μm thick. Thicker skin slices may be used as long as excessive thickness is avoided (ca < 1 mm).

22.2.4 Integrity Testing

Although it is possible to damage the skin during preparation, the need for integrity testing has been the subject of some debate because the setting of a pass/fail criterion could exclude some pieces of skin at the extreme of the normal range. Although damaged membranes will be obvious when the penetration data is analysed as an immediate and rapid increase in receptor penetrant concentration to a constant value, this will only be known when the experiment is complete and damaged cells cannot contribute to the results. An early indication of damaged cells may allow them to be replaced and more complete data sets can be obtained. Guideline 428 mandates the testing of integrity but does not make any recommendation as to the method to be used.

A number of techniques have been utilised to test the physical integrity of the skin mounted in a diffusion cell [10]. The diffusion of tritiated water across the skin is regarded as a good test of the permeability of the preparation, but this can take several hours to perform, requiring equilibration before and after [11]. The possibility that the skin might be damaged when the tritiated water is removed and this damage not detected until after the test should also be considered. Alternatively, the measure of the resistance of the skin preparation to electrical current (resistivity) can be performed more rapidly but is only suitable for receptor solutions containing salts that can conduct current [12]. The natural loss of water from the diffusion cell can also be measured using a transepidermal water loss (TEWL) monitor [13]. Though there is some evidence that TEWL may not be completely determined by barrier function *in vitro* [14], the method is quick and reliant on only the presence of water in the receptor and an intact membrane to prevent its free evaporation.

22.2.5 Application of the Test Substance

The guideline recommends that the test penetrant should be radiolabelled. This certainly aids sample analysis but has the disadvantage that parent compound and breakdown products cannot be distinguished. If the analysis of metabolism is a part of the study, another method of chemical analysis is required even if radiolabelling is used. Equally, for many chemicals the preparation of radiolabelled material may be prohibitively expensive, if technically possible. Liquid chromatography (LC) is a good alternative if suitable methods can be identified. LC has the advantage that it will quantify parent compound and may quantify breakdown products simultaneously. It does, however, take longer to perform on the large number of samples analysed during an *in vitro* diffusion test, so LC may not be less expensive.

How the application of test penetrant to the surface of the skin is critical to the characteristics of penetration measured (i.e. lag time and maximum rate) because it can affect the area of skin covered by the penetrant and the concentration of the chemical in the donor chamber. Guideline 428 does not prescribe specific methods

for chemical application, allowing investigators to use the method that is applicable to the exposure scenario they are investigating. Nonetheless, the way in which the penetrant is applied to the skin should be given very careful consideration to minimise artefactual measures of penetration. When making measurements of the steady-state flux, the amount of penetrant applied should be sufficient so that not more than 10 % of the chemical is absorbed during the experiment and the whole of the available area for diffusion should be covered, or the area should be accurately measured to enable calculations of flux rates per unit area. When finite doses as small droplets are used, the area of spread should be confirmed in some way so that this can be used in flux rate calculations. If a volatile solvent is used to apply the penetrant and subsequently evaporates to leave the penetrant on the surface in a thin layer, the solvent should be chosen to minimise any effects on the lipids in the skin, and hence, perturbation of penetration. In summary, the method of applying the penetrant to the surface of the skin in an *in vitro* experiment should be determined by the exposure scenario being investigated, but the effect of the method of application on the calculation of penetration rates should be carefully considered.

22.2.6 Conditions During the Test

During an experiment *in vitro* diffusion cells should be kept at a constant temperature equivalent to the skin surface temperature (32 ± 1 °C). Even in hot or cold conditions, the skin will be kept at this temperature by homeostasis, and since temperature can significantly change rates of diffusion, measurements made outside of this range have little physiological relevance. There are various methods by which this temperature control can be achieved using water jackets integrated into the diffusion cell, heated plates or water baths. Whatever method is used, it is a good practice to confirm the temperature at the surface of the skin by some means (e.g. thermocouple or thermal imaging).

When investigating the penetration of volatile chemicals and the experiments are not carried out under occluded conditions, the effect of airflow should be taken into account when interpreting the results. If possible, airflow should be measured and steps taken to ensure that it is uniform across all the diffusion cells used to minimise experimental variation. Some workers capture the evaporated chemical on an activated charcoal cap, as described in Chap. 5. If this is used, the effect that it has on free evaporation should be carefully considered.

During the test, the receptor fluid should be constantly stirred to ensure that high concentrations of penetrant do not accumulate in a layer just below the skin and reduce the diffusion rate across the skin resulting in a falsely low penetration being measured.

Over the period of the test, the receptor fluid is sampled at regular intervals and replaced with fresh receptor fluid to maintain the volume of the fluid in the receptor chamber.

22.2.7 After the Test

A critical part of an *in vitro* diffusion test is the mass balance at the end of the test. The method for doing this is summarised in Chap. 5 but this may be complicated if the penetrant is not radiolabelled. In this case suitable methods for extracting and quantifying the penetrant remaining in the skin need to be developed and validated.

The guideline makes several recommendations about reporting of *in vitro* diffusion measurement results, which are generally good practice. Though the precise format of any reporting will be determined by national regulatory requirements, what the customer for the work wants and any requirements for publication, a graph of amount penetrated against time to allow assessment of the nature of the penetration, maximum flux rates, lag times and a mass balance are the minimum required to adequately describe the experimental results. The amount of penetrant in the samples taken throughout the test needs to be accounted for the calculation of amount penetrated. If maximum flux rates are calculated, the method of the calculation should be described.

22.2.8 Summary

The method recommended by OECD Guideline 428 can be summarised as follows.

Transcutaneous diffusion should be measured *in vitro* using a diffusion cell comprised of a donor chamber and receptor chamber which can be clamped together either side of a skin membrane to achieve an effective seal. The receptor chamber should be filled with a receptor solution in which the penetrant is soluble and which is compatible with the integrity of the membrane (structural and/or biochemical) with a suitable facility for sampling the receptor solution and its structural integrity checked before the test. Candidate penetrant should be applied to the surface in a way consistent with the exposure scenario being investigated and the diffusion cell held at skin surface temperature with the receptor solution being constantly stirred for the duration of the measurement period. Samples of the receptor fluid should be taken at suitable time intervals during the period of the experiment and analysed for penetrant content using the most appropriate method. At the end of the experiment, the amount of the penetrant in each of the diffusion cell compartments should be measured to express a mass balance.

22.3 Conclusion

The scientific debate that resulted in the publication of OECD Guideline 428 and its associated guidance notes highlighted the need for some flexibility in the methods used to measure percutaneous absorption *in vitro*. Though the wide variety of exposure scenarios that *in vitro* percutaneous absorption measurement is used to

define, requires this flexibility; there are many aspects which can be defined as a good experimental practice. The guideline is a very good starting point for the definition of methods used in any new study and as guidance for those new to the field. It should not, however, be read in isolation from the guidance notes on the subject published, and referenced above, or the extensive scientific literature on the subject described elsewhere in this book. In short, when combined with *in vivo* animal studies and clinical investigations, *in vitro* diffusion measurements can provide a good description of percutaneous absorption which can be used to predict topical bioavailability in man, define bioequivalence of similar products and aid safety and efficacy testing.

References

1. Tregear RT. Molecular movement: the permeability of the skin. In: Tregear RT, editor. Physical functions of the skin. London:Academic; 1966. pp. 1–52.
2. Franz TJ. Percutaneous absorption on the relevance of in vitro data. J Invest Dermatol. 1975;64(3):190–5.
3. Franz TJ. The finite dose technique as a valid in vitro model for the study of percutaneous absorption in man. Curr Probl Dermatol. 1978;7:58–68
4. Howes D, Guy R, Hadgraft J, Heylings J, Hoeck U, Kemper F, Maibach H, Marty JP, Merk H, Parra J, Rekkas D, Rondelli I, Schaefer H, Tauber U, Verbiese N. Methods for assessing percutaneous absorption—the report and recommendations of ECVAM workshop 13. Atla-Alternatives Lab Anim. 1996;24(1):81–106.
5. Skelly JP, Shah VP, Maibach HI, Guy RH, Wester RC, Flynn G, Yacobi A. FDA and AAPS report of the workshop on principles and practices of in vitro percutaneous penetration studies—relevance to bioavailability and bioequivalence. Pharm Res. 1987;4(3):265–7. doi:10.1023/a:1016428716506.
6. Commission E: Guidance document on dermal absorption. (2004) http://ec.europa.eu/food/plant/protection/evaluation/guidance/wrkdoc20_rev_en.pdf. Accessed March 2014.
7. OECD: ENV/JM/MONO (2004) 2 Guidance document for the conduct of skin absorption studies. (2004) http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono (2004) 2&doclanguage=en. Accessed March 2014.
8. OECD: ENV/JM/MONO (2011) 36 guidance notes on dermal absorption. OECD. (2011) http://www.oecd.org/chemicalsafety/testing/48532204.pdf. Accessed March 2014.
9. OECD: Skin absorption: in vitro method. OECD. (2004) http://www.oecd-ilibrary.org/docserver/download/9742801e.pdf?expires=1399461731&id=id&accname=guest&checksum=278607C8396913D78801C3661F6E89D6.
10. Heylings JR, Clowes HM, Hughes L. Comparison of tissue sources for the skin integrity function test (SIFT). Toxicol In Vitro. 2001;15(4–5):597–600.
11. Scott RC, Dugard PH, Ramsey JD, Rhodes C. Invitro absorption of some ortho-phthalate diesters through human and rat skin. Environ Health Perspect. 1987;74:223–7.
12. Davies DJ, Ward RJ, Heylings JR. Multi-species assessment of electrical resistance as a skin integrity marker for in vitro percutaneous absorption studies. Toxicol In Vitro. 2004;18(3):351–8.
13. Nangia A, Patil S, Berner B, Boman A, Maibach H. In vitro measurement of transepidermal water loss: a rapid alternative to tritiated water permeation for assessing skill barrier functions. Int J Pharm. 1998;170(1):33–40. doi:10.1016/s0378-5173(98)00137-9.
14. Chilcott RP, Dalton CH, Emmanuel AJ, Allen CE, Bradley ST. Transepidermal water loss does not correlate with skin barrier function in vitro. J Invest Dermatol. 2002;118(5):871–5. doi:10.1046/j.1523-1747.2002.01760.x.

Chapter 23
Bioequivalence, Quality, and Novel Assessment Technologies for Topical Products: Current Challenges and Future Prospects

Vinod P. Shah, Avraham Yacobi and Majella E. Lane

23.1 Introduction

This chapter provides a critical review of current methodologies used to develop, evaluate, and maintain quality of semisolid products. The review relies on presentations and discussions at the workshop, "Evaluation of Topical Drug Products—Current Challenges in Bioequivalence, Quality, and Novel Assessment Technologies." This workshop was organized by the Product Quality Research Institute (PQRI) and was cosponsored by the American Association of Pharmaceutical Scientists (AAPS), the European Federation for Pharmaceutical Scientists (EUFEPS), the International Pharmaceutical Federation (FIP), and the United States Pharmacopoeia (USP), held on March 12–14, 2013, Rockville, Maryland, USA. The in depth discussions of various issues in evaluation of semisolid products was very useful in further development of methods for assessment of bioequivalence (BE) of topical dosage forms. Therefore, it was deemed useful to provide a review of the important discussions in this chapter.

For details, please see Expert Review article in *Pharmaceutical Research, 31(4): 837–846, 2014*.

Topical dermatological drug products are semisolid preparations such as creams, ointments, and gels. They deliver drugs to the skin to prevent or treat skin diseases. The onset, duration, and magnitude of therapeutic response for any topical drug product depend on the relative efficiency of three sequential processes: (1) release

V. P. Shah (✉)
11309 Dunleith Place, North Potomac, MD 20878, USA
e-mail: dr.vpshah@comcast.net

A. Yacobi
DOLE Pharma, LLC, 13 Oak Trail Rd., Englewood, NJ, 07631, USA
e-mail: avraham.yacobi@gmail.com

M. E. Lane
University College London School of Pharmacy, London, UK
e-mail: majella.lane@btinternet.com

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6_23

of the drug substance from the product; (2) penetration/diffusion of the drug through the stratum corneum (SC); and (3) activation of the desired pharmacological effect at the site of action. Determining BE of topical dermatologic products has been a challenge for the regulatory authorities as well as the pharmaceutical industry.

In the US, the generic product is required to be pharmaceutically equivalent (PE) and BE so that when approved it is considered therapeutically equivalent and interchangeable with the brand name product [1]. BE may be documented by performance of different types of studies [2] comparing the test and reference product, in declining order of preference: (1) pharmacokinetic (PK) measurements (2) pharmacodynamic (PD) measurements (3) comparative clinical trials and (4) *in vitro* tests.

As the target site of dermatological formulations is the skin, most topical products produce very low measurable amounts of drug in blood or plasma. Currently, comparative clinical endpoint trials are used to establish BE for most dermatological formulations except in the case of topical corticosteroids, which are tested for BE by PD measurements. The Food and Drug Administration (FDA) has recently issued a draft guidance for generic acyclovir ointment utilizing *in vitro* characterization to establish BE [3].

23.2 Characteristics of Topical Products

A topical dermatologic product such as a cream, ointment, gel, and lotion, is designed to deliver drug into the various layers of skin for treating dermal disorders with skin as the target organ. More recently, a number of patches have been developed which contain the active in a suitable adhesive matrix for topical vs. systemic delivery. For all topical products delivery of most of the active ingredient is locally to the skin, rather than through the skin. Depending on the disease, the target site for the topical drug product will vary. Fungal infections require treatment of the stratum corneum (SC); eczema and psoriasis require drug action in the viable epidermis; amelioration of muscle strains and sprains requires drug penetration to the deeper tissues.

23.3 Topical Generic Products and Reference Product

The generic product is expected to be Q1 and Q2 with respect to the reference listed drug (RLD), where Q1 means qualitative similarity and Q2 means quantitative similarity ($\pm 5\%$) in composition of the individual ingredients (as defined in 21 CFR 314.94 (a)(9)(v)). Q3 represents the microstructure of the formulation that is the arrangement of matter and state of aggregation of the topical drug product. For most topical formulations, their Q3 depends on the manufacturing processes and the conditions of storage. Differences in Q3 could manifest themselves as differences in physical properties such as rheology or *in vitro* release (dissolution) rate.

23.4 Assessment of BE

23.4.1 Clinical Endpoint Studies

For the majority of topical drug products comparative clinical endpoint studies are used to demonstrate BE to the RLD. Clinical endpoints are associated with high variability and low sensitivity that make such studies less reliable and inefficient. In general, a clinical response to a drug is known to be quite variable largely due to pathophysiological and environmental factors, which influence the pharmacological performance of a given agent. In these studies, formulation differences may not be detected efficiently and the number of patients enrolled can be quite large making the studies tedious, expensive, and sometimes impossible.

23.4.2 PK Studies

The use of PK studies to demonstrate BE for topical products is limited to some special cases where significant systemic absorption of the drug occurs [4]. PK studies are largely used to determine the safety of topical products.

23.4.3 PD (Vasoconstriction Assay)

When applied topically, corticosteroids produce skin blanching. This blanching response is caused by vasoconstriction, and has been correlated with clinical efficacy of the drug product. Blanching is used to measure bioequivalence of topical formulations in healthy volunteers, but not in patients. The original skin blanching methodology, commonly referred to as the Stoughton–McKenzie vasoconstriction assay, employed visual evaluation of the degree of blanching by trained observers [5]. To address the highly subjective nature of the assessment, instrumentation such as the chromometer has subsequently been recommended and used to evaluate the blanching response. Guidance developed by the FDA suggests the use of a chromometer to quantify the blanching response and to allow measurement of dose-response, onset, duration, and decline of response [6]. Demonstration of BE is based on statistical analysis showing that the 90% confidence interval on the test/reference ratio is within 0.80–1.25.

While this method works well with semisolid formulations, a number of practical problems have been reported with the vasoconstriction assay (VCA) using gels and sometimes collapsible foams with difficulty in determining the correct ED_{50}. One of the most commonly reported issues is that of high inter- and intra-subject variability which may be >50% in some studies. With some products, large numbers of subjects have to be enrolled (e.g., over 100) in the study to achieve the required number of evaluable subjects for BE assessment.

23.5 Alternative/Novel Methods to Assess BE

23.5.1 Dermatopharmacokinetics (DPK)

Since the target organ of topical products is the skin, it would seem logical that determining drug concentration in skin should provide an indication of topical BE between products. This is the underlying principle of the DPK technique. Using this approach, only the SC and not the deeper epidermal and dermal skin layer is evaluated for drug content. In a draft guidance, FDA had proposed a DPK test as an universal method for demonstrating BE of all topical drug products in June 1998 [7]. The draft guidance was withdrawn when evaluation of the DPK method with commercially available tretinoin gel products in two different laboratories showed contradictory results ([8–10]). The studies were conducted with different protocols, rendering any comparison improper. Efforts to refine the DPK approach continued post 2002 with FDA initiated studies [11, 12] to identify and evaluate sources of variability in the method and to optimize the protocol procedure(s). A number of other limitations associated with the DPK approach were addressed in these studies. The drug concentration at the site of action is not measured for drugs whose target is not the SC and thus bioavailability is not assessed. Even where the target site of the drug is the SC, the DPK approach will not distinguish between the amount of drug which is therapeutically available and that which may have crystallized out or been "stranded" in the skin. In addition, DPK evaluation is conducted on healthy skin which is a poor model of diseased skin. The application area must be standardized as this contributed to the variability observed in the earlier DPK studies. Attention must also be focused on the tape employed to remove the SC. A need for assuring "tape equivalence" from batch-to-batch, and manufacturer-to-manufacturer is evident but has not been addressed to date.

23.5.2 Microdialysis

Microdialysis consists of placing a probe, an ultrathin, semipermeable hollow fiber, in the dermis and perfusing it with a sterile buffer at a very low rate (between 0.1 and 5 μl/min) by means of a microdialysis pump [13]. The technique permits direct and continuous measurement of unbound drug. Recently, dermal microdialysis (DMD) has been used to assess drug permeation in both healthy and damaged skin and to determine the BE of certain topical formulations [14–16]. In DMD, the perfusate is most often an isotonic saline solution or Ringer's solution. Depending on the lipophilicity of the drug, the perfusate medium may have to be modified to allow more lipophilic substances to enter the probe. Calibration is necessary for quantitative DMD.

DMD is comparatively more invasive than DPK. On the other hand, for most drugs, DPK data may not correlate with the amount of drug at the site of action whereas DMD can provide detailed chronological PK data. More importantly, these

observations can be obtained in subjects with the disease itself, without relying on extrapolations from normal skin, as is the case for DPK. In addition, for DMD, several sampling sites can be studied simultaneously in the same volunteer as with DPK. The DMD method can be challenging when used for sampling very lipophilic or highly protein-bound drugs because of low recovery of these molecules. One option for the measurement of lipophilic molecules is the use of a lipid emulsion as the perfusate instead of conventional aqueous buffers. Training of laboratory personnel is necessary to ensure low variability. The FDA has actively sought information, presentation, and cooperation with DMD researchers and submission of DMD studies.

23.5.3 Open Flow Microperfusion

The probe design for open flow microperfusion (OFM) in dermal studies has an open exchange area and can sample interstitial fluid directly [17]. For this reason, OFM does not have the same limitations as DMD, specifically, with reference to molecular size, drug protein-binding, and drug lipophilicity. The OFM probes require active push-pull pumps in order to avoid the loss of perfusate to the tissue and the risk of edema formation. While OFM may offer advantages in relation to the range of sampled substances possible, OFM samples are generally more complex than DMD samples and require pretreatment before analysis.

23.5.4 Confocal Raman Spectroscopy

Confocal Raman Spectroscopy (CRS) combines the principles of confocal microscopy and Raman spectroscopy. The device comprises a high-performance dispersive spectrometer with laser excitation and a confocal measurement stage. This technique is capable of providing detailed information concerning the molecular composition of the SC and specifically the water gradient across the SC [18]. CRS has been used to profile endogenous skin components and to probe the influence of formulations on the SC. It was used to examine the disposition of ibuprofen after application from simple formulations which were previously investigated using the DPK approach [19]. The results confirmed that ibuprofen distribution profiles in the SC were comparable to previously published data from tape stripping experiments. CRS is a noninvasive alternative to DPK for evaluation of topical pharmaceutical formulations.

23.5.5 In Vitro *Skin Permeation Studies*

In vitro skin permeation studies are routinely carried out in topical formulation development and to evaluate how a generic product compares with the RLD. These skin permeation studies have been evaluated and compared with the relevant clinical

data from BE trials for a number of drugs [20, 21]. However, the variabilities in the quality and performance of the skin may compound the issues governing any *in vitro* testing and render the test unreliable. Although there was an interest in the use of cadaver skin for evaluation of topical products, this approach has never been considered a viable avenue to determine BE of products.

23.5.6 In Vitro *Release Testing*

An *in vitro* release test (IVRT) for semisolid preparations using *in vitro* diffusion cell (VDC) has been recognized in the FDA's SUPAC-SS guidance as a test for product sameness after certain manufacturing related changes [22]. Recently, this IVRT has also been recognized as a reasonable and useful test to be considered as a product release and stability test [23]. When the active and inactive ingredients in a topical dosage form between the test and reference product are the same (Q1 and Q2), the only possible difference between the two is the method of manufacture and the micro structure of the formulation (Q3). Q3 can be assessed using IVRT. The draft guidance for BE of acyclovir ointment 0.5 % indicates that if the generic product is Q1, Q2, and Q3 to RLD, a biowaiver can be granted. The issue going forward is whether this principle can be extended to other semisolid drug products when Q_1, Q_2, and Q_3 are the same. Just as lower strengths of oral dosage forms are eligible for biowaiver when they are dose-proportional to higher strengths and meet dissolution profile similarity (f_2) criteria, can the same principles be applied for lower strengths of topical dosage forms using IVRT? These are important points which need to be explored.

23.6 Assessment of Quality and Performance of Topical Drug Products

Product development should use the principles of quality-by-design (QbD) and should try to build quality into the product. Testing of the quality and performance of semisolid products provides assurance of batch-to-batch quality, reproducibility, reliability, and performance throughout the shelf-life. To determine physical integrity, the following assessments are recommended: determination of visual separation and/or chemical separation; colour change; pH; presence of crystals; general appearance (lumps, air, smell, etc.); viscosity and spreadability for semisolid products. For chemical stability the following should be conducted: assays of the active content or potency; tube/container uniformity, assay of the preservative content or potency; determination of the presence of degradation products; evaluation of any impurities of the active, inactive ingredients, or product. Product performance evaluation for semisolid dosage forms may be conducted using an IVRT.

23.7 Proposed Decision Tree for Assessment of Bioequivalence

It is evident that a "one size fits all" approach is not suitable for assessment of BE of all topical dosage forms. Various methods need to be explored for BE determination. The method will need a rational linkage to the clinical endpoint and/or site of action. The process of decision making should start with the Q1, Q2, Q3 evaluation of the generic and RLD products. For further details of the suggested approach, please see the workshop report in pharmaceutical research [24].

Under this rubric (1) if the generic product is Q1 and Q2 with the RLD, *in vitro* characterization and performance testing showing Q3 equivalence may be conducted and *in vivo* testing may be waived depending on the *in vitro* results; (2) if the generic is Q1 equivalent but not Q2 then *in vivo* tests are required; (3) if the product is not Q1 and Q2, *in vitro* and *in vivo* tests will be required to demonstrate no formulation effect on absorption.

The VCA method is available where the product is a corticosteroid. Pharmacokinetic measurements can be an option if the product results in significant plasma or tissue levels. Where the target site of action of the drug is the SC, clinical endpoint evaluation, or methodologies based on DPK and confocal Raman spectroscopy may be appropriate. For products intended to act in the dermis or epidermis, clinical endpoint evaluation, or microdialysis, or confocal Raman spectroscopy methods can be appropriate. Determination of topical BE will require a multifaceted approach, tailored to the drug, disease, product interface. This is evident from the following examples.

23.8 BE of Topical Dermatology Drug Products: Case-By-Case Solutions

Due to the complexities of demonstrating BE for topical dermatologic drug products, the FDA has allowed different approaches on a case-by-case basis as evident from the following examples:

- Application of PK approach: Lidocaine topical patch 5%: Lidocaine concentration in plasma can be easily measured. Lidocaine plasma concentration is proportional to its presence in dermal tissue, at the site of action.
- Application of pharmacodynamics approach: Fluocinolone acetonide 0.01% topical oil: The vasoconstrictor assay can accurately detect the rate and extent of availability in skin. If a generic product is Q1 and Q2, then a biowaiver can be granted.
- Application of clinical approach: 5-flourouracil (5-FU) cream 5%: A clinical endpoint BE study in AK lesions patients is recommended.
- Application of *in vitro* approach: Acyclovir ointment 5%: If the generic product is Q1 and Q2 with the brand name product, approval can be granted based on

Q3, that is similar *in vitro* release rate, and similarity in particle size, viscosity, morphic form, PEG molecular weight distribution.

- Application of PK endpoint study and a clinical endpoint study: Diclofenac sodium gel 1 %: Two BE studies are required. Topically applied diclofenac is well absorbed, and it acts locally also. A PK study and a BE study with clinical endpoint is recommended.

23.9 Looking Toward the Future

It is clear that a "one size fits all" approach will not work for BE determination of all types of topical dosage forms, it will require a multifaceted approach, tailored to the drug, disease, and dosage form. It is possible that more than one approach may be needed for BE determination. In the future, more regulatory applications of *in vitro* drug release tests, a standardized protocol for DPK, use of DMD and Confocal Raman microscopy may be expected.

23.10 Conclusions

At the outset, the main goals of the workshop were to: (1) develop a science based regulatory approach for development and evaluation of topical dermatologic products—product quality, performance, and BE determination (2) identify and suggest the methodologies that may be used by the regulatory agencies to assess BE as part of registration dossiers, and (3) determine the value of *in vitro* drug release in semisolid dosage form development and in assessment of product quality and BE. The scientific presentations and discussions at this workshop clearly underlined the need to reevaluate the present methods and approaches to determine BE of topical dermatologic products. The need for new creative approaches to optimize the existing available methodologies and the possibility of exploring alternative methodologies which may facilitate the development, registration, and ultimately approval of semisolid products was also evident. The tools discussed, and others that may be developed in the future, should not be viewed in isolation, but as part of a larger "complimentary toolkit of methods."

References

1. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Guidance for industry. Bioavailability and bioequivalence studies for orally administered drug products-general considerations. 2003.
2. Code of Federal Regulations. Title 21 Part 320.24 (b) (1–4).
3. www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm296733.pdf. Accessed 14 Nov 2014.

4. www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm086293.pdf. Accessed 14 Nov 2014.
5. McKenzie AW, Stoughton RB. Method of comparing percutaneous absorption of steroids. Arch Dermatol. 1962;86:608–10.
6. Office of Generic Drugs, Center for Drug Evaluations and Research, FDA. Guidance: topical dermatologic corticosteroids: in vivo bioequivalence. 1995.
7. US FDA. Guidance for Industry: topical dermatological drug product NDAs and ANDAs-in vivo bioavailability, bioequivalence, in vitro release, and associated studies. Draft Guidance, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research; June 1998.
8. US FDA. Guidance for industry on special protocol assessment; availability. Fed Regist. 2002;67:35122.
9. Pershing LK. Bioequivalence assessment of three 0.025 % tretinoin gel products: dermatopharmacokinetic vs. clinical trial methods, Transcribed presentation to the Advisory Committee for Pharmaceutical Sciences Meeting, Center for Drug Evaluation and Research, FDA; 2001 Nov 29; Rockville.
10. Franz TJ. Study #1, Avita Gel 0.025 % vs Retin-A Gel 0.025 %, Transcribed presentation, Advisory Committee for Pharmaceutical Sciences Meeting, Center for Drug Evaluation and Research, FDA; 2001 Nov 29; Rockville.
11. Bunge AL, N'Dri-Stempfer B, Navidi WC, Guy RH. Dermatopharmacokinetics: improvement of methodology for assessing bioequivalence of topical dermatological drug products, Revised Final Report, Award No. D3921303, Submitted to Department of Health and Human Services, FDA, Colorado School of Mines; 2006 Sept 2; Golden.
12. Bunge AL, N'Dri-Stempfer B, Navidi WC, Guy RH. Therapeutic Equivalence of Topical Products, Final Report, Award No. 223-04-3004, Submitted to Department of Health and Human Services, FDA, Colorado School of Mines; 2007 Jan 30; Golden (Revision submitted June 2008).
13. Petersen LJ, Kristensen JK, Bülow J. Microdialysis of the interstitial water space in human skin in vivo: quantitative measurement of cutaneous glucose concentrations. J Invest Dermatol. 1992;99:357–60.
14. Ortiz PG, Hansen SH, Shah VP, Menné T, Benfeldt E. The effect of irritant dermatitis on cutaneous bioavailability of a metronidazole formulation, investigated by microdialysis and dermatopharmacokinetic method. Contact Dermat. 2008;59:23–30.
15. Ortiz PG, Hansen SH, Shah VP, Menné T, Benfeldt E. Impact of adult atopic dermatitis on topical drug penetration: assessment by cutaneous microdialysis and tape stripping. Acta Derm Venereol. 2009;89:33–8.
16. Benfeldt E, Hansen SH, Vølund A, Menné T, Shah VP. Bioequivalence of topical formulations in humans: evaluation by dermal microdialysis sampling and the dermatopharmacokinetic method. J Invest Dermatol. 2007;127:170–8.
17. Bodenlenz M, Hoefferer C, Priedl J, Dragatin C, Korsatko S, Liebenberger L, Zahiragic S, Raml R, Magnes C, Pieber T, Sinner F. A novel certified dermal sampling system for efficient clinical research. J Invest Dermatol. 2011;131 (Suppl 2):S44–4.
18. Caspers PJ, Lucassen GW, Bruining HA, Puppels GJ. Automated depth-scanning confocal Raman microspectrometer for rapid in vivo determination of water concentration profiles in human skin. J Raman Spectrosc. 2000;31:813–8.
19. Mateus R, Abdalghafor H, Oliveira G, Hadgraft J, Lane ME. A new paradigm in dermatopharmacokinetics—confocal Raman spectroscopy. Int J Pharm. 2013;444:106–8.
20. OECD (2004) Test No. 428: skin absorption: in vitro method. OECD, p. 8.
21. Franz TJ, Lehman PA, Raney SG. Use of excised human skin to assess the bioequivalence of topical products. Skin Pharmacol Physiol. 2009;22:276–86.
22. FDA. SUPAC-SS nonsterile semisolid dosage forms, scale-up and postapproval changes: chemistry, manufacturing, and controls; in vitro release testing and in vivo bioequivalence documentation.U.S. Department of Health and Human Services, Washington, DC. 1997. http://www.fda.gov/cder/guidance/1447fnl.pdf. Accessed 09 April 2007.

23. Chang R-K, Raw A, Lionberger, Yu L. Generic development of topical dermatologic products: Formulation development, process development, and testing of topical dermatologic products. AAPS J. 2013;15:41–52.
24. Yacobi A, Shah VP, Bashaw ED, Benfeldt E, Davit B, Ganes D, Ghosh T, Kanfer I, Kasting GB, Katz L, Lionberger R, Lu GW, Maibach HI, Pershing LK, Rackley RJ, Raw A, Shukla CG, Thakker K, Wagner N, Zovko E, Lane ME. Current challenges in bioequivalence, quality, and novel assessment technologies for topical products. Pharm Res. 2014;31(4):837–46.

Index

V. P. Shah et al. (eds.), *Topical Drug Bioavailability, Bioequivalence, and Penetration,* DOI 10.1007/978-1-4939-1289-6