Effect of Continuous Phase Drug Concentration, Evaporation and Partitioning on Transdermal Drug Permeation Kinetics with Lipophilic Vehicles

Inauguraldissertation

zur
Erlangung der Würde eines Doktors der Philosophie
vorgelegt der
Philosophisch-Naturwisschenschaftlichen Fakultät
der Universtität Basel

von

Miriam Regina Luise Reiser
aus Stein bei Nürnberg
DEUTSCHLAND

Basel, 2008

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät

auf Antrag von

Herrn Prof. Dr. phil. nat. Georgios Imanidis (Fakultätsverantwortlicher)

Herrn Prof. Dr. phil. nat. Christian Surber (Korreferent)

Basel, den 14. Oktober 2008

Prof. Dr. Eberhard Parlow

Dekan

In Liebe und Dankbarkeit

Mama und Papa

Uli

Stefan

Heike

Frank

Acknowledgements / Danksagung

Herrn Prof. Dr. Dr. h.c. Hans Leuenberger danke ich für die Möglichkeit, meine Dissertation am Institut für Pharmazeutische Technologie der Universität Basel durchführen zu können.

Frau Prof. Dr. Gerda Huber danke ich für die spontane Übernahme und Unterstützung des Projekts nach dem Wechsel zur School of Life Sciences, FHNW.

Bei Herrn Prof. Dr. Christian Surber möchte ich mich für das Interesse an meiner Arbeit und die Übernahme des Korreferats recht herzlich bedanken.

Ein ganz besonderer Dank gilt meinem Doktorvater Prof. Dr. Georgios Imanidis für die ausserordentliche tolle wissenschaftliche und kollegiale Betreuung. Für die Gestaltung meiner Arbeit nach meinen Vorstellungen und Ideen waren mir stets alle erdenklichen Möglichkeiten und Freiheiten gegeben. Ich danke für die zahlreichen spannenden Diskussionen, die wir in den letzten Jahren geführt haben, da sie für mich immer eine grosse und unschätzbare Hilfe waren. Unvergessen wird für mich sicherlich auch die Zugfahrt von Montpellier nach Basel bleiben, bei der die Köpfe qualmten, die aber meine Arbeit ein grosses Stück vorwärts gebracht hat.

Ursula Stäger und Melanie Albert gebührt ein ganz besonderer Dank für das unglaubliche Engagement im Rahmen ihrer Masterarbeit und das mir entgegen gebrachte Vertrauen als Betreuerin.

Stefan Winzap und Sonja Reutlinger danke ich für jegliche technische und praktische Unterstützung, welche immer einen reibungslosen Ablauf des Laboralltags gewährleistete sowie meinem Kollegen Marcel für den wissenschaftlichen Austausch.

Marcel Düggelin und Daniel Mathys vom Zentrum für Mikroskopie der Universität Basel danke ich für die wunderschönen und faszinierenden rasterelektronenmikroskopischen Aufnahmen meiner Formulierungen.

Heiko danke ich besonders für die grosse Hilfe und die Anregungen gerade zu Beginn meiner Arbeit.

Ich möchte mich hiermit auch bei all meinen Kollegen am Pharmazentrum der Uni Basel sowie der School of Life Sciences, FHNW, für die freundliche Atmosphäre und die fröhliche Gesellschaft, vor allem während der Cafépause und dem Mittagessen, bedanken. Besonders

bedanken möchte ich mich an dieser Stelle bei David, Dana, Ervina und Murad für die tatkräftige Unterstützung bei der Durchführung des Praktikums der halbfesten und dispersen Arzneiformen. Katja, Carolina, Elaine und Ivana danke ich für die tolle gemeinsame Zeit auch ausserhalb des Labors, ebenso wie meinem lieben bayerischen Kollegen Christoph.

Danken möchte ich hier auch meiner langjährige pharmazeutischen Weggefährtin Karin für die tolle Freundschaft, sowie allen pharmazeutischen und sportlichen Höhen und Tiefen, die sie mit mir nunmehr schon seit 1998 durchlebt.

Die ganze hier vorliegende Arbeit konnte nur entstehen, weil ich immer wunderbare Menschen um mich hatte, die mich motivierten, unterstützen und mir Rückhalt gaben.

Besonderer Dank gebührt meinem besten Freund Stefan, für die tagtägliche (mehrfache) Korrespondenz, die stets objektiven, ehrlichen Ratschläge und die lustige Zeit zusammen.

Von Herzen danke ich meiner besten Freundin Heike mit Jens und Familie, deren Anregung, Motivation und Beistand in allen Lebenslagen massgeblich zum Gelingen der hier vorliegenden Arbeit beigetragen hat.

Ein ganz grosses und herzliches Dankeschön geht an meinen lieben Frank, der immer für mich da war, mir zuhörte und für all meine Problemchen sofort eine Lösung wusste. Nagyon örülök, hogy vagy!

Zu aller letzt bedanke ich mich bei meinen Eltern und meinem Bruder Uli für die Geduld und die Zusprache. Danke, dass ihr mir das alles ermöglicht habt und mich auf meinem langen Weg zum Ziel immer unterstützt habt!

List of Abbreviations

β	Buffer capacity
BTA-Cl	Benzyltrimethylammonium Chloride
C_L	Drug concentration in the continuous lipid phase
C_W	Drug concentration in the dispersed water phase
C_{tot}	Overall drug concentration of the vehicle
D	Diffusion Coefficient
DLVO	Theory named after Derjagin, Landau, Verwey and Overbeek to describe forces between charged surfaces in liquid medium
DSC	Differential scanning calorimetry
E30	w/o-emulsion comprising 30% of water phase dispersed in oil
E50	w/o-emulsion comprising 50% of water phase dispersed in oil
E70	w/o-emulsion comprising 70% of water phase dispersed in oil
Ibu	Ibuprofen
IPM	Isopropyl myristate
Iso	Emulsifier Isolan PDI
$K_{L/W}$	Drug partition coefficient between oil and water phase
$K_{W/L}$	Drug partition coefficient between water and oil phase
$K_{SC/L}$	Drug partition coefficient between stratum corneum and oil phase
Migl	Miglyol 812N
P	Permeability coefficient
P_{app}	Apparent permeability coefficient
P_{dbl}	Permeability coefficient of the diffusion boundary layer / vehicle
P_m	Permeability coefficient of the membrane / skin
Para	Paraffinum Liquidum
Ph.Eur.	European Pharmacopoeia
Φ_L	Phase fraction of continuous oil phase
Φ_W	Phase fraction of dispersed water phase
SC	Stratum Corneum
SD	Standard Deviation
SEM	Standard error of mean
SEM	Scanning electron microscopy
TEWL	Transepidermal water loss
V_L	Volume of continuous oil phase
V_W	Volume of dispersed water phase
V_{tot}	Total volume

Abstract

1 Abstract

In this work the dependence of transdermal drug permeation kinetics on continuous phase drug concentration, partitioning between formulation phases, partitioning between stratum corneum and continuous oil phase and evaporation of volatile formulation components for a hydrophilic (caffeine) and a lipophilic (ibuprofen) model drug incorporated into w/o-emulsions of varying composition was investigated.

The studied w/o-emulsions consisted of an oil phase into which water phase was dispersed in mass fractions of 70%, 50% and 30% (E70, E50 and E30, respectively). The oil phase consisted of a single oil component (isopropyl myristate, miglyol 812N or paraffinum liquidum) and the polymeric emulsifier Isolan PDI. Water phase contained sodium chloride and was buffered to pH 4.5 in all emulsions containing ibuprofen. Pure oil with and without emulsifier were selected as reference formulations. Transport experiments were carried out in Franz-type diffusion cells across pig ear skin at 32°C under occlusive and non-occlusive conditions with an infinite dosing of 0.3 g/cm^2 and 0.7 g/cm^2. Continuous phase drug concentration was determined experimentally by ultracentrifugation and theoretically by calculation taking into account drug partitioning between distinct phases.

A concept for the interpretation of drug permeation was proposed that considered continuous phase drug concentration as the driving force for transdermal permeation. Drug distribution within the formulation and partitioning between stratum corneum and continuous oil phase were determined in order to gain a full understanding of the examined absorption processes.

Dependence of apparent permeability coefficient P_{app} on fraction of drug concentration in the continuous phase was analyzed with a model taking into account the permeability coefficient of the skin P_m and the permeability coefficient of the diffusion boundary layer P_{dbl}. P_{dbl} reflects the diffusivity of the drug in the vehicle. By fitting this model to the experimental data using non-linear regression, parameter values for P_m and P_{dbl} were deduced. P_m values were consistent with the drug partitioning between stratum corneum and continuous oil phase. For isopropyl myristate a permeation enhancement was found in agreement with literature. P_{dbl} values were compared with calculated values using a literature model for diffusion in heterogeneous matrix systems. These were found in most cases to be in fairly good agreement with the P_{dbl} values.

Free emulsifier present in the continuous oil phase affected neither saturation concentration nor continuous phase drug concentration nor transdermal absorption of the model drugs. Thickener Aerosil 200 tremendously decreased transdermal permeation of caffeine, but did not show any interaction with ibuprofen. A reduction of applied dose (0.3 g/cm^2 instead of 0.7 g/cm^2) did not significantly affect apparent permeability coefficient P_{app}. Evaporation pattern of all examined formulations revealed that relative water loss was independent of the dispersed mass fractions and the employed experimental setup, but increased as the applied formulation dose was reduced.

For implementing continuous phase drug concentration concept to non-occlusive conditions, a formula was derived that considered observed water loss and permeated drug amount in order to calculate the resulting drug concentration in the continuous formulation phase over time. An increase of the drug concentration in the continuous oil phase was estimated which, however, did not lead to a measurable increase of the apparent permeability coefficient.

To conclude, the proposed concept considering continuous phase drug concentration can be used to explain experimentally measured apparent permeability coefficient P_{app} for lipophilic vehicles. Applying this concept to w/o-emulsions comprising varying mass fractions provides a predictive tool in order to delineate the effect of physicochemical formulations parameters and transdermal drug delivery rate, if occlusive conditions are assumed. In case of non-occlusive transport conditions, however, evaporation will lead to compositional changes and consequently changes in continuous phase drug concentration. How these alterations will affect apparent permeability coefficient using a finite dose requires further investigations.

Introduction and Objectives

2 Introduction and Objectives

2.1 Introduction

In clinical practice, a drug is rarely applied to the skin in form of a pure chemical, but instead, is incorporated into a carrier system, the vehicle, to guarantee efficient topical or systemic therapy. Suitable applicability, compatibility, adequate stability and above all, efficacy with respect to duration and strength of the desired pharmacological action are requirements directly related to the employed vehicle. Thus, the development and optimization of these dermatological vehicles is a challenging task [1].

Common vehicles usually comprise several components that are often not mutually miscible, thus, separate phases are formed that, on the microscopic lever, are intermixed with each other. From the macroscopic point of view a homogeneous system is apparent that disguises different microstructures arising inside the formulation [2, 3].

It is widely acknowledged that transdermal permeation is regulated by the formulation of the drug product. This regulation may take place not only based on physicochemical principles such as diffusion and partitioning of the active ingredient, but also by an interaction with the absorptive epithelium, i.e. the epidermis, affecting the permeability of the drug. Hence, a solid knowledge of the composition, including its physico-chemical properties and present microstructures, is crucial in terms of achieving optimal topical delivery [4].

However, if a finite dose of a dermatological formulation is applied onto the skin, the physico-chemical and thermodynamic conditions of the freshly applied emulsion change radically. Initial structural matrix and quantitative composition will most likely change during and after mechanical agitation associated with the application of the product (e.g. rubbing) and/or evaporation of ingredients. Possible evaporation of the volatile components of some vehicles, for instance o/w-emulsions, can result in an appreciable increase in solute drug concentration, first leading to saturation conditions and then to supersaturation or drug precipitation. Yet, research is still in the beginning of elucidating the complicating effects of vehicle metamorphosis on the entire clinical picture. On the contrary, if an infinite dose is applied, these effects should be less pronounced [1, 3, 5].

To date, there is no uniform and comprehensive recommendation or guideline available that takes into consideration the multifaceted complexity present in dermatological formulations in

order to quantitatively understand the mechanisms being decisive for transdermal absorption processes [1, 6].

In all likelihood, drug concentration in the vehicle is the most crucial physicochemical parameter governing permeation kinetics [7]. Increasing concentration of a drug within a vehicle, for instance, due to evaporation processes can strongly affect overall drug permeation [2, 3, 8].

Little is known about the relationship between diffusivity in a formulation and skin permeation in practical studies. Drug permeation through the skin out of vehicles has to be divided into diffusivity and partitioning in the formulation and diffusivity and partitioning in the skin and it has to be clarified which parameter has larger effect on skin permeation. Yamaguchi et al., for example, studied the in vitro skin permeation of 22-oxacalcitriol from ointments having differing compositions and considered the diffusion coefficients of the drug inside the vehicles. Drug diffusion coefficients within the ointment differed significantly depending on the amount of medium chain triglycerides present [9].

Permeation parameters such as drug diffusivity in a formulation and drug partitioning from the formulation to the skin are easily altered by the composition of the formulation [10]. For example, the microstructure of modified water containing hydrophilic ointment DAB 1997 with suspended hydrocortisone-21-acetate on in vitro drug release showed distinct vehicle effects. Both, the colloidal structure of the cream and the presence of specific vehicle compounds, i.e. penetration enhancers, are likely to influence the drug permeation through the skin more than the total drug concentration in the cream dose [11].

Previous work of our group proposed the continuous phase drug concentration concept to delineate regulation of drug skin permeation considering drug distribution among distinct phases of multi-phase hydrophilic formulations. Validity of the concept was confirmed for occlusive and non-occlusive conditions even though vehicles underwent considerable changes in their composition and microstructure due to evaporation of volatile ingredients [2, 3].

The idea of this work was to implement the proposed concept to lipophilic vehicle systems applied onto skin under occlusive and non-occlusive conditions. Concerning these systems, apparent permeability coefficient is dependent on drug diffusion in the vehicle and drug permeability through the skin. To date, drug diffusivity inside the vehicle and its overall contribution to total transdermal permeation has received little attention. Diffusion inside the

vehicles is mainly dependent on the properties of the drug and the excipients, but also on phase fractions intermixed with each other and on arising microstructures. Additionally, drug concentration in the external oil phase can be influenced by the presence of free emulsifier, interactions with additives like thickeners or changes vehicles undergo in the course of their application like evaporation of volatile components. The extent of water loss and consequently the degree of occurring compositional changes is furthermore dependent on the applied dose.

2.2 Objectives

The goal of this PhD thesis was to implement continuous phase drug concentration concept to lipophilic vehicle systems under occlusive and non-occlusive conditions, i.e. w/o-emulsions of varying phase fractions and oil phases, in order to confirm its validity and, importantly, use it as a tool to delineate the contribution of diffusivity within the vehicle and permeability through the skin to overall apparent permeability. The combined efficiency of different parameters influencing the transdermal absorption process was to be clarified in order to establish a methodology to quantitatively understand the dependence of transdermal drug permeation on composition, dispersed phase fractions and microstructure of w/o-emulsions.

Drug permeation was studied in vitro across full-thickness pig ear skin in order to control the experimental environment and so to elucidate individual factors that may modify permeation. Representative w/o-formulations consisted of emulsifier, oil, buffer or water, sodium chloride and were designed in order to receive diversity of their composition and variety of their phase ratio of dispersed to continuous phase. Drug distribution within the vehicles was determined by ultracentrifugation and calculated taking into account the drug partitioning between hydrophilic and lipophilic phase. Continuous phase drug concentration was postulated to describe quantitatively drug permeation rate. The use of a hydrophilic model drug that is mainly present in the dispersed phase and a lipophilic model drug which is mainly present in the continuous oil phase were the basis to test this concept and to detect possible interactions of formulation ingredients with the skin. To delineate the effect of formulation ingredients on skin barrier function more detailed, drug distribution experiments between isolated stratum corneum and continuous oil phase were performed. With respect to clinical practice, permeation was further studied non-occlusively considering changes formulations undergo due to evaporation of volatile components. Emphasis was laid on the investigation of changes in the phase ratio between dispersed to continuous phases and accordingly changes in continuous phase drug concentration in order to expand the proposed concept to the situation of non-occlusive application.

Drug skin permeation was further investigated in dependence of formulation parameters like emulsifier content, thickener content and dose.

Caffeine (hydrophilic) and ibuprofen (lipophilic) were applied as model drugs with different solubility properties The studied w/o-emulsions consisted of an oil phase into which water phase was dispersed in mass fractions of 70%, 50% and 30% (E70, E50 and E30, respectively). Oil phase consisted of a single oil component (isopropyl myristate, miglyol 812N or paraffinum liquidum) and polymeric emulsifier Isolan PDI. Water phase contained sodium chloride and was buffered to pH 4.5 in all emulsions containing ibuprofen. Pure oil with and without emulsifier was selected as reference formulation. The formulations were designed specifically for elucidating the effect of different continuous phase drug concentrations, microstructures and oil bases on transdermal delivery.

THEORETICAL SECTION

3 Theoretical Section

3.1 THE SKIN

3.1.1 Structure and Function of the Skin

Human skin (cutis, integumentum commune) is one of the most extensive and readily accessible organs of the human body covering a surface area of approximately $2m^2$ with a thickness of 1.5 to 4 mm and a weight of 3 kg. Generally, it can be distinguished between an outer stratified, avascular epithelial (epidermis) and an inner vascularized connective tissue (dermis). Epidermis comprises of viable cellular epidermis and the outermost layer, called stratum corneum. The vascularized layer is formed by the dermis and the subcutis with the function to physiologically support the epidermis [12-15].

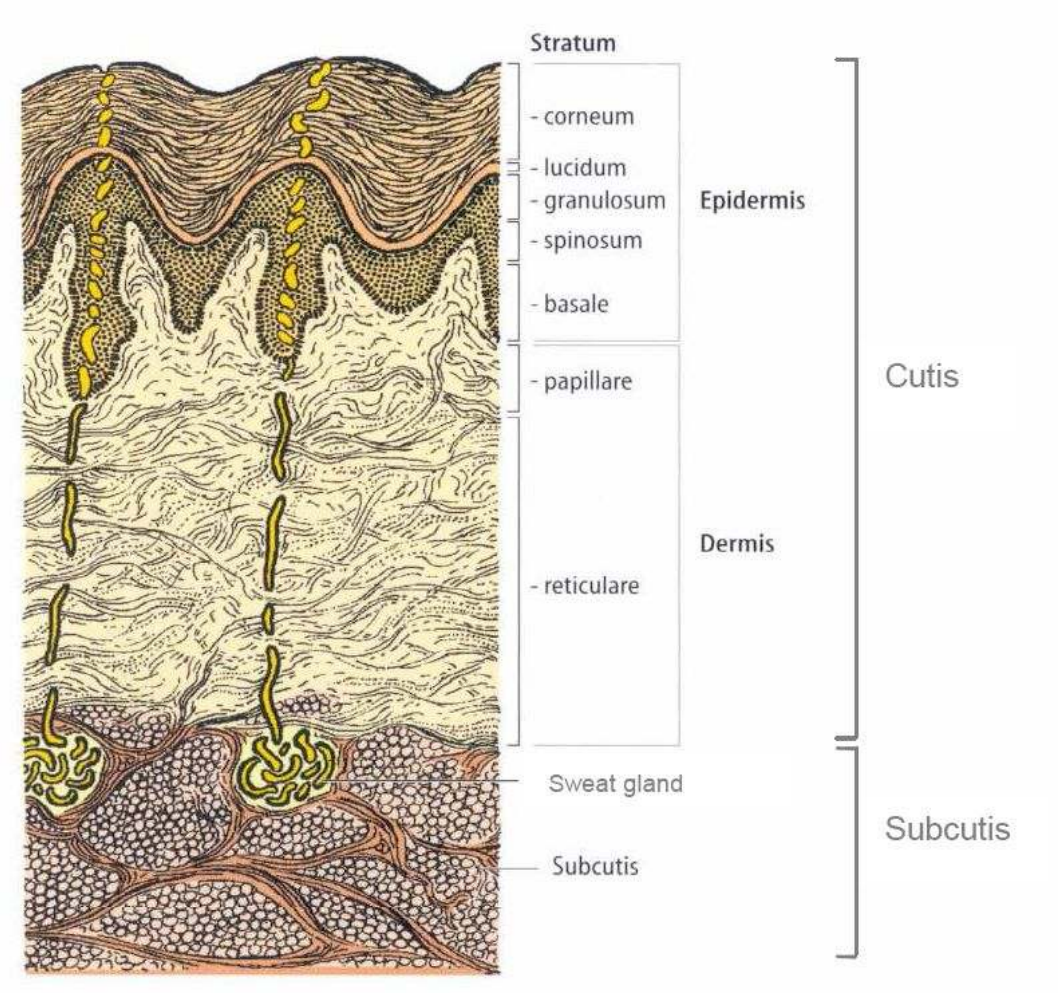


Figure 1. Cross-section of the skin [16]

Due to its complex structure and composition the skin is able to ensure various vital functions. The predominant task is to prevent the loss of water, electrolytes and body constituents while barring the ingress of harmful or unwanted molecules form the external environment. This protection is primarily provided by the stratum corneum. Additionally, the skin provides mechanical protection due to a strong fibre network and a pillow function of fatty tissues in the dermis. Melanin pigments in epithelial cells are protecting against damaging UV radiation and skin is strongly involved in the thermoregulation of the body. Besides, the skin offers a good protection against infections because parts of the immune system are located within the epidermis and dermis. Sensory functions are mainly due to the location of receptors for heat, pain, pressure and touch within the epidermis, dermis and subcutis. The skin is active in the synthesis, processing and metabolism of proteins, lipids, glycans and signalling molecules, it performs endocrine functions like vitamin D synthesis and peripheral conversion of prohormones. On the surface of the epidermis lies a thin film made up of skin lipids that are secreted from the sebaceous glands. These lipids are mixed with ingredients of sweat and act as water-repellents [17-21].

Two kinds of cells form the epidermis: First, the keratinocytes (90%) which are responsible for keratin production and are kept together by desmosomes and second, the dendritic cells (10%): melanocytes (pigment cells), Langerhans cells (immuno competent cells) and Merkel cells (responsible for perception) [16].

3.1.2 Epidermal Differentiation and Viable Epidermis

The generation of the extracellular lipid compartment and the transformation of the keratinocytes into corneocytes are the main features of epidermal differentiation. However, equally important is the continuous renewal of the stratum corneum, which is insured by a careful balance between the replenishment of new keratinocytes form the proliferating basal layer and the well-orchestrated loss of the most superficial cells after the so-called “epidermal programmed cell death” [22]. Thus, the epidermis owns a multi-lamellar structure that represents the different stages of cell differentiation. Generally, viable epidermis can be sectioned into three distinct layers: stratum basale, stratum spinosum (together forming the stratum germinativum) and stratum granulosum [13]. Viable epidermis undergoes continuous differentiation in order to form the outermost layer of the skin, the straum corneum [15].

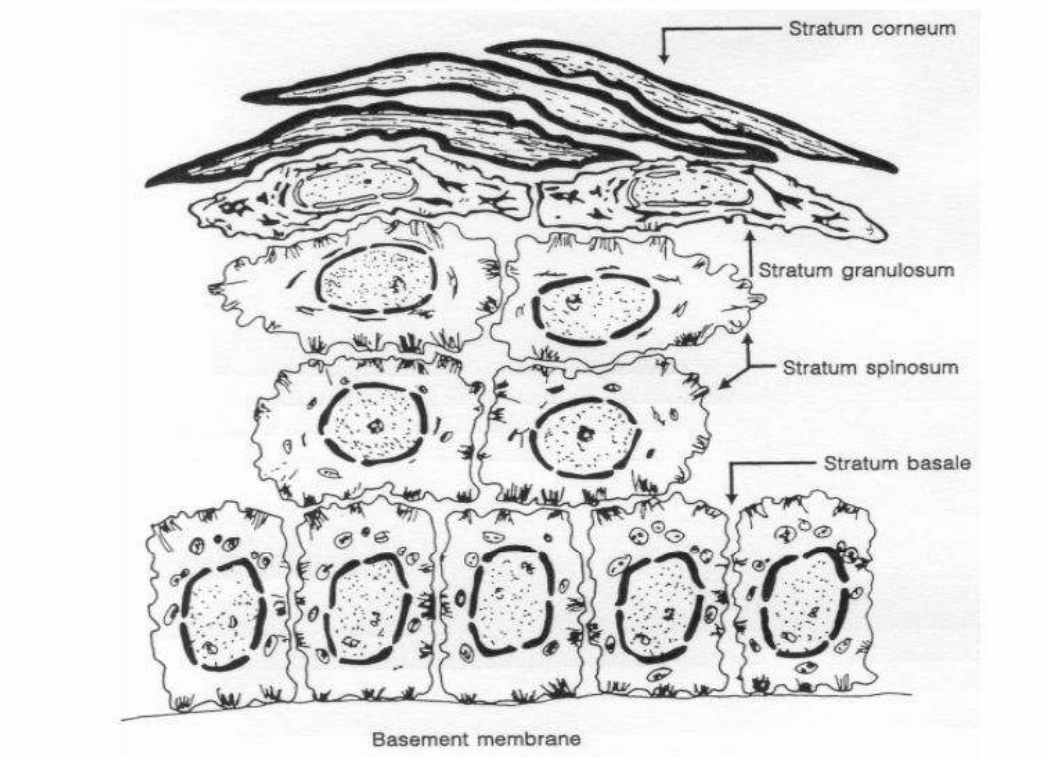


Figure 2. Viable epidermis [14]

The stratum basale forms a single layer of columnar amplifying cells derived from epidermal stem cells and is anchored to a basement membrane that separates epidermal tissue from underlying dermis. The constant renewal and proliferation balance the loss of dead horny cells from the skin surface and it is assumed that total turnover from basal layer to shedding is about an average of 28 days. Basal cell layer also includes melanocytes which produce and distribute melanin granules to keratinocytes in a complex interaction. Cells produced in the basal layer alter morphologically and histochemically in the course of cornification [13, 17, 23].

Cylindric cells of stratum basale undergo a horizontal reorientation in the stratum spinosum, followed by a continuing flattening and shrinking of their nuclei as they move outwards. Stratum spinosum has a spiny appearance and reveals lipid-enriched lamellar bodies (Odland bodies) and an increase in keratin filaments, indicating the dual character of differentiation, protein and lipid synthesis. These Odland bodies own several tasks in epidermal differentiation like intercellular adhesion and dyshesion, membrane thickening, keratinocyte starvation and deposition of lipid-rich material necessary to form the epidermal permeability barrier [13, 17, 23].

On their way to skin surface, the cells begin to flatten and elongate, forming the stratum granulosum. Keratinocytes start to manufacture keratohyalin granules, representing the early form of keratin. The increased keratin synthesis is accompanied by an increasing number of lamellar bodies [24]. The granular layer is often referred to as “transitional zone” due to its high biochemical activity and morphological change from living cells to dead keratin filled corneocytes, later forming the stratum corneum [13]. The formation of the cornified envelope also starts within this layer by the synthesis of an immature type of envelope underneath the plasma membrane which then undergoes maturation by the covalent attachment of preformed dedicated molecules to produce a rigid structure [25].

In the stratum corneum, cellular organelles and cytoplasm have disappeared and remaining proteins constituents are remodelled to form the dead keratin-filled corneocytes that are surrounded by a cornified lipid cell envelope which is stabilized by cross-linked proteins and covalently bound lipids. The intercellular regions of stratum corneum are filled with lipids primarily generated from the exocytosis of the lamellar bodies during the terminal differentiation process of keratinocytes. These intercellular lipids are required for a competent skin barrier and form the only continuous domain in the stratum corneum [14]. The bottom part of the stratum corneum (stratum compactum) is very firmly bound together by corneo(desmo)somes and intercellular lipids and owns an important protective function. The top part is looser in its structure (stratum disjunctum) and undergoes desquamation by enzymatic digestion of the corneo(desmo)somes [26]. Figure 3 points out the differences in lipid composition for the stratum basale, the stratum granulosum and the stratum corneum.

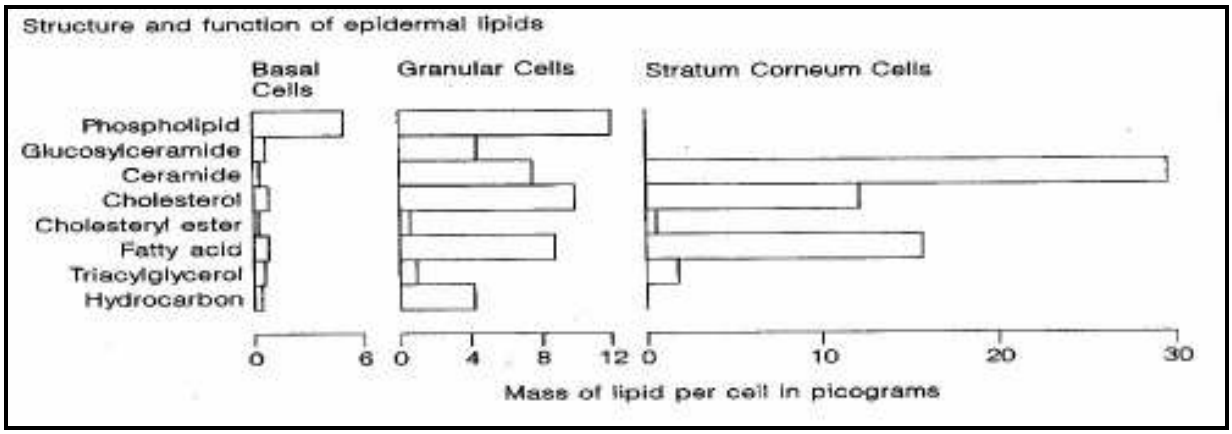


Figure 3. Lipid composition of different skin layers [14]

3.1.3 Stratum Corneum

The stratum corneum (SC) is the major source of resistance to penetration and permeation of the skin and consists of dead, flattened, keratin-filled cells that are embedded in an extracellular interlocking structure of multiple lipid bilayers, consisting of ceramides, fatty acids, cholesterol and cholesterols esters, all of that somewhat akin to bricks and mortar [15, 27].This classic model, first established by Peter Elias in 1975, is still the most simplistic organization description for the outermost layer of the skin reducing it to a two-compartment system of strongly heterogeneous composition [23, 28, 29].

A better insight into the SC lipid matrix is a prerequisite for understanding the skin barrier properties. Knowing the internal structure and hydration behaviour on the molecular level is essential for studying drug penetration through the SC and for a more rational design of transdermal drug delivery systems. During the last decades, various molecular skin barrier models have been developed, such as the stacked monolayer model, the domain mosaic model of Forslind, the sandwich model of Bouwstra and the single gel phase model of Norlen [29-32].

Nevertheless, to date, a detailed picture of the molecular organization of lipids in the SC, in particular of the function of each ceramide subclass, has not been fully elucidated. It is clear that a profound knowledge of the physical properties of the SC lipids and of their interaction is essential for a deeper understanding of the impact of each ceramide species on the barrier function of the SC [33].

For scientific purpose, stratum corneum often has to be separated from underlying epidermal structures. For more than 50 years, proteolytic enzymes such as trypsin have been extensively used for epidermal separation and keratinocyte isolation. The unique ability of proteases to cause selective epidermal separation has been in part explained by the proteolytic degradation of desmosomal proteins in the stratum corneum, which leads to cell dissociation. Recently, several endogenous proteases occurring in the epidermis have been found to play important roles in regulating epidermal cell desquamation [34, 35].

3.1.4 The Dermis and Subcutis

The dermis is folded into ridges that project into the upper unvascularized epidermis and comprises of stratum papillare and stratum reticulare. Main components of the dermis are collagen and elastin fibres that form a vast network of filamentous and amorphous connective tissue that ensure flexibility and strength of the skin. The skin is nourished by a

strong network of blood and lymph vessels that are situated in the utmost level of the dermis directly adjacent to the epidermis. Hair follicles, sebaceous glands and sweat glands originate in the dermis and perforate the stratum corneum. At the bottom of the dermis lies the subcutis that consists mainly of loose connective tissue and adipocytes [12, 14, 15].

3.2 DERMATOLOGICAL VEHICLES

3.2.1 Overview and Classification

In dermatology, the drug is rarely applied to the skin in the form of a pure chemical but instead is incorporated into a carrier system, a so-called vehicle. A formulation may be classified by its pharmaceutical nomenclature used in pharmacopoeias (e.g. cream, ointment, gel, paste), by the principal of structural matrix (e.g. emulsion, liposome, gel, suspension, transdermal patch) or by associated appearance (e.g. paint, milk, foam, shakes). However, until today no uniform and comprehensive classification is currently available [1]. In the European pharmacopoeia (Ph.Eur.) dermatological vehicles are subdivided following galenical points of view like rheology (liquid or semisolid), polarity (hydrophilic or lipophilic) and physico-chemical points of view (one-phase or multiple-phase). Generally, vehicles consist of a hydrophilic and/or lipophilic base, emulsifier, thickener, antioxidants and conserving agents [36].

Buhse et al. tried to obtain a scientifically based systematic classification of dosage forms for topical drugs. Several methods were applied in their studies to distinguish topical dosage forms, among which rheology, loss on drying, thermogravimetric analysis, appearance and composition were the most auspicious ones. Rheology is the most discriminating property separating creams and lotions. Lotions can be classified as liquid emulsions and creams as emulsions with a semisolid appearance. Liquids possess Newtonian or pseudoplastic flow behaviour, whereas semisolids show plastic flow behaviour. Ointments and creams differ in their composition, mainly in the presence/absence of water and/or other volatile ingredients. Gels are distinguished from other dosage forms by their composition and especially by their thermal behaviour.

Furthermore, dosage forms can be classified into liquids (solutions, suspensions, lotions) and semisolids (creams, gels, pastes, ointments). Ointments can be emulsions or suspensions and are separated from creams and gels on the basis of their composition, followed by the loss on drying. Generally, water plus volatiles content of less than 20% characterizes ointments. The low level of water and volatiles is due to a high hydrocarbon and/or polyethylene glycol content (>50%). Gels are semisolids and contain a gelling agent [37].

Table 1. Simplified characteristics and properties of dermatological formulations [adapted from [1, 36-38]]

Dermatological Formulation	Properties and Characteristics
Paste	• Ointment with a high percentage of insoluble particulate solids (>50%) • Semisolid
Ointment	• Hydrocarbon based semisolids containing dissolved or suspended drug • High molecular weight hydrocarbons form a fine crystalline matrix in which short chain hydrocarbons are entrapped • Non-aqueous hydrophobic (single-phase) • Water-emulsifying: can absorb larger amounts of water, leading to w/o or o/w - systems • Hydrophilic: contain bases that are miscible with water, may contain water
Cream	• Semisolid emulsions (w/o or o/w) • Multiphasic
Gel	• Liquid phase is immobilized in a three-dimensional polymeric matrix of a gelling agent (0.5 – 2%) • Semisolid
Rigid Foam	• Air or other gases are emulsified in a liquid phase to the point of stiffening

Modern pharmaceutical (and cosmetic) formulation development is based upon stability and compatibility of excipients and active agents (pharmaceutical-technological criteria), cosmetic acceptability, usage criteria and bioavailability of the agents at the target site (biopharmaceutical and therapeutic criteria).

Table 2. Pharmaceutical-technological, biopharmaceutical and cosmetic criteria for dermatological formulations [adapted from [1]]

Pharmaceutical-technological criteria	Biopharmaceutical criteria
• Stability of active drugs and ingredients	• Enhanced drug delivery and retention in the skin
• Rheological properties: consistency, extrudability	• Controlled drug delivery and retention in the skin
• Loss of water and other volatile components	• Targeted drug delivery and retention in the skin
• Phase changes, “bleeding”	**Cosmetic and usage criteria**
• Particle size and particle size distribution of dispersed phase	• Visual appearance
• Apparent pH	• Odour and colour
• Microbial contamination / sterility	• Sampling and dispensing characteristics
• Enhanced or controlled drug release from the vehicle	• Application properties
	• Residual impression after application

To increase the flux of a given drug, the selection of the vehicle is extremely important. An increase of the drug escaping tendency, achieved by selecting an ointment base with low ability to dissolve the drug, leads to enhanced penetration rates. The maximal possible flux can be achieved by incorporation of the drug at its maximal thermodynamic activity. However, the resistance of the stratum corneum is not a constant parameter and may be

reduced by specific vehicle effects, thus, penetration enhancers and hydration must be taken into consideration [39].

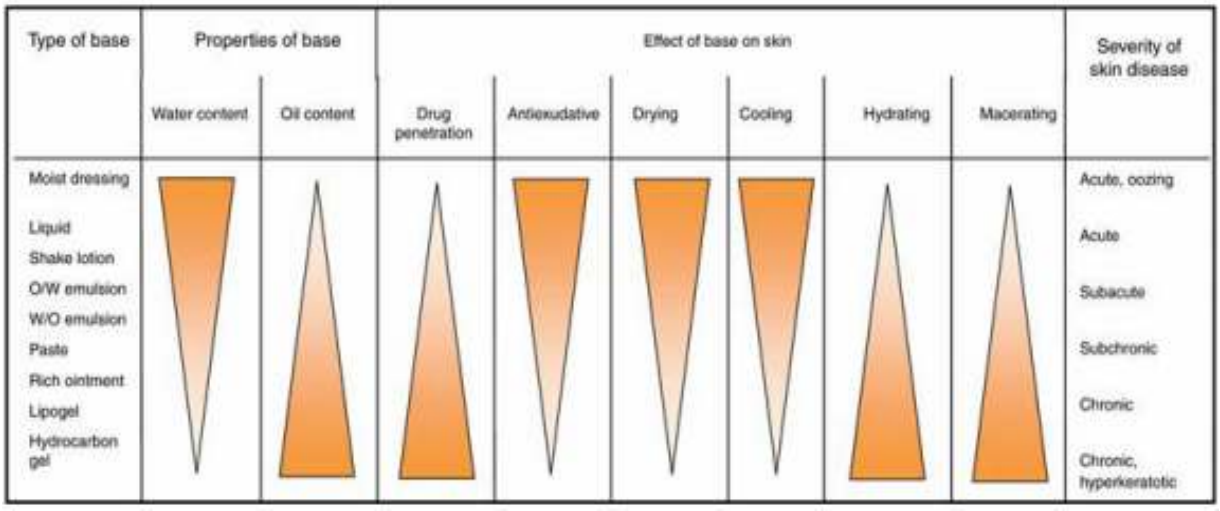


Figure 4. Use of vehicles on diseased skin [36]

3.2.2 Emulsions

3.2.2.1 Definition

Emulsions are heterogeneous mixtures of at least one immiscible liquid dispersed in form of droplets in another liquid. In general, the droplet diameters are greater than 0.2 µm and broadly distributed. It can be distinguished between oil-in-water (o/w) or water-in-oil (w/o) systems, where the first phase mentioned refers to the dispersed fraction and the second phase mentioned refers to the continuous fraction. The volume fraction of dispersed material in emulsions is seldom less than 10% and sometimes as high as 90%. The majority of emulsions have a white, milky appearance due to the fact that the dispersed and continuous phases have different refractive indexes [36]. Besides o/w and w/o-emulsions, more complicated systems may arise, generally referred to as multiple emulsions. They are composed of droplets of one liquid dispersed in larger droplets of a second liquid, which is then dispersed in a final continuous phase. Such systems may be w/o/w-emulsions where the internal and external phase is hydrophilic or o/w/o which have the reverse composition [40, 41].

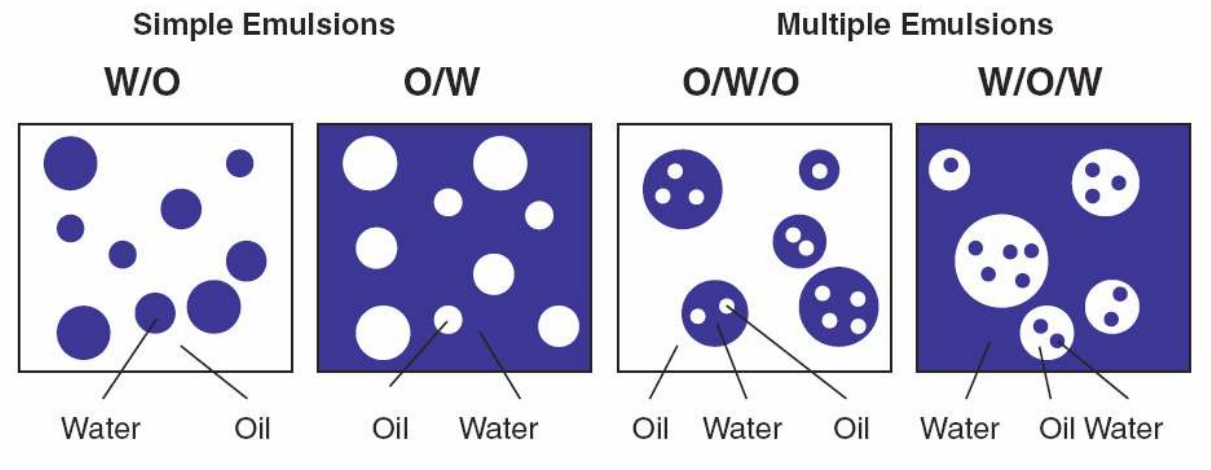


Figure 5. Schematic drawing of various types of emulsions [36]

The preparation of emulsions requires the formation of a very large amount of interfacial area between two immiscible liquids. The work required is given by

$$W = \sigma_i \cdot \Delta A$$

where σ_i denotes the interfacial tension between the two liquid phases and ΔA the change in interfacial area. Since the amount of work that is required to increase the interfacial area remains in the system as potential energy and minimum energy levels (equivalent to minimum interfacial area) are generally favoured, the system rapidly undergoes whatever transformations possible to reduce that energy, in this case, e.g. by reducing the interfacial area and separation. In order to prevent this coalescence or at least reduce its rate to negligible proportions and, hence, guarantee stability, in almost all practical emulsions, the inclusion of additives, such as surfactants, finely divided solids and polymers, is obligatory. The additive may perform two primary functions: (I) lower the energy requirements of drop formation (i.e. lower the interfacial tension) and (II) retard the process of drop reversion to separate bulk phases. Additionally, stability is affected by the dispersing process (manufacture method), the characteristics and quantities of additives employed, mixing temperature and order of mixing [40, 41].

In principal, flocculation, sedimentation/creaming, coalescence and breaking are the forms of instability that can occur. Flocculation is the mutual attachment of individual emulsion drops to form flakes or loose assemblies of particles in which the identity of each is maintained. It is a reversible process that can be compensated by simply shaking the emulsions. Coalescence refers to the joining of two (or more) drops to form a single drop of greater

volume, but smaller interfacial area. Creaming is related to flocculation in that it occurs without the loss of individual drop identities. Creaming occurs over time with almost all emulsion systems in which there is a difference in the density of the two phases. If the dispersed phase happens to be denser than the continuous phase, the separation process is termed sedimentation. Finally, the breaking of an emulsion refers to a process in which a gross separation of two phases occurs [40, 41].

The maximum volume fraction of dispersed phase which can be obtained is 74.02 wt%, if it is assumed that the emulsion is composed of rigid, spherical droplets of equal size. However, it is possible to prepare emulsions of dispersed volume fractions far exceeding this "theoretical" limit because droplets generally are not monodispers. Smaller droplets will locate themselves in-between close packed, larger droplets. Besides, emulsion droplets are not rigid spheres, but highly deformable so that their shape can be changed from spherical to elongated or polyhedral shapes [41]. The particle size distribution of emulsions is changed easily by adjustment of the phase volume ratio, method of manufacture, temperature and viscosity. In 1933, Langevin was the first to notice that variation of the proportion of emulsion ingredients influences the size of oil globules [42].

3.2.2.2 W/O-Emulsion

The lipid portion in w/o-emulsions is usually constituted of a mixture of lipids of different chain lengths. Fluid short-chain lipids are intercalated between longer-chain lipids responsible for the structural framework. Generally, hydrophobic skin emulsion bases are emulsions with a high proportion of the water phase (up to about 70%). Besides, these emulsions tend to break on the skin. Thus, water is released and a lipid layer remains on the skin in which there are many gaps which arise during the demulsification [36, 43].

w/o-emulsions offer a series of significant benefits compared to traditional o/w-emulsions. By forming an occlusive layer on the skin, they are efficiently reducing the evaporative water loss from the skin. They are excellent water repellents which makes them a very attractive formulation basis for sun care and colour cosmetic formulations. Despite of these attractive benefits, the use of w/o-emulsions has been limited due to an association with stability issues and a heavy skin fell resulting from high oil content. Typical w/o-emulsions have oil phase contents (emollients, waxes, emulsifiers) of 25-30 wt% and a water phase content of 70-75 wt%. Formulating stable w/o-emulsions with oil phase content below 25 wt% is still a big challenge due to low oil content, currently available emulsifiers and the resulting high emulsion viscosity. A reduction of the oil phase content of w/o-emulsions means reducing

continuous phase and, by doing so, increasing the interaction between water droplets in the emulsion. An increase in viscosity is the direct consequence of that. Additionally, the risk of coalescence of water droplets increases as the average distance between the water droplets further decreases. However, it is possible to formulate so-called concentrated emulsions owning a dispersed phase content bigger than 74 wt-%. Here, the droplets cannot be spherical any more and assume some transitional form between spheres and polyhedra [44, 45]. As indicated by the phase ratio, a w/o-emulsifier has to provide excellent stability to succeed in formulating stable emulsions with such a high internal phase content [46].

It is widely accepted that electrolytes dissolved in the aqueous phase of w/o-emulsions dramatically increase emulsion stability [45].

Opawale and Burgess examined the influence of sodium chloride on the stability of w/o-emulsions using different span surfactants. Surface-active substances lower interfacial tension and form an interfacial film, but for long-term stability to coalescence and phase separation, it has been reported that strength of the interfacial film is more important than its effect on interfacial tension. Possibly, the addition of salt promotes interfacial elasticity, and decreases adsorption and/or molecular interactions of surfactants, as Na and Cl ion are preferentially hydrated over the surfactant molecules. This apparently results in a reduced interaction of water with the surfactant polar head groups and/or salting out of the surfactant molecules present at the interface. The use of high salt concentration (>1M) in emulsion formulations, however, is not recommended as this decreases the elastic behaviour of the interfacial film and therefore emulsion stability [47].

Kent and Saunders studied the influence of added magnesium sulphate on the properties of water-in-oil (inverse) emulsions with respect to coalescence. The average droplet size of the emulsions increased with increasing salt concentration. These apparently contradictory results were attributed to differences in their relative importance. It was proposed that magnesium sulphate decreased/retarded the rate of surfactant adsorption at the oil-water interface which resulted in an increased non-equilibrium interfacial tension during emulsification and increased droplet size. While stability of o/w-emulsions in the presence of electrolyte can be described by the conventional DLVO-theory, stability of inverse emulsions cannot be simply described by these conventional theories of colloid stability (e.g. DLVO) as continuous phase possesses a very low dielectric constant. Nevertheless, electrostatic effects should not be ignored as they may contribute to emulsion stability. To conclude, there have been a number of conflicting reports in the literature concerning the role of electrolytes on the interfacial properties of the oil-water interface. The study revealed that datas obtained

within one study can be contradictory and that kinetic effects involving surfactant adsorption are very important for inverse emulsions [48].

3.2.2.3 Methods of Preparation

Ointments, pastes and creams tend to be produced by one or the other of the two general methods. Either they are made at high temperature by blending the liquid and heat-liquefiable components together and then dispersing other solids (often including the drug) within the oily melt, or, in the instance of emulsions, within the aqueous phase of the emulsion or the freshly formed emulsion itself (fusion methods); or the drug is incorporated in the already solidified base (cold incorporation).

In the fusion method for ointments, the ingredients are heated together somewhere between 60℃ and 80℃, depending on the components, and mixed to a uniform composition while in the fluidized state. Cooling is then effected using some sort of a heat exchanger. Systems in preparation are always cooled with mild stirring until they are close to solidification.

The fusion methods for preparing creams are a bit more complex. In this instance the aqueous and oil phases are heated separately to somewhere between 60℃ and 80℃. As a general rule, the water phase is heated to 5℃ above the temperature of the oil phase, the latter to prevent premature solidification during the emulsification process. Water-soluble ingredients are dissolved in the heated aqueous phase, and oil-soluble ingredients are dissolved in the oily melt, but only as long as they are heat-stable and not too volatile. If an o/w-system is to be made, the emulsifiers are added to the aqueous phase and the emulsion is formed by slow addition of the oil phase. If a w/o-emulsion is to be made, the addition steps are usually reversed. Therefore and generally, the discontinuous phase is added to the continuous, external phase containing the emulsifier [38].

Each method of preparation requires that energy is put into the system in some form. The energy may be supplied in a variety of ways, such as trituration, heat, agitation or homogenization. The rotor stator technology, for example is a well-established method for time-saving and easy emulsion preparation. A typical dispersing aggregate consists of two teeth rings, one of them is fixed and does not move - the stator; the other is driven by a motor through the shaft and turns around inside the stator - the rotor. The shear forces and bounce effects which are created between the running rotor and the stator treat the product mechanically, so for example 2 phases can be homogenized in short time [3].

Figure 6. Functional principle of rotor-stator system

3.2.2.4 Emulsifier and Isolan PDI®

There are four general classes of materials that can act as emulsifiers and/or stabilizers for emulsions. It includes common ionic materials, colloidal solids, polymers and surfactants. Each class varies greatly in its effectiveness and its mode of action. Adsorbed non-surfactant ions impose a slight electrostatic barrier between approaching drops. They may also affect the stability of the system by orienting solvent molecules in the neighbourhood of the interface, altering some local physical properties such as dielectric constant, viscosity and density, thereby producing a small stabilizing effect. Small colloidal materials (sols) stabilize an emulsion by forming a physical barrier between drops, thereby retarding or preventing drop coalescence. Particles should partially be wetted by both liquid phases, but with a slight preference for the external phase. Polymeric additives may change surface properties, but mostly are used as stabilizers. Their action may result from steric or electrostatic interactions, from changes in the interfacial viscosity or elasticity or from changes in the bulk viscosity of the system. Of the possible emulsifiers, most are true surfactants in that they are effective at lowering significantly the interfacial tension between the two liquid phases. The rule of Bancroft states that the liquid in which the surfactant is most soluble, will form the continuous phase in the final emulsion [41]. Often a mixture of surfactants with widely differing solubility properties leads to emulsions with enhanced stability. Synergistic effects will lead to a tremendous reduction of interfacial tensions and the formation of cooperative surfactant “complexes” impart greater strength, possibly leading to higher rigidity of the interfacial layers [41]. Emulsifiers can be classified according to the hydrophilic-lipophilic balance (HLB) system of Griffin. This system calculates a HLB number for every surfactant based on its chemical structure in a range from 0 to 20. It is proposed to mix emulsifiers with an oil phase of corresponding HLB value. At the high end of the scale (8 - 18) lie hydrophilic surfactants, whereas at the low end (3 - 6) are surfactants with low water

solubility acting as w/o-emulsion stabilizers. Nevertheless, the HLB system does not always provide a clear-cut answer for a given system [41].

The IUPAC Commission for nomenclature defines an emulsifier as “a surfactant which is positively adsorbed at interfaces and lowers the interfacial tension. It facilitates, when present in small amounts, the formation of an emulsion, or enhances its colloidal stability by decreasing either or both of the rates of aggregation and coalescences” [49, 50]. Surfactants are classified based on their structure and physico-chemical behaviour. They are amphiphilic and can form aggregates such as micelles or lamellar liquid crystals. An important distinguishing factor is the charge of the hydrophilic head-group of the molecule. Accordingly, surfactants are categorized as anionic, cationic, amphoteric and non-ionic [36].

Other additives such as polymers and sols function primarily as stabilizers, rather than emulsifiers. In addition, because of their molecular size, the adsorption process for polymers is generally very slow relative to the timescale of the emulsification process. The primary function of polymers and sols in emulsions is the retardation of droplet flocculation and coalescence [41].

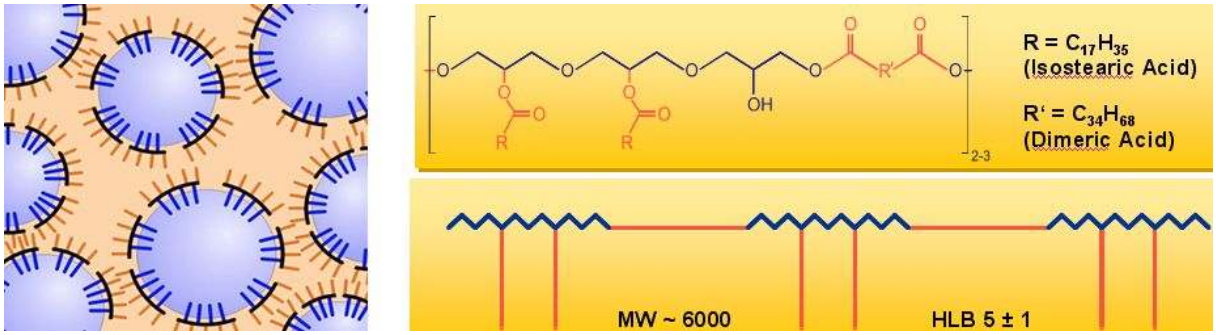


Figure 7. Isolan PDI®: Structure and Mode of Action

The selected emulsifier of this work for manufacturing stable w/o-emulsions of varying phase ratios and oil bases is Isolan PDI®. It is a polymeric and polyfunctional emulsifier for cosmetic applications which mode of action is illustrated in figure 7. Isolan PDI® accumulates at the interface between water droplets and continuous oil phase, thus, interfacial tension is lowered, and a barrier (electrostatic and/or steric) between drops is established. Furthermore, viscosity of the oil phase and consequently the whole system is increased due to interactions of the hydrocarbon chains. So to say, the effective adsorbed layer thickness is increased and concomitantly also interfacial viscosity. Stable lipophilic lotions and creams can be manufactured requiring only low amounts of emulsifier (up to 3%) and no further co-surfactants [41, 51].

Isolan PDI® belongs to the class of polymer surfactants that, quantitatively and qualitatively seen, often achieve considerably more if compared to low-molar-mass surfactants. This is due to the plentiful possibilities of tailoring polymer architectures, taking advantage of the large pool of monomeric building blocks as well as of modern synthesis strategies. Compared to low molecular mass surfactants, polymer surfactants offer the possibility to combine different properties (ionic, non ionic, hydrophilic, hydrophobic) within the same molecule in such a way that almost every polar – non-polar gradient can be realized. The critical micelle concentration (CMC) is extremely low or even not existent, micelles/aggregates are stable at even high dilutions and aggregation numbers are adjustable by the macromolecular design. Properties such as foaming or viscosifying can be varied broadly, the polarity of the hydrophobic polymer block is adjustable and, thus, its compatibility and solubilization capacity can be tuned for a given solute. The required amount of surfactant needed can be decreased and surfactant behaviour can be controlled by external stimuli, e.g. changing of pH, temperature [52].

3.2.2.5 Characterization

3.2.2.5.1 Lipophilic and Hydrophilic Character

In order to distinguish between the types o/w and w/o of dermatological vehicles, there are several physical methods available like optical observation after blending the sample with a hydrophilic or lipophilic dye or simple dilution experiments with oil or water bulk phase [53, 54]. Electric conductivity is based on the measurement of movable particles with electric charge that can carry electricity when a difference of electric potential is placed across a conductor. The conductance C in Siemens (S) is the reciprocal of the resistance R in ohms (Ω) of the conductor:

$$C = \frac{1}{R}$$

The resistance R is given by:

$$R = \rho \cdot \frac{l}{A}$$

where ρ is the specific resistance of the conductor, l denotes its length and A its cross-section area in cm^2. The specific conductance or electric conductivity κ in S/cm is the reciprocal of the specific resistance and described by the term:

$$\kappa = C \cdot \frac{l}{A}$$

A pair of electrodes connected to an external electric source is immersed into the dermatological formulation in order to measure its electric conductivity. The electrodes possess the distance l and a specific area A each. The ratio l/A is referred to as cell-constant of a specific measuring cell. If the water phase is continuous, a current will pass through the emulsion, while a continuous oil phase will fail to carry the current. The electric conductivity of o/w formulations was found to be in the same order of magnitude to the bulk continuous phase [55].

3.2.2.5.2 Optical Methods

Structures within dispersed systems may range in their size from about 10 nm (microemulsions) over several hundred nm (liposomes) up to more than several microns (emulsions). The overall appearance of such systems inherently involves some obvious information about comprising structures. Potential physical stability of a colloidal system can often be understood on the basis of particle size. In particular, smaller colloids, especially those made up to like-charged particles, are appreciably more resistant to flocculation, sedimentation etc. than systems containing larger or uncharged entities. For formulations made up of immiscible fluids, such as emulsions or microemulsions, reduced particle size is an indicator of improved kinetic stability or the attainment of a thermodynamic equilibrium, respectively. Second, the mechanisms at work during processing can be better understood by quantitatively characterizing the products generated under various operational parameters. Third, performance of the formulation in vivo sometimes can be controlled if not completely understood on the basis of size consideration. Finally, the safety of multiphase parenteral formulations containing submicron particles can be better assessed if the absence of larger particles can be assured. It should be noted that Haskell provided a good minireview on characterization of submicron systems via optical methods [56].

For visualization of dispersed structures that are smaller in size than several hundred nanometers and are optically isotropic, transmission electron microscopy (TEM) or scanning electron microscopy (SEM) is generally applied. Scanning electron microscopes have many advantages over traditional microscopes. The large depth of field allows more of a specimen to be in focus at one time. SEM possesses a much higher resolution, so closely spaced specimens can be magnified at much higher levels. The degree of magnification can be controlled as lenses are replaced by electron beams. Actual strikingly clear images are

achieved and, thus, SEM has become one of the most useful instruments in research today [57-60].

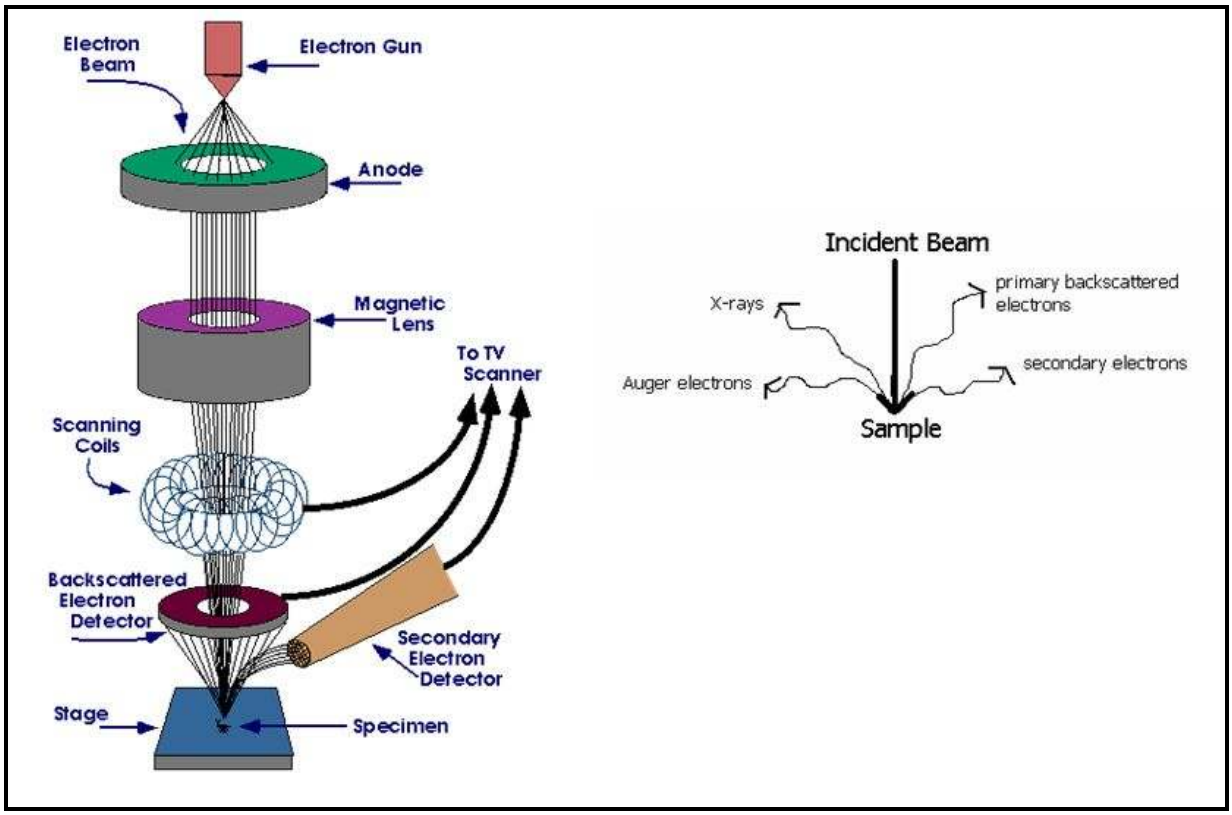


Figure 8. Scanning electron microscopy [61]

In case of freeze fracture scanning electron microscopy, original formulation is fractured in a frozen-hydrated state and then observed. Specimen preparation for this method involves the following steps: First, a small droplet of the formulation is placed on a specimen table and rapidly frozen in a mixture of liquid propane and nitrogen. The samples must be rapidly cryo-transferred into a freeze-fracturing device where the fracture is performed at low temperature under high vacuum. Due to this treatment, the sample will cleave along a fracture plain with the least cohesion. In case of an emulsion, for example, these are the hydrophobic areas of lipids stabilized by van-der-Waals forces, which are much weaker that the hydrogen bonds of the water domains. By further sublimation of ice to a depth of several nanometers, referred to as etching, structural details otherwise hidden in deeper ice layer can be additional exposed. This fracture plane is finally coated with a metal-carbon film before scanning electron microscope detects electrons, emitted from an electron source, that are back-scattered from the specimen surface. To achieve this, the specimen surface must be coated with a layer of metal, for example platinum with a thickness of a few nm, to render the surface conductive. If the metal is evaporated unidirectional at a fixed angle, usually 45° onto the specimen, a

shadow effect is generated that gives the image of a three dimensional appearance. A carbon film finally reinforces this metal layer.

The SEM is an instrument that produces a largely magnified image by using electrons instead of light to form an image. A beam of electron is produced at the top of the microscope by an electron gun. The electron beam follows a vertical path through the microscopes which is held within a vacuum. The beam travels through electromagnetic fields and lenses which focus the beam down toward the sample. Once the beam hits the sample, electrons and x-rays are ejected from the sample. Detectors collect these x-rays, backscattered electrons and secondary electrons and convert them into a signal that is sent to a screen similar to a television screen. This produces the final image (see figure 8 and 9).

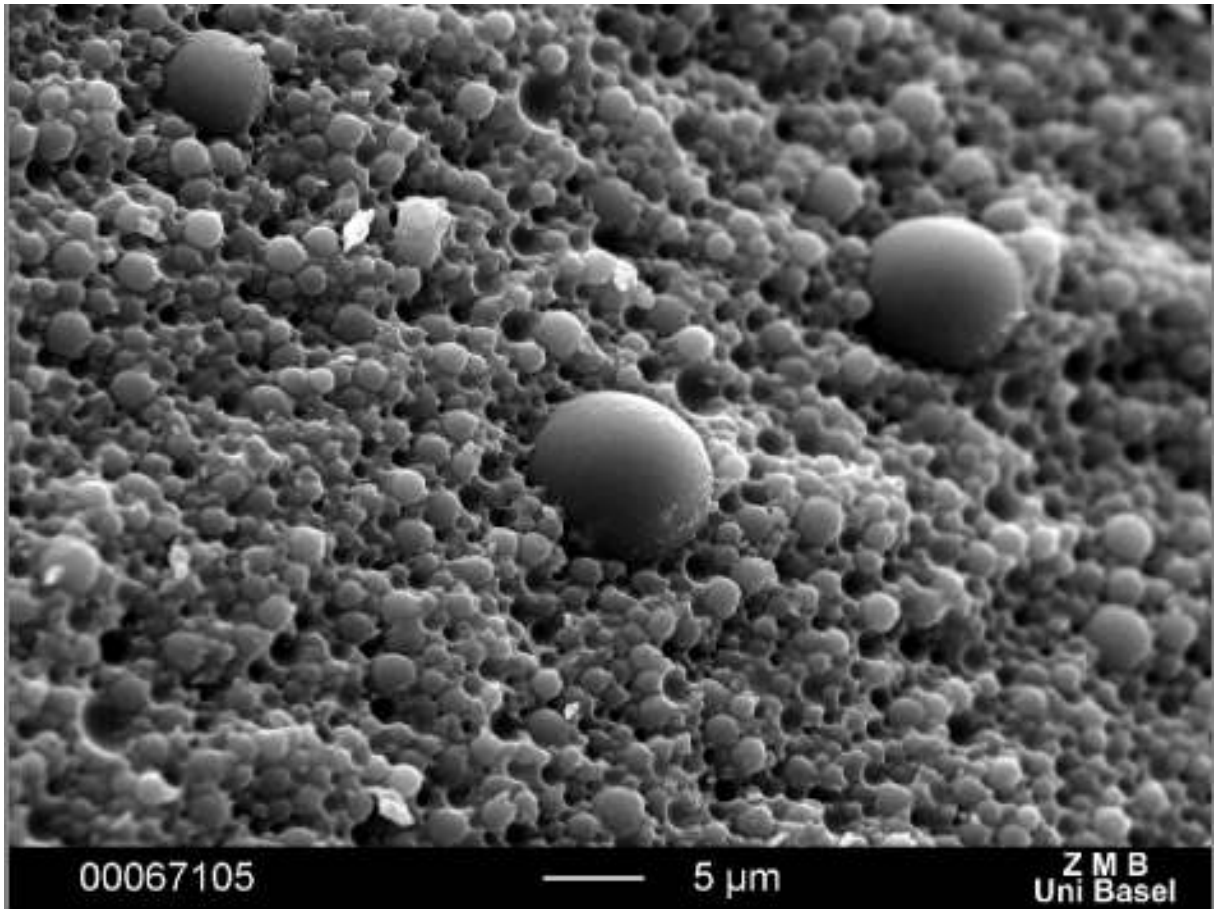


Figure 9. Freeze fracture scanning electron microscopy of a w/o-emulsion comprising 70% of water phase dispersed in 30% oil phase (Miglyol 812N)

3.2.2.5.3 Ultracentrifugation

Ultracentrifugation of dermatological vehicles like emulsions is a precious tool to gain information about the nature, the composition and, if the density is known, about the size of dispersed structures. It is a conventional method to purify and concentrate liposomal formulations and also used for stability testing of emulsions [62-64]. Besides, ultracentrifugation is applied for structural analysis of semisolid multi-phase formulations, typically in combination with further physicochemical methods [2, 3, 65].

The sedimentation and creaming processes are dependent on the specific density of each single vehicle component and are accelerated by the application of centrifugal forces. Stoke's law describes the velocity of a creaming or sedimenting particle. An (ultra)centrifuge is an instrument designed to apply a rotational force to a mass (particle) and if the mass is unrestricted, it will move away from the centre of rotation. Hence, acceleration of gravidity g is replaced by acceleration of centrifugation $\beta=\omega^2 x$, where ω is the angular velocity and x is the distance of the particle form the centre of rotation.

Stoke's law is accordingly modified to:

$$v = \frac{dx}{dt} = \frac{2 \cdot r^2 \cdot (\rho - \rho_0) \cdot \omega^2 \cdot x}{9 \cdot \eta_0}$$

where v is the velocity of sedimentation, ρ and ρ_0 are the density of the sedimenting spherical particle and the medium, respectively and η_0 is the viscosity of the medium. The instantaneous velocity v = dx/dt of a particle in a unit centrifugal field is expressed in terms of Svedberg sedimentation coefficient s

$$s = \frac{dx/dt}{\omega^2 \cdot r}$$

The force at which a centrifuge is operated is often expressed in terms of the number of times that the force of gravity is exceeded. For example, the ultracentrifuge Centricon T-1075 with the rotor TFT 7013 (Kontron Instruments, Mailand, Italy) used in the present work to fractionate the formulations produces a force in between 221290 and 448610g, depending on the distance from the center of rotation.

Lalor, Hummel and Nalenz, for instance, applied (ultra)centrifugation for separation of emulsions into their respective phases and subsequently determined the concentration of active ingredients present in each phase [2, 3, 6].

3.2.3 Oil and Organogel

3.2.3.1 Definition

Organogels are semi-solid systems in which an organic liquid phase is immobilized by a three-dimensional network composed of self-assembled, intertwined gelator fibres in low concentrations (<15%). Based on physical and chemical interactions, gelator fibres form an extensive mesh network that prevents solvent flow as a result of surface tension. Phase separations into crystalline and liquid layers are avoided owing to the balance between gelator aggregating forces and solubilizing solvent-aggregate interactions.

Organogels can be distinguished from hydrogels by their predominantly organic continuous phase and can then be further subdivided based on the nature of the gelling molecule: polymeric or low molecular weight organogelators.

Despite the large abundance and variety of organogel systems, relatively few current applications in drug delivery exist, owing mostly to the lack of information on the biocompatibility and toxicity of organogelator molecules and their degradation products. A number of organogel vehicles have been developed for transdermal delivery. Important advantages are the permeation enhancement if a suitable liquid base is selected and their general ease of preparation, consisting in simple dissolution of drug and gelator in the liquid medium. Often fatty acids, alcohols, essential oils, fluid wax or liquid paraffin are used as components. Also, organogels can be applied as a drug reservoir/matrix in transdermal patches. Advantages of this gel vehicle include the capacity to accommodate polar and non-polar drugs, thermo-reversibility, high degree of stability to moisture and temperature, and the ability to control drug release. Besides (trans)dermal delivery organogels are used for parenteral depot formulations as well as for oral and trans-mucosal formulations. Interest in the field of organogels has increased due to the strikingly rise of the discovery of substances that are able to gel organic solvents, but still medicinal lipophilic solutions for use on the skin are very uncommon [66-69].

One of the very famous examples is the pluronic lecithin organogel developed by Jones and Kloesel in the 1990s that provides a base for nonsteroidal anti-inflammatory drugs, hormones, selective serotonin reuptake inhibitors and opiates among others [70, 71].

3.2.3.2 Physicochemical Properties

Lipids can be roughly divided into four chemical classes: The least polar substances are hydrocarbons such as petrolatum or soft and liquid paraffin. Moderately polar lipids include many types of wax such as yellow wax and fluid wax esters (isopropyl myristate and ethylhexyl palmitate), which typically spread over the skin very well. Among the more polar lipids are most of the glycerides such as medium-chain triglycerides (neutral fat) and olive oil. Silicone oils are hydrophobic substances that spread extremely well. These oils include various compounds that contain a polyorganosiloxane as the characteristic functional group. Important substances include dimethylpolysiloxane (dimethicone), phenyl ethyl polysiloxane and cyclic methyl siloxane (cyclomethicone) [36].

Isopropyl myristate (IPM) is an important solvent in studies of permeation of solutes through skin. First, it has been suggested as a model for the stratum corneum and partitioning from water to IPM, P_{IPM}, have been compared to partitioning from water to the stratum corneum or to permeation rates through skin. Second, IPM has been used as a vehicle for permeation through skin, usually hairless mouse skin [72]. Saket et al. found a good correlation between partitioning into the stratum corneum and partitioning into IPM, but this was limited to cortisone, hydrocortisone and their esters [73]. Abraham et al. showed that the water-IPM system is a poor chemical model for partitioning from water to the stratum corneum and for permeation from water through human skin. The solute factors that influence partitioning into IPM are quantitatively not the same as those that control partitioning into the stratum corneum [72].

3.2.3.3 Thickener and Stabilizer

Gelling agents are usually organic, rarely inorganic macromolecules that enable formation of a three-dimensional frame-work by intermolecular interactions and, thus, form viscous solutions in aqueous or oily systems or, in higher concentrations, gels [36].

There are several types or organogelators which include steroid derivatives, anthryl derivatives, amino acid-type organogelators and organometallic compounds. Aggregation in organogels results from a different set of interactions. In non-aqueous liquids, the binding forces are primarily dipolar interactions, intermolecular hydrogen bonds or metal-coordination bonds [66].

Table 3. Organogel formulations used in drug delivery [68]

Organogelator used formulations	Route of administration
Lecithin	Transdermal
Glyceryl fatty acid esters	Transdermal
N-lauroy-l-glutamic acid di-n-butylamide	Transdermal
Poly(ethylene)	Transdermal
Sorbitan monostearate or monolaureate	Nasal Oral Subcutaneous intramuscular
N-stearoyl l-alalnine methyl or ethyl ester	Subcutaneous
Poly(methacrylic acid-co-methylmethacrylate) [and crosslinked poly(acrylic acid)]	Rectal, buccal

3.3 TRANSDERMAL ABSORPTION

3.3.1 Drug Delivery across the Skin

The application of dermatological formulations may be targeting the drug to three different anatomical locations, namely the skin itself (topical delivery), deeper tissue layers (endoderm or diaderm delivery) and the systemic circulation (transdermal delivery) [1].

Transdermal delivery has several advantages with regard to e.g. oral delivery. It circumvents variables that rest on the anatomical and physiological properties along the gastrointestinal tract, like pH gradient and nutrition, it bypasses first pass metabolism and due to the fact that only small drug amounts reach systemic circulation, it possesses less adverse effects. Special drug delivery systems like transdermal therapeutic systems (TTS) provide controlled administration and duration of drug action [15].

Figure 10 gives an overview about the drug flux that may arise following application of a suspension vehicle. Generally, the process of percutaneous absorption is an action of several consecutive steps. The drug may undergo any or all of the depicted events: First, the drug molecules must dissolve in the vehicle to enable its diffusion inside the formulation to the vehicle-stratum corneum interface. The drug diffuses passively out of its carrier and partitions into either the almost impermeable stratum corneum or the sebum-filled ducts of the pilosebaceous glands. For most hydrophilic and amphiphilic penetrants, diffusion through the stratum corneum will be the rate-limiting step, whereas lipophilic substances are favoured to penetrate this layer. Inward diffusive movement continues from these locations to deeper

tissues of the viable epidermal and dermal points of entry. For very lipohphilic drugs, the viable epidermis will act as the rate-limiting factor and clearance rate will govern its percutaneous absorption. For systemic delivery, drugs encounter the capillary of the cutaneous microvasculature and accordingly gain access to the systemic circulation. Taken together all of these steps, a concentration gradient is established across the skin up to the outer reaches of the skin's microcirculation, where the drug is swept away by the capillary flow and rapidly distributed throughout the body. Systemic drug levels are usually low and inconsequential. If the stratum corneum is not intact, many chemicals can gain systemic entrance at alarming rates [38, 74, 75].

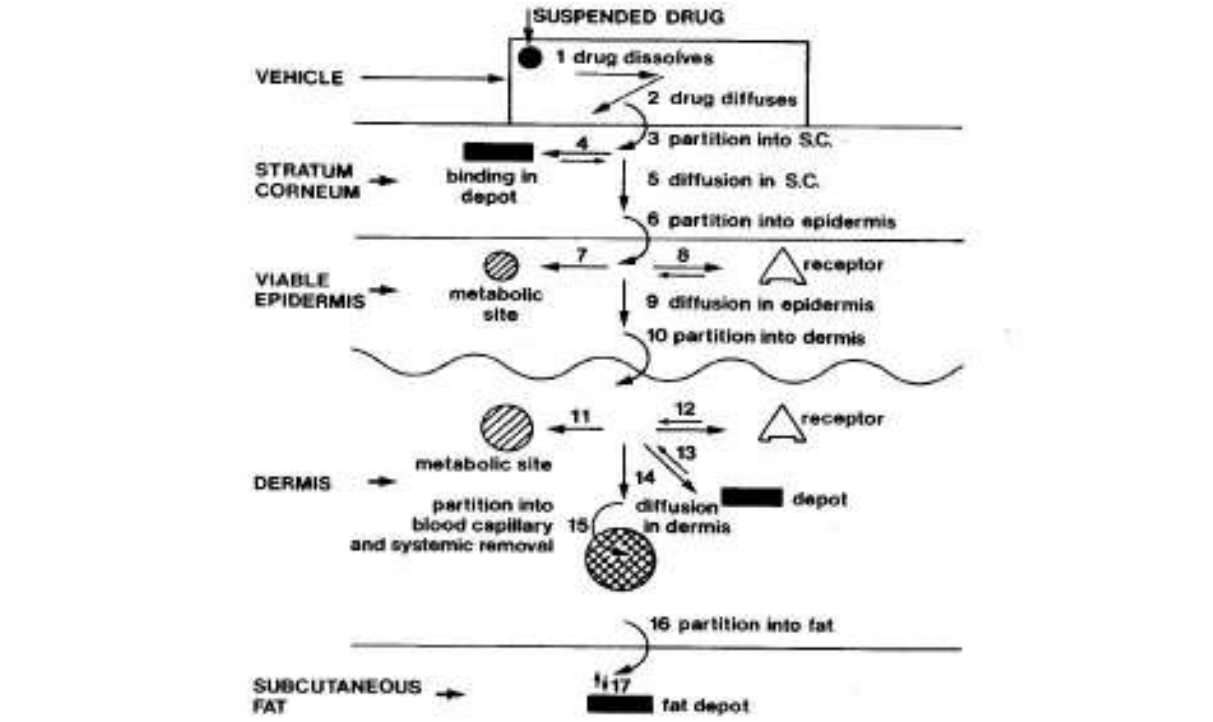


Figure 10. Different stages of percutaneous absorption [13]

Percutaneous absorption is a process controlled by simple passive diffusion and Fick's law can be used to analyse permeation data:

$$J = \frac{dm}{dt \cdot A} = -D \cdot \frac{dc}{dx}$$

where J is the amount of drug permeated through the skin per unit time and per unit area, A is the effective diffusion area and D is the diffusion coefficient (cm^2/sec). dc/dx denotes the concentration gradient over a distance x. Assuming the conditions of a perfect sink (receiver concentration negligible, therefore zero) an infinite donor concentration C_D, rate limiting membrane (skin) diffusion and partitioning between donor vehicle and rate limiting membrane, Fick's first law of diffusion can be expressed as:

$$J = \frac{dm}{dt \cdot A} = -D \cdot \frac{K \cdot C_D}{h}$$

K represents the distribution coefficient of the diffusant between the vehicle and the membrane (skin) and h is the thickness of this membrane. As an exact determination of h and D is difficult, these parameters may be summarized in term of the permeability coefficient P (cm/sec):

$$P = D \cdot \frac{K}{h}$$

This permeability coefficient can be regarded as a characteristic for a specific substance-membrane system and is, beside the drug flux and the totally permeated drug amount after a specific time, a common measure for steady state drug permeation. Fick's law of diffusion shows that the flux J should increase linearly with concentration until the solubility limit is reached. The important physicochemical determinants that control diffusion of xenobiotics through the skin are therefore partitioning, diffusion and solubility [3, 76].

In clinical practice, a finite dose of 1-3 mg/cm^2 is applied [77]. However, extent and rate of absorption of an agent is determined by vehicle properties, the agent itself and their interactions and resulting influences. In order to cross the epidermal barrier, drug molecules must possess certain characteristics including: low molecular weight (< 500 Dalton), moderate lipophilia (octanol-water partition coefficient between 10 and 1000) and a moderate melting point (< 200°C) indicating good solubility [36]. Generally, substances with greater hydrophobicity are absorbed more readily by the skin than less hydrophobic. Dermal

absorption increases as log P does from -1 to 3.5. Highly lipophilic substances (log P > 5) can pass easily through the stratum corneum but are generally too water insoluble to pass through the remaining sub-layers and to enter the bloodstream. On the other hand, the rate of absorption of substances through the skin is inversely proportional to molecular mass and size. Thus, small molecules (>150 Dalton) that are both lipid- and water-soluble are the most readily absorbed [78, 79]. Even when active substances exhibit such properties, it is usually necessary to find additional means to increase its transport across the skin and until today there are only few drugs on the market that fulfil the requirements of a potent topical active.

During permeation changes in the physical environment of the skin layers occur as depth increases from the stratum corneum through viable epidermis into dermis. These changes may affect the residence time and distribution of solutes during the penetration process. Partitioning into the stratum corneum intercellular lipids and protein binding, i.e. binding to keratin, may occur and are the factors responsible for the residence time of drugs in the skin [80]. To summarize, for the prediction of the maximum flux of a drug, its solubility in the rate-limiting barrier is decisive [81].

3.3.2 Permeation Routes

Due to the heterogeneous structure in the stratum corneum, several pathways can be considered for drug permeation and it is difficult to specify the extent to which drug utilizes each pathway [21, 82].

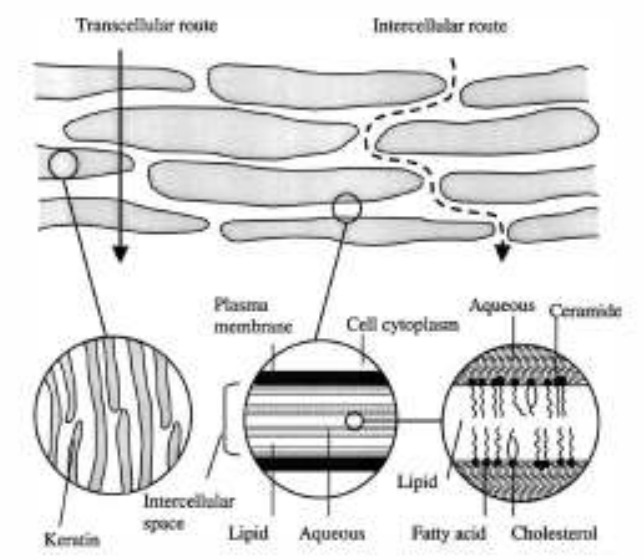


Figure 11. Permeation routes [27]

A molecule may use two diffusional routes to penetrate normal intact human skin: the appendageal route and the transepidermal route. The appendageal route comprises transport via the sweat glands and the hair follicles with their associated sebaceous glands. These routes circumvent penetration through the stratum corneum and are therefore known as shunt routes. Although these routes offer high permeability, they are considered to be of minor importance because of their relatively small area, approximately 0.1% of total skin area. Transepidermal transport means that molecules cross the intact horny layer. Two potential micro-routes of entry exist, the transcellular (or intracellular) and the intercellular pathway. The principal pathway taken by a penetrant is decided mainly by the partition coefficient (log K). Hydrophilic drugs partition preferentially into the intracellular domains, whereas lipophilic permeants (log P > 2) traverse the stratum corneum via the intercellular route. Most molecules pass the stratum corneum by both routes. However, the tortuous intercellular pathway is widely considered to provide the principal route and major barrier to the permeation of most drugs.

In general, the transcellular route is not very likely because substances have to pass through areas of high lipophilicity as well as through areas of high hydrophilicity. Recently, it has been discussed that drugs either diffuse laterally along the lipophilic carbon chains of stratum corneum lipids or that they diffuse along the corneodesmosomes (hydrophilic route). The intercellular spaces contain structured lipids and a diffusing molecule encounters significant resistance to permeation because it has to cross both lipophilic and hydrophilic structures before it reaches the junction between the stratum corneum and the viable epidermis [83, 84]. Substances like estradiol with an extreme high lipophilicity further build up a depot in the lipophilic bilayers [85-87].

3.3.3 Factors Affecting Drug Permeation through the Skin

3.3.3.1 Skin Hydration and Occlusion

Occlusion refers to skin covered directly or indirectly by impermeable films or substances. Healthy stratum corneum typically has a water content of 10-20%, but if diffusional water loss from the skin surface is blocked by occlusion, stratum corneum hydration can be raised up to 50%. Hydration can have several impacts on percutaneous absorption: The partitioning between the surface chemical and the skin is altered due to the increasing presence of water, corneocytes swell and possibly the intercellular lipid phase organization changes, accompanied by an increase of skin surface temperature and an increase of blood flow. Thus, skin barrier function is compromised. If hydrophilic character of the stratum corneum is increased, it follows, in turn, that stratum corneum – viable epidermis distribution coefficient

is reduced. An increase in skin volume can induce internal stresses, especially in keratin fibres, which need to enlarge to accommodate absorbed water. Furthermore, occlusive covering prevents evaporation of volatile vehicle components, maintaining its solvency for the drugs. All is all, occlusion is a complex event producing profound alterations, but its impact is also dependent on the anatomic site, the vehicle (e.g. volatility) and the properties of the penetrant (polarity, partition coefficient, aqueous solubility) [38, 75, 88-92].

Wagner et al. claimed that the hydration state of the stratum corneum obviously is more decisive for the drug penetration than examined lipophilic liquid compounds of the ointment bases and their tendency to diffuse into the stratum corneum, where they could interact with the stratum corneum lipids so as to change barrier properties [93].

Nevertheless, permeation enhancement by occlusion is not similar to all kinds of drugs. Penetration of lipid-soluble, non-polar molecules seems to be more likely than for polar molecules. So to say, a trend of occlusion-induced absorption enhancement with increasing penetrant lipophilicity is apparent [89]. Several studies proved the enhancing, non-enhancing or even retarding effect. Treffel et al. compared permeation profiles of two molecules with different physicochemical properties under occluded versus non-occluded conditions in vitro: Occlusion increased the permeation of citropten 1.6 times (lipophilic compound; partition coefficient 2.17), but permeation of caffeine (amphiphilic compound; partition coefficient 0.02) remained unchanged and, thus, confirmed that not necessarily occlusion leads to an increased absorption of all drugs [94]. In contrast, Walters reported on a permeation enhancement of caffeine when human skin was occluded. Makki et al. underlined that occlusion impact is a function of drug polarity, suggesting that absorption is increased only up to moderately lipophilic molecules, whereas it is almost ineffective in molecules of high lipophilicity and not or only slightly affecting permeation of amphiphilic molecules like caffeine. Thus, hydration induced alteration on permeation rates appear to be a function of the physicochemical nature of the permeant [15, 95].

3.3.3.2 Evaporation of Volatile Components Following Application

In clinical and experimental situations, most dermatological vehicles undergo considerable changes following application to the skin, most likely due to evaporation of volatile components, supported by mechanical agitation associated with application of the product [1]. These alterations in the vehicle composition may change the activity of a drug in the residual vehicle phase and are possibly necessary for adequate, although generally low, percutaneous absorption and efficiency. Most studies on this topic report on the changes in

solvent concentration after application, often investigated with volatile solvent mixtures. Here, the flux of a given drug will change as the vehicle evaporates and is expected to be proportional to both the concentration of the drug in the vehicle and its activity until saturation is achieved. The drug may supersaturate and flux levels above those set by a saturated solution may be obtained. Nevertheless, when drug concentration is pushed up to a level where prompt crystallization is unavoidable, the drug delivery can be markedly depressed relative to a supersaturated system even tough the concentration is high [96].

Coldman et al. studied the enhancement of percutaneous absorption by the use of suitable volatile:non-volatile systems as vehicles and observed an increase in permeation rate due to the evaporation of the volatile component that lead to an increase in the solute concentration and, thus, a higher thermodynamic activity. A marked depression of drug delivery relative to the supersaturated state, attributed to occurring precipitation, has been observed by Chiang et al. who examined permeation of minoxidil form water/ethanol/propylene glycol mixtures as volatile vehicle systems [8, 96]. An interesting publication reports on a drug delivery device where the evaporation of ethanol from an ethanol-water mixture increased the vehicle-skin partition coefficient of the active substance, compensating for the loss of drug due to skin permeation. The consequence is a near zero order flux over the entire application time and an absence of a large excess of drug in the donor reservoir [97].

To summarize, the evaporative concentration effect may even force the drug out of the solution and superimpose a dissolution dependency in the delivery rate. Other investigations focus more detailed on possible changes dermatological formulations may undergo following application, but without correlating the observation with drug delivery [98-100]. The general, but valuable conclusion of these studies is that respective phase diagrams may reflect the arising structure during evaporation. The dependence of drug delivery on alterations dermatological formulations and colloidal structures undergo due to evaporation of volatile components, however, does not appear to have been considered to any great extent in the literature.

Müller-Goymann and Alberg related evaporative changes of water containing hydrophilic ointment that was modified by the incorporation of ethanol with permeation of hydrocortisone-21-acetate in vitro [11]. They reported on a reduced loss of volatile components after the incorporation of ethanol, likely due to fixation of the alcohol in the microstructure of the ointment. The liberation kinetics across an artificial membrane could be related to the arising drug concentration, but permeation kinetics, however, was equal from both formulations. This was accredited to penetration enhancing effects of the ethanol. The

effect of percutaneous absorption of hydrophilic model drugs on the emulsion type after non-occlusive infinite dose application is presented by Ferreira et al. [101]. Hummel and Imanidis demonstrated that obviously varying in-vitro skin permeation of ibuprofen from several non-occlusively applied multi-phasic dermatological formulations was only governed by the continuous phase drug concentration of the vehicles, independently of existing dispersed structures. The back diffusion of the lipophilic model drug into the dispersed phases compensated for the influence of the rising overall drug concentration due to evaporation of volatile components, so that linear drug flux with time was observed [102]. Nalenz and Imanidis studied sodium nicotinate permeation out of multi-phasic o/w-formulations that were applied non-occlusively taking especially into consideration alterations these formulations may undergo due to evaporation of volatile components. During evaporation of volatile vehicle compounds, several phase transitions were detected, such as vesicle to microemulsions, phase inversion from o/w to w/o and drug precipitation. Independently of structural changes, continuous phase drug concentration could quantitatively describe permeation kinetics without the need to consider formulation effect on skin barrier function [3].

3.3.3.3 Drug-Skin Interactions

In order to achieve a satisfying therapeutic effect, drugs have to reach their destination in sufficient amounts. The permeation and, consequently, the pharmacological action may be affected by skin metabolism, vascular and hydration effects. Especially highly lipophilic compounds are often adsorbed within the skin with the result of a decreased and delayed drug flux. The keratin-drug-binding owns a crucial importance in this point. The formation of reservoirs within the skin is mainly based on van-der-Waals forces and H-bonds.

The stratum corneum reservoir is defined by three independent variables: (a) the diffusivity of the drug in the stratum corneum, (b) the amount of drug in the stratum corneum and (c) clearance of the drug from the epidermis. The diffusivity of solutes in the stratum corneum determines the time to reach steady state or to desorb from the stratum corneum. The amount of drug in the stratum corneum is defined by the affinity of the drug for the stratum corneum. Partitioning occurs into both the lipid and protein domains of the stratum corneum. Removal of solutes from the stratum corneum depends on clearance into the viable epidermis and hence into the dermis. Whilst clearance is normally assumed not to be rate limiting, a sufficiently low clearance may lead to a reduced flux through the stratum corneum and an increased amount retained in the stratum corneum. Steroids are famous for their so-

called reservoir effect, but it also occurs with other substances like nicotine, caffeine or cationic β-blocking agents [103].

Al-Saidan revealed a self-permeation enhancement of ibuprofen, possibly acting as an ionic surfactant that disrupts stratum corneum barrier. Furthermore, it is possible that some drugs which quickly penetrate into the skin to yield tissue concentrations high enough to exert an osmotic effect may increase hydration [13, 103, 104].

3.3.3.4 Drug-Vehicle Interactions

In topical and transdermal formulations, selection of a suitable vehicle is crucial as it can affect both drug release and percutaneous absorption. Factors that contribute to the selection are: (i) solubility of the drug in the vehicle, (ii) release of the drug from the vehicle into the skin and (iii) enhancement of drug penetration through the stratum corneum [105]. Regarding the vehicle, it is the interaction between the vehicle components and the drug that finally determine in as much topical delivery of the formulation is efficient or not. Vehicles can comprise different structures like micelles or liquid crystalline stages like hexagonal phase or lamellar phase. Depending on where the drug is located and how strong binding forces are, vehicle structure will have a strong influence on its liberation [106]. Furthermore, liberation extent and velocity are dependent on partition coefficient, solubility of the drug in the vehicle and concentration of the active ingredient (see 3.3.1). In general, it can be assumed that polar substances are more easily liberated from non-polar vehicles and vice versa [107]. Vehicle-drug interactions also include thermodynamic activity of the drug in the vehicle which is related to its concentration in the vehicle and its activity (see 3.3.3.6.2) [1]. The maximum drug transfer into the skin takes place when the vehicle is saturated with the drug at the vehicle-skin interface. In this situation, the thermodynamic activity is 1. In some cases, however, thermodynamic activity may be greater than unity, for example in a supersaturated state due to vehicle evaporation (see 3.3.3.2). Hence, when the system deviates from ideality, the drug concentration must be replaced by activity α_D:

$$\alpha_D = \gamma_D \cdot C_D$$

where C_D denotes the donor concentration and γ_D is the activity coefficient [1]. Schwarb et al. reported on a supra-proportionally increased flux of fluocinonide through a silicon membrane with increasing drug concentration, likely due to increasing thermodynamic activity, while in

vivo skin penetration of the drug was only increased by the factor of which the magnitude of concentration in the respective formulation was increased [7].

A further drug-vehicle interaction that may affect drug permeation is the formation of ion pairs of charged drugs with counter ions present in the vehicle. For instance, Valenta et al. found that fluxes of charged lignocaine salts were significantly increased in the presence of organic counter ions, while inorganic salts did not influence the permeation kinetic [108, 109]. Several publications that deal with cationic substances and fatty acids as counter ions support this ion pair approach as a possible enhancing effect of fatty acids [110, 111]. In some cases drug permeation through the skin is not governed by the poor permeability through the stratum corneum. For example, when the horny layer is damaged or drug diffusion within the vehicle is exceptionally slow, the release rate of the drug from the vehicle provides the rate-limiting step in overall diffusion and the skin functions as a perfect sink [13].

3.3.3.5 Vehicle-Skin Interactions

In general, skin surface is equilibrated with the surrounding environment and application of a dermatological formulation can perturb this balance and consequently affect skin barrier function. For example, lanolin, isopropyl myristate, carbon hydrates with long chain lengths and w/o-formulations are famous for their hydrating effects, but also dehydration and temperature changes may occur, dependent on the formulation selected. Concerning the lipids of stratum corneum, they may be extracted, fluidized or stabilized and solubility properties of the skin may be changed. It may be hypothesized that if the solubility parameter of the vehicle alters that of the skin so that it is closer to the solubility parameter of the drug, permeation may be enhanced [106, 112]. Gaps in the desquamating layers of the horny layer allow the vehicle components to enter until a depth of 2/3 to 3/4. Besides, the vehicle may undergo profound changes after application due to evaporation and admixing with the lipid surface layer of the skin [77].

Dias et al. studied the transdermal permeation of caffeine out of several saturated vehicles (same thermodynamic activity), but different solubility parameters, different lipophilicities and varying saturation concentrations. Mineral oil and isopropyl myristate among others enhanced permeation by modulation of the barrier properties. It was concluded that the vehicles permeate into the skin lipids and alter the solubility properties in a favourable way. The degree to which the vehicles can achieve this depends on their uptake into the skin and their solubility of caffeine in the modified environment. The study indicated that although vehicles promote changes in partitioning and diffusion, it is likely to be the partitioning that is

the dominant factor. Drugs with a log P of about 2 have a higher permeation rate due to their lipophilicity [112].

Jaeckle et al. studied the permeation of the model drug ketoprofen through heat-separated human epidermis and artificial membranes with and without pre-treatment of ointment bases to test the influence of those dermatological ointments. It was possible to demonstrate an easy and cheap methodology to search for even relatively weak vehicle effects to influence drug permeation [113]. Tape stripping technique and in vitro skin permeation experiments are useful for comparing the percutaneous absorption kinetics of different formulations. The permeation parameters diffusivity inside a vehicle and partitioning from formulation to skin are easily altered by the composition of the formulation and are thus important factors affecting permeation kinetics. Diffusivity within the vehicle can be determined from in vitro release studies, whereas partitioning from a formulation to the skin can by measured by the tissue distribution of the drug (see 3.3.4.4). However, little is known about the relationship between these two parameters in practical study because mostly skin permeation of the drug has been analyzed assuming a well-stirred condition in the formulation.

To summarize, the cause of skin permeation alteration from formulation has to be divided into diffusivity and partitioning in a formulation, diffusivity and partitioning in the skin and it has to be clarified which parameter has more effect on skin permeation – as presented in the work of Yamaguchi et al. [9].

3.3.3.6 Permeation Enhancer

3.3.3.6.1 Definition and Mode of Action

Methods for improving cutaneous delivery rely either on the use of chemical penetration enhancers, novel vehicle systems (e.g. microemulsions), liposome-based delivery systems, supersaturated formulations or more complex physical enhancement strategies such as iontophoresis, sonophoresis and electroporation. Skin penetration enhancers are molecules which reversibly remove the barrier resistance of the stratum corneum and, thus, allow drugs to penetrate more readily to the viable tissues and systemic circulation [114, 115]. Potential substances used need to fulfil several requirements [116].

Table 4. Requirements of an ideal enhancer [116]

Ideal Enhancer
• Pharmacological and chemical inertness • Chemical stability • High degree of efficiency with specific activity and reproducibility • High compatibility with formulation and system ingredients • Possess no allergizing, sensitizing, phototoxic potential • Possess solubility parameters of the skin • Modification of barrier function in a reversible manner • Possess no odour, taste or colour: Acceptable for cosmetical purposes

Generally, based of Fick's first law of diffusion, the permeation enhancement strategies may be delineated: (i) increase of the diffusion coefficient, (ii) increase of the solubility of the drug in the stratum corneum, i.e. increase of the drug partition coefficient into the skin and, finally, (iii) increase of the drug concentration gradient vehicle-skin, that mainly acts as driving force for permeation, i.e. increase of the drug's concentration inside the vehicle up to saturation or above (supersaturation approach) [115].

Barry tried to classify accelerant action by rationalizing various mechanisms responsible in the so-called lipid-protein-partitioning theory: Enhancers may interact with intercellular lipids, they may interact with intracellular protein (mainly keratin) and they may penetrate in high amounts as so-called co-solvents into stratum corneum with a resulting improved dissolving capacity of the barrier for the drugs [27, 117, 118].

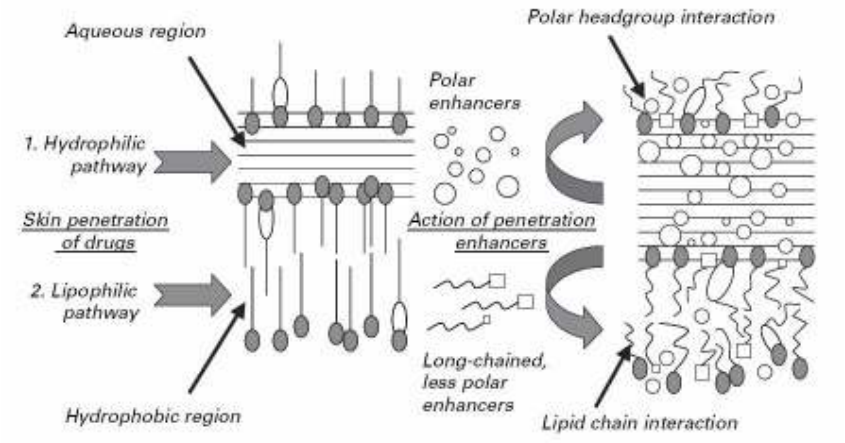


Figure 12. Mode of action of permeation enhancers [84]

Drug diffusion coefficient can be increased by disordering the stratum corneum lipids. It is assumed that accelerant molecules penetrate into and mix homogeneously with the lipids of the bilayers. Enhancers interact either with the polar head groups of the lipids (hydrophilic pathway) or with the long carbon chains (lipophilic pathway). Interaction with polar head

groups of the lipids modifies hydrogen-bonding and ionic forces, disturbs hydration spheres and subsequently leads to an upset of the packing at the polar plane. The domain becomes more fluid and starts to promote diffusion, mainly of polar penetrants. More aqueous fluid may enter the tissue and increase the water volume between the lipid layers. An important secondary feature is that disruption of interfacial structure will also alter packing of lipid chains. The lipid hydrophobic route, thus, becomes more disordered and more readily traversed by lipid-like penetrants. Also, it is possible that accelerants insert themselves between the hydrophobic tails of the bilayers, upsetting their packing and, thus, increasing their fluidity, permitting easier diffusion. Through rotation, vibration and translocation micro cavities can be formed and free volume available for drug diffusion can again be increased. Fatty acids, azone, DMSO, oleic acid and terpenes are good examples for substances acting this way [27, 84, 115, 117].

Many solvents enter the stratum corneum, change its solution properties by altering the chemical environment and, thus, increase partitioning of a second molecule into the horny layer, i.e. increase of drug partitioning. Ethanol and propylene glycol are solvents that are known for this action, but, nevertheless, partition promotion is always specific for certain kinds of drugs. In theory, nonsolvent enhancers that mainly act by interaction with the lipids in the bilayer should also increase the partition coefficient for drugs. That is, by disordering the lipid interfacial domain they increase free volume and make a larger fraction of bilayer available for solute partitioning [27].

The drug concentration gradient vehicle-skin may be increased by increasing the degree of saturation inside the vehicle. This leads to an enhanced thermodynamic activity and therefore to an increased skin permeation. It is the drug's thermodynamic activity that is more important than its absolute concentration. A saturated solution implies a thermodynamic activity of unity. An increase in the degree of saturation to greater than one can be achieved via supersaturation. However, such formulations are thermodynamically unstable and drug crystallization occurs over time [115].

Additionally, the stratum corneum can be made more permeable for drug substances by the extraction of its lipids as the result of an interaction with chemical penetration enhancers. Polar penetration enhancers can also interact with intracellular keratin, leading to changes in the protein conformation, mainly opening up the dense structure and making it more permeable. However, intracellular route is usually not important in drug permeation and that is why this feature usually stays neglected in literature [27, 84].

Panchagnula et al. tried to increase transdermal permeation of highly lipophilic paclitaxel (MW>500) by modifying stratum corneum with terpenes and fatty acids, but although bilayer was disrupted, permeation was not facilitated and this suggest that permeation of high-molecular-weight can not be enhanced through bilayer and partitioning alteration [119].

3.3.3.6.2 Supersaturation

As already mentioned above (3.3.3.4) supersaturation is a simple approach to increase drug permeation without alteration of stratum corneum structure. Higher drug concentrations in the vehicle lead to an increase in the concentration gradient of the Fick's law and, thus, force the active principle out of the formulation and into or across the stratum corneum. Nevertheless, gaining a thermodynamic activity greater than 1 is always accompanied with thermodynamic unstable formulations and drug crystallization. The stability of supersaturated formulations can sometimes be prolonged by the addition of certain polymers but, even then, these systems are not stable enough for long-time storage. Supersaturation can be achieved by different methods directly before of during application of the formulation: (i) by water uptake from the skin, (ii) through evaporation of a volatile formulation components during application, (iii) by using the method of mixed cosolvent systems wherein vehicle changes are produced immediately prior to administration of the formulation and (iv) heating and subsequent cooling. Theoretically, supersaturation offers an advantage over "traditional" enhancers in which the enhancement is specific to the compound of interest: There is no breakdown of the barrier function and the absorption of other compounds (e.g. excipients) is not enhanced [7, 86, 115].

3.3.3.6.3 Water as Penetration Enhancer

The simplest means of penetration enhancement involves influencing the barrier function of the skin by increasing hydration of the stratum corneum. Retention of water in the horny layer leads to a loosening of its compact structure and, thus, makes it more permeable. The water content of the horny layer can either be increased by release of water from the vehicle or by (partial) occlusion that blocks transepidermal water loss [36, 86, 117].

Table 5. Effect of carrier system on the stratum corneum water content and the penetration of active ingredients [120]

Vehicle	Example / Constituents	Effects on skin hydration	Effect on skin permeability
Occlusive dressings	plastic film, unperforated waterproof patch	Prevent water loss, full hydration	Marked increase
Lipophilic vehicles	paraffins, oils, fats, waxes, fatty acids, fatty alcohols, esters, silicones	Prevent water loss, may produce full hydration	Marked increase
Absorption bases	unhydrous lipids plus w/o emulsifiers	Prevent water loss, marked hydration	Marked increase
Absorption bases	Unhydrous lipids plus o/w emulsifiers	Prevent water loss, marked hydration	Marked increase
W/O systems	W/O creams, W/O emulsions	Retard water loss, raised hydration	Increase
O/W systems	O/W creams, O/W emulsions	Can donate water, slight hydration increase	Slight increase
Humectants	Water-soluble vehicles, glycerol, glycols	Can withdraw water, decreased hydration	Possible decrease or at as chemical enhancer
Powder	Clays, shake lotions	As water evaporation, decreased excess hydration	Negligible effect on stratum corneum

Water as well as propylene glycol is thought to hydrogen bond to the polar head groups of the lipids in the stratum corneum. This leads to an enlargement of the distance between the lipid molecules in combination with a lateral extension of the alkyl chains. By hydration of the stratum corneum, the penetration of most of the drugs can be increased. Normally, the stratum corneum water content is 5-10%, but it can be in raised up to 50% under occlusive conditions. Furthermore, moisturizers such as urea can be used to increase the hydration of the stratum corneum and in consequence improve the diffusion of hydrophilic drugs [84, 93].

3.3.3.6.4 Chemical Enhancers

Several excipients are able to promote the transport of an active substance across the skin barrier by a variety of mechanisms (see 3.3.6.3.1). Table 6 briefly summarizes the most commonly used enhancers and their mode of action [84].

In general, permeation enhancers act by more than one mechanism and combing different enhancers can lead to synergistic effects. Nevertheless, one has to be aware that with an increasing potential to enhance permeation, also the risk for skin irritation is raised.

In this section, special emphasis should be given to isopropyl myristate as permeation enhancers as it was one of the lipophilic bases selected for the w/o-emulsions examined in this work.

Isopropyl myristate belongs to the group of esters that may influence drug penetration by various mechanisms. Brinkmann et al. were able to show that isopropyl myristate incorporates within the densely packed bilayer lipids of stratum corneum leading to a loss of order of the corneocyte-bonded lipids and, thus, an increased fluidity with reduced diffusional resistances. The influence of isopropyl myristate on intercellular lipids can be demonstrated by several methods such as DSC, x-ray diffraction, SAXD and WAXD. While SAXD measurements show a slight decrease in intensity of the bilayer packing in the presence of isopropyl myristate, WAXD of isopropyl myristate pre-treated stratum corneum demonstrates a complete loss of order of the corneocyte-bonded lipid fraction and a decrease in the lateral packing distance of the hexagonally arranged lipid fraction. From these findings, an isopropyl myristate insertion into stratum corneum lipids has to be concluded, affecting both lipid fractions mentioned above. Isopropyl myristate insertion within the hydrocarbon chains results in a more densely packed arrangement and therefore in a higher permeation barrier. Obviously this strengthening of the stratum corneum barrier function counteracts the loss of order of the corneocyte-bonded lipid fraction [121-123].

Table 6. Chemical enhancers and their mode of action [84]

Enhancer	Mode of Action
Ethanol and higher alcohols	• Increases solubility of the drug in the vehicle • Increases partition coefficient into the skin • Extract lipids and proteins, thereby increase porosity of stratum corneum • Disrupts bilayers structure of intercellular lipids • Displaces bound water at the lipid head-groups
Sulphoxides, e.g. DMSO	• Creates solvent-filled spaces in stratum corneum due to outstanding dissolving properties • Disturbs bilayers structure of stratum corneum (concentration above 60% necessary) • Denaturises intercellular structural proteins • Changes intercellular keratin confirmation • High irritating potential • Extracts stratum corneum lipids
Azone and derivatives	• Low irritating potential • Low toxicity • No pharmacological activity • Mechanism under ongoing research • Intercalation into structured lipids of the stratum corneum • Disturbs lipid backing order of intercellular lipids • Increases fluidity of hydrophobic stratum corneum regions
Pyrrolidones	• Enhance hydrophilic and lipophilic drugs • Adverse effects like erythema and irritancy
Urea and derivatives	• Hydrating agent • Keratolytic properties • Moisturizing effects inducing hydrophilic diffusion channels
Alkyl-N,N-disubstituted aminoacetates	• Interaction with stratum corneum keratin • Increases hydration
Propylene glycol	• Penetrates into skin and thereby drags drugs • Increases partition coefficient of the drug into the stratum corneum
Surfactants	• Potential to solubilize stratum corneum lipids • Interactions with keratin • Cationic surfactants are more effective than anionic and non-ionic compounds
Terpens and terpenoids	• Interact with intercellular lipids • Influences non-polar penetration route • Low toxicity and irritancy
Fatty acids, e.g. oleic acid	• Enhancement strongly dependent on fatty acid structure • Oleic acid forms pools within lipid bilayers of stratum corneum
Esters	• Enhance by affecting diffusion of the drug
Cyclodextrines	• Form inclusion complexes with lipophilic drugs • Increase solubility of the drug • Cannot penetrate skin

3.3.3.6.5 Liposomes and Microemulsions

Liposomes are colloidal particles, typically consisting of phospholipids and cholesterol, with other possible ingredients such as niosomes (non-ionic surfactant), ethosomes (ethanol) and transferosomes (surfactants as “edge activators”). Most reports cite a localizing effect, whereby vesicles accumulate drugs in the stratum corneum or other upper skin layers. Generally, liposomes are not expected to penetrate into viable skin. Traditionally they are thought to confine themselves to the surface or upper layers of the stratum corneum, where they dehydrate and fuse with skin lipids [27, 124].

There has been a lot of controversial discussion and scepticism on the question whether vesicles penetrate the skin. Some authors claim that skin treated with liposomes reveals vesicle fusion, yielding a structural breakdown of the liposomal bilayers and hence, fusion of the phospholipids with the intercellular lipids of the stratum corneum. This can cause ultrastructural changes in the region with the consequence of reduced skin barrier function and permeation enhancement. A crucial factor may be the organization of phospholipids within the vesicle: highly ordered state (gel structure) or liquid crystalline state. Several studies underline the trend that more flexible liquid state liposomes are favoured to penetrate the skin. To summarize how liposomes interact with the skin, it is still not well understood whether they penetrate as entire liposomes or not [125-127].

Microemulsions are modern drug carrier systems for topical delivery that are thermodynamically stable, low viscous, transparent and optical isotropic with a dynamic microstructure that forms spontaneously by combining appropriate amounts of a lipophilic and a hydrophilic ingredients, as well as a surfactant and a co-surfactant. While emulsions consist of roughly spherical droplets of one phase dispersed in another (see 3.2.2), microemulsions may constantly evolve between various structures ranging form droplet like micelles to bicontinuous structures. The “particle” size in microemulsions lies in the range of 10 -200 nm, whereas macroemulsions are at least one order of magnitude bigger (1-20 µm).

Different mechanisms are responsible for the ability of microemulsions to increase the transdermal delivery: Important features are their high drug solubilization capacity which leads to high concentration gradients towards the skin and a microstructure that allows free and fast drug diffusion. Furthermore, the surfactants in the microemulsion may reduce the diffusional barrier of the stratum corneum altering both the lipophilic and the polar pathway by synergistic interaction of vehicle components with the stratum corneum. Due to their ultra-low interfacial tension, an excellent surface contact between the skin and the vehicle is

ensured with the result of a faster penetration and permeation into deeper skin layers and a decreased lag-time, compared to conventional formulations, e.g. emulsions. However, the correlation between microemulsion structure/composition and drug delivery potential is not yet fully elucidated.

It is assumed that the high content of lipophilic and aqueous phase fluctuate continuously, facilitating transition of both lipophilic and hydrophilic drugs from their typically hydrophilic-vehicle to the lipophilic stratum corneum [128-131].

3.3.3.6.6 Stratum Corneum bypassed or removed

Stratum corneum can be bypassed by injection and one development of this approach is a device of 400 microneedles which insert drug just below the barrier. The solid silicon needles (coated with drug) or hollow metal needles (filled with drug solution) penetrate the horny layer without breaking it or stimulating nerves in deeper tissues [27].

As the horny layer usually provides the permeation barrier, one could consider simply removing it. Chemical peels, micro-dermabrasion using a stream of aluminium oxide crystals and dermabrasion using a motor-driven abrasive fraise or cylinder are available. Adhesive tape can remove stratum corneum prior to drug application. Tape stripping techniques is also a frequently used method to determine drug uptake into the skin [27].

3.3.4 In Vitro Permeation Experiments

3.3.4.1 Pig Ear Skin

In search of a suitable animal model, two main options have to be considered: (a) use of an animal phylogenetically as close as possible to man or (b) use of an animal in which the process under investigation is as close as possible to that in man, considering its physiology, biochemistry and that the anatomy is similar. Various anatomical and physiological characteristics indicate that pig is a good model for man, but also dissimilarities do exist like vascularization (rich in man, poor in pig) and in the sebaceous glands. Humans have mostly eccrine sweat glands whereas pigs possess only apocrine glands. Percutaneous absorption in man and most probably in animals also varies depending on the area of the body on which the chemical resides. Most of the permeation studies conducted in pigs were performed on their back, on the ear and few on the flank or abdomen [132].

Porcine skin, however, is a well-accepted and readily available model for human skin; especially excised porcine ears have become increasingly used for the assessment of dermatological formulations in vitro. The structure of porcine tissue, including hair follicles, has been studied qualitatively and quantitatively in comparison with human skin and results obtained underline the suitability of porcine tissues as a model for human skin.

Significant similarities were found for lipid composition, epidermal thickness and permeabilities of the membranes to diverse compounds. Porcine ear skin shows even a good agreement in its histology as studied by Jacobi et al. [133, 134].

In addition, Lopez et al. investigated the composition and structure of pig stratum corneum tissues based on the action of different solubilizing agents and reported a strongly similar lipid composition and structural organisation of pig stratum corneum compared to human stratum corneum, described in 3.1.3 [135].

Table 7. Lipid composition of porcine and human stratum corneum (expressed in weight-%) [14]

Component	Epidermis	Stratum Corneum	
	pig	pig	human
Cholesterol esters	4.1	1.7	10
Triglycerides	1.3	2.8	0.0
Fatty acids	1.5	13.1	9.1
Cholesterol	8.9	26.0	26.9
Ceramide 1	21.8	4.1	3.2
Ceramide 2	2.6	16.7	8.9
Ceramide 3	9.1	6.9	4.9
Ceramide 4	3.5	4.4	6.1
Ceramide 5	2.4	4.5	5.7
Ceramide 6	3.1	7.6	12.3
Glucosylceramides	8.1	1.0	0.0
Phospholipids	17.6	-	-
Cholesterol sulphate		3.9	1.9
Protein-bound lipids	12.8		
Other	0.8	5.7	11.1

Table 8. Comparison of porcine ear skin with human skin [133]

		Pig ear skin	Human skin
Thickness [µm]	Stratum Corneum	21	6 -19
	Viable epidermis	72	70 (shoulder)
			82 (buttock)
	Dermis	1860	1800 - 1900 (back)
Hair density [number / cm^2]		20	14 - 32
Diameter Hair [µm]		82	16 - 18
Hair extension into skin [mm]		1.20	> 3

Schmook et al. compared in vitro percutaneous absorption of human, pig, rat, Graftskin™ LSE™ (living skin equivalent) and Skinethic™ HRE (human reconstructed epidermis) for dermatological drugs of widely varying polarity and concluded in agreement with already published data that pig skin appeared as the most suitable model [136].

In order to predict skin permeability and metabolism of human skin by extrapolating datas derived on animal skin, differences in skin metabolism have to be taken into consideration. Different dermal esterase activities and, subsequently, varying metabolism and transport rates were subject of studies conducted by Ngawhirunpat et al. in various species. Their investigations revealed that there was no significant difference in skin permeation and metabolism between human and pig skin [137].

Sekkat et al. examined stratum corneum barrier function during its progressive removal by adhesive tape-stripping using the techniques of transepidermal water loss (TEWL) and impedance spectroscopy. Comparing the results with in vivo data obtained from human strongly support the validity of the porcine membrane as a good in vitro model [134].

In general, rodent skin is more permeable than human skin. This can lead to an overestimation of permeation relative to that in human. This problem is partly associated with the effect of hydration wherein prolonged (generally 24h and more) exposure of skin to aqueous donor and receiver phases brings about a marked diminution in the barrier properties [138].

3.3.4.2 Franz-type Diffusion Cells

There has been a wide variety of diffusion cells designed for in vitro measurements of skin permeation. Side-by-side diffusion cells (bichambers) can be used to delineate mechanisms of permeation under controlled conditions (e.g. iontophoresis). Vertical cells, like the Franz

diffusion cell, are more versatile because a wide variety of experimental conditions can be tested. The diffusion cell consists of a donor and a receiver compartment separated by a membrane, e.g. skin, that is mounted horizontally. The cell is suitable for measuring absorption from a range of dosage forms, including solutions, suspensions, powders, creams, gels, ointments, aerosols and solids. Furthermore, the delivery vehicle can be applied non-occlusively and in small amounts (finite dose) to simulate clinical situations as close as possible. For hydrophilic compounds, the receptor compartment should be a buffer solution, whereas for lipophilic compounds the use of solubilizing additive is sometimes necessary (e.g. cyclodextrine, bovine serum albumin) [115]. Sink conditions must be assured. The great advantage of performing in vitro permeation experiments is the avoidance of animal experiments and costs, experimental environment can be controlled and thereby individual factors modifying penetration can be elucidated. Nevertheless, only little information on metabolism and distribution after absorption can be gained, shedding as well as physiological and pharmacological responses do not occur. All of this can lead to an overestimation of total absorption [15, 138, 139].

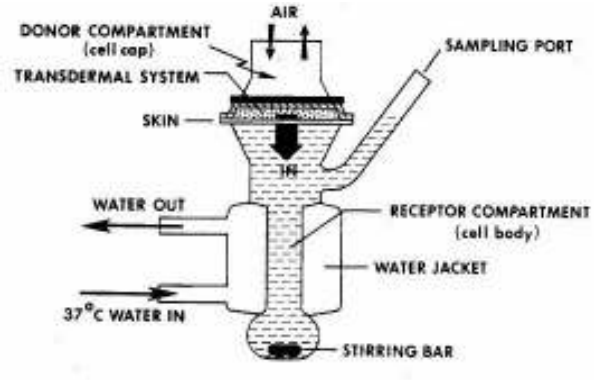


Figure 13. Franz Diffusion Cell [140]

3.3.4.3 Assessment of Skin Barrier Integrity

In the course of performing in vitro percutaneous absorption studies, it is important to ensure the integrity of the stratum corneum. Based on OECD guidance document 28 three different methods are recommended for integrity testing: (a) measurement of transepidermal water loss (TEWL), (b) measurement of electrical resistance or (c) the use of tritiated water as permeation marker [141-143]. The principal of the TEWL-measurement is based upon an open chamber system with two humidity and two temperature sensors, placed at 2 and 4 mm

distance to the skin surface. This setup allows estimation of the evaporation gradient on the skin surface.

The physical basis for this non-invasive and rapid alternative to tritiated water permeation is the first diffusion law discovered by Adolf Fick in 1855:

$$\frac{dm}{dt} = -DA\frac{ds}{dl}$$

where dm/dt denotes the diffusion stream across the open chamber, expressed as transported mass of water per time. A is the exposed area and ds/dl the change of density over the entire distance l. D is the diffusion coefficient of water vapour in the air.

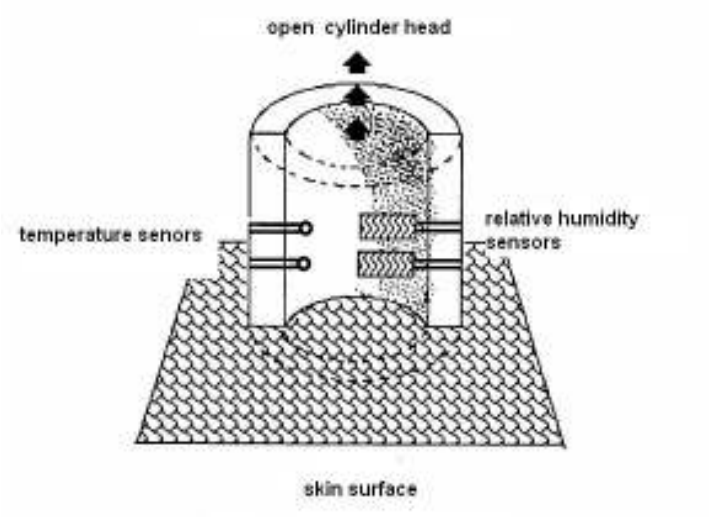


Figure 14. Sensor head for measurement of TEWL

In general, TEWL values vary widely, dependent on the body site and the environmental conditions. Nevertheless, disturbance of the integrity of the skin barrier, leads to drastically increase in the loss of endogenous water [144]. TEWL measurements allow only the detection of severe damage of the stratum corneum, but not of small changes that already may influence drug diffusion as demonstrated by Netzlaff et al. [145].

3.3.4.4 Skin-Vehicle Distribution Coefficient

The outer layer of the skin has been recognized for some time as the rate controlling membrane that acts predominantly as a lipophilic barrier and, thus, partitioning into the stratum corneum is a dominant physico-chemical determinant in controlling absorption [146].

The partition coefficient for compounds between stratum corneum and water K_{SC} can be estimated based on the drug octanol-water partition coefficient K_{oct}:

$$\log K_{SC} = -0.024 + 0.59 \log K_{oct}$$ according to Pugh et al. [147]

$$\log K_{SC} = 0.104 + 0.514 \log K_{oct}$$ according to Abraham et al. [148, 149].

If diffusion is straight through the cells, the pathlength is approximately 15µm, for transfer through the intercellular channels, the pathlength has been estimated to be between 350 and 880 µm. It is possible to calculate K_{SC} and D using various values for the pathlength. For a pathlength of 15 µm, for example, K_{SC} for caffeine is assumed to be 1.79, for a pathlength of 500 µm (tortuous pathway) K_{SC} of caffeine is assumed to be 0.05 [150, 151].

One problem in the dermal field of research is inter- and intra-individual differences of the lipid composition of the skin that makes it very difficult to compare results obtained form numerous skin donors. In the last years, it was shown that the penetration of drugs into the stratum corneum mostly corresponds with the presence of various components of the lipid composition present in each skin specimen. For instance, Wagner et al. found a direct linear correlation between the stratum corneum/water partition coefficients and the amount of drug (flufenamic acid) penetrated into the stratum corneum [152]. Van der Merwe et al. tested various agricultural and chemicals with regard to their partitioning into and their permeability through stratum corneum. They concluded that, for the compounds tested, partitioning into the stratum corneum was determined by the relative solubility of the solute in the donor solvent and the stratum corneum lipids. However, composition of the lipoidal phase of the stratum corneum can be changed by the diffusion of e.g. components of an ointment base into the stratum corneum. This might have an impact on the drugs distribution coefficient and its solubility in stratum corneum, although they tend to parallel. The skin-vehicle distribution coefficient might be useful to delineate penetration and permeation, but it has to be considered that permeability always reflects the result of successive, complex processes and is not always predictable form stratum corneum partitioning alone [152, 153].

Several techniques can be used to determine the drug amount penetrated into stratum corneum and deeper skin layers and, thus, stratum corneum/drug-in-solution partition coefficient can be computed. Typically, isolated, accurately weighted stratum corneum pieces are incubated with the specific substance dissolved in the vehicle over a validated time (equilibration). Afterwards remnants in the vehicle and/or drug amount in the stratum corneum piece are quantified. Drug amount in stratum corneum can be received by tape-

stripping technique and the subsequent quantification of each tape or by the extraction of cleaned, but whole stratum corneum piece with a suitable solvent (e.g. hexane). Furthermore, radiolabelled drugs can be used as the facilitate quantification, both in solution and skin sheets later on. A common method to isolate stratum corneum is based upon the treatment of excised skin with trypsin solution for several hours. Then, the stratum corneum may be carefully peeled off from the underlying epidermal cells and, after treatment with trypsin inhibitor solution in order to prevent further degradation, dried and stored in a desiccator over silica gel. For deeper skin layers, e.g. for the determination of stratum corneum/dermis partition coefficient cryo-sections can be used [152, 154, 155]

PART II

4 Transdermal Drug Skin Permeation with Lipophilic Vehicle: The Continuous Phase Drug Concentration Concept

Abstract

The purpose of this study was to investigate the influence of phase fraction, oil base and drug partitioning of occlusively applied w/o-emulsions on transdermal drug permeation for a hydrophilic and a lipophilic model drug (caffeine and ibuprofen) across pig ear skin in vitro. A concept for the interpretation of drug permeation is proposed that considers continuous phase drug concentration as the driving force for transdermal permeation. Drug distribution within the formulation and drug distribution between stratum corneum and continuous oil phase are determined in order to gain a full understanding of the examined absorption processes.

The studied w/o-emulsions consisted of an oil phase into which water phase was dispersed in phase fractions of 70%, 50% and 30%, respectively (E70, E50, E30). Oil phase consisted of a single oil component (isopropyl myristate, miglyol 812N or paraffinum liquidum) and the polymeric emulsifier Isolan PDI. Water phase contained sodium chloride and was buffered to pH 4.5 in all emulsions containing ibuprofen. Pure oil with emulsifier was selected as reference formulation. Transport experiments were performed in Franz-type diffusion cells across pig ear skin with an infinite dose of 0.7 g/cm^2.

Dependence of apparent permeability coefficient P_{app} on fraction of drug concentration in the continuous phase was analyzed with a model taking into account the permeability coefficient of the skin P_m and the permeability coefficient of the diffusion boundary layer P_{dbl}. P_{dbl} reflects the diffusivity of the drug in the vehicle. By fitting this model to the experimental data using non-linear regression, parameter values for P_m and P_{dbl} were deduced. P_m values were consistent with the drug partitioning between stratum corneum and continuous oil phase. For isopropyl myristate a permeation enhancement was found in agreement with literature. P_{dbl} values were compared with calculated values using a literature model for diffusion in heterogeneous matrix systems. These were found in most cases to be in fairly good agreement with the P_{dbl} values.

The proposed concept can be used to explain experimentally measured apparent permeability coefficient P_{app} for lipophilic vehicles. Applying this concept to w/o-emulsions

comprising varying mass fractions provides a predictive tool to delineate the effect of physicochemical formulations parameters and for transdermal drug delivery rate.

4.1 Introduction

In clinical practice, a drug is rarely applied to the skin in form of a pure chemical, but instead, is incorporated into a carrier system, the vehicle, to guarantee efficient topical or systemic therapy. This carrier system, however, has to fulfil a host of requirements like suitable applicability, compatibility, adequate stability and foremost efficacy that comprises duration and strength of the desired pharmacological action. Thus, development and optimization of these dermatological formulations is a challenging task [1].

Common vehicles usually comprise several components that are often not mutually miscible, thus, separate phases are formed that, on the microscopic level, are intermixed with each other. From the macroscopic point of view a homogeneous system is apparent that disguises different microstructures arising inside the formulation [2, 3].

It is widely acknowledged that transdermal permeation is regulated by the formulation of the drug product. This regulation may take place not only based on physico-chemical principles such as diffusion and partitioning of the active ingredient, but also by an interaction with the absorptive epithelium, i.e. the epidermis, affecting the permeability of the drug. However, a variety of factors combine to complicate a correct interpretation of how drug transport across the skin depends on the delivery formulation. A solid knowledge of the composition, including its physico-chemical properties, dynamic properties (evaporation) and present microstructures, is therefore crucial in terms of achieving optimal topical delivery.

However to date, there is no uniform and comprehensive recommendation or guideline available that takes into consideration the multifaceted complexity present in dermatological formulations in order to quantitatively understand mechanisms being decisive for transdermal absorption processes [1, 6].

The group of Coldman et al. noticed that increasing concentration of a drug within a vehicle due to evaporation processes can strongly affect overall drug permeation. Thus, total drug concentration of a formulation seems to be a crucial parameter for transdermal delivery [8].

Little is known about the relationship between drug diffusivity inside the formulation and drug skin permeation in practical studies, although molecular mobility of a drug within a formulation is reportedly a further aspect that may contribute to drug delivery [131, 156]. The cause of skin permeation alteration from formulation has to be divided into partitioning and diffusivity within the formulation and partitioning and diffusivity within the skin and it has to be clarified which parameter has a larger effect on skin permeation. Yamaguchi et al., for

example, studied the in vitro skin permeation of 22-oxacalcitriol from ointments having different compositions and considered the diffusion coefficients of the drug inside the vehicles. Drug diffusion coefficient within the ointment differed significantly depending on the amount of medium chain triglycerides present [9]. However, permeation parameters such as diffusivity in a formulation and partitioning from formulation to skin are easily altered by the composition of the formulation [10]. For instance, the microstructure of modified water containing hydrophilic ointment DAB 1997 with suspended hydrocortisone-21-acetate on in vitro drug release showed distinct vehicle effects. Both, the colloidal structure of the cream and the presence of specific vehicle compounds, i.e. penetration enhancers, are likely to influence the drug permeation through the skin rather than the total drug concentration in the cream dose [11].

Previous work of our group proposed the continuous phase drug concentration concept as a model to delineate regulation of skin permeation considering drug distribution among distinct phases of multi-phasic hydrophilic formulations. Validity of this concept was confirmed for lipophilic (ibuprofen) and hydrophilic (sodium nicotinate) drugs applied under occlusive and non-occlusive conditions even though vehicles underwent considerable changes in their composition and microstructure due to evaporation of volatile ingredients [2, 3].

The goal of the present work was to implement this continuous phase drug concentration concept to lipophilic vehicle systems under occlusive conditions, i.e. w/o-emulsions of varying phase fraction and oil phases, in order to confirm its validity and, importantly, use it as a tool to delineate the contribution of drug diffusivity within the vehicle and drug permeability through the skin to overall apparent permeability coefficient. The combined efficiency of different parameters influencing the transdermal absorption process was to be clarified.

The studied w/o-emulsions consisted of an oil phase into which water phase was dispersed in phase fractions of 70%, 50% and 30%, respectively (E70, E50, E30). Oil phase consisted of a single oil component (isopropyl myristate, miglyol 812N or paraffinum liquidum) and polymeric emulsifier Isolan PDI. Water phase contained sodium chloride and was buffered to pH 4.5 in all emulsions containing ibuprofen. Pure oil with and without emulsifier was selected as reference formulation. These formulations were designed specifically for elucidating the effect of differing continuous phase drug concentrations, microstructures and oil bases on transdermal delivery. Benzyltrimethylammonium chloride (salt, hydrophilic), caffeine (hydrophilic) and ibuprofen (lipophilic) were applied as model drugs, owning different solubility properties. Permeation experiments were carried out in vitro using pig ear skin as a

model for human skin [134]. A practically infinite amount of formulations was applied in order to determine steady state flux values that facilitate the data evaluation. This was deemed appropriate for reaching the specific goals of this study. Additionally, drug partitioning between distinct phases of transdermal absorption processes were considered. Continuous phase drug concentration was determined experimentally by ultracentrifugation of the corresponding formulations and theoretically by calculation. Gaining deeper understanding of the mode of interaction and the dependence on physicochemical structures of the formulations rounds up the goal of the present work.

In a separate manuscript (Part III), the continuous phase drug concentration concept was applied to w/o-emulsions considering non-occlusive transport conditions. Evaporation pattern and consequently water loss of w/o-emulsions was studied over time and implemented on changes in the continuous phase drug concentration. Microstructure of the formulations was additionally examined by freeze fracture scanning electron microscopy.

4.2 Materials and Methods

4.2.1 Materials

Caffeine was a gift from Boehringer Ingelheim (Ingelheim, Germany) and ibuprofen was obtained from Glatt GmbH (Binzen, Germany). Miglyol 812N® (Migl), medium chain triglycerides, was a gift from Hüls AG (Witten, Germany) and the polymeric emulsifier Isolan PDI (INCI: Diisostearoyl Polyglycerly-3 dimer dilinoleate) was donated from Goldschmidt GmbH (Essen, Germany). Isopropyl myristate (IPM), paraffinum liquidum (Para), sodium chloride, sodium dihydrogenphosphate dihydrate and phosphoric acid 85% were purchased from Haenseler AG (Herisau, Switzerland). Benzyltrimethylammonium chloride (BTA-Cl), sodium azide, sodium acetate, 1-octanesulfonic acid sodium salt monohydrate, puriss.p.a., and trypsin inhibitor from soybean were purchased from Fluka Chemie GmbH (Buchs, Switzerland). Methanol was obtained from Merck KGaA (Darmstadt, Germany). Acetonitrile (HPLC, gradient grade) was ordered from Biosolve (Valkeensward, Netherlands). Trypsin 2.5% was received from LuBioScience (Lucerne, Switzerland). All other chemicals and reagents used in this study were of analytical grade. Bidistilled water was used in all cases.

4.2.2 Methods

4.2.2.1 Manufacturing of the Formulations

The study formulations (for composition see Table 9, 10 and 11) were prepared by first mixing water and oil phase separately. In case of hydrophilic drugs the water phase consisted of the model drug (benzyltrimethylammonium chloride BTA-Cl or caffeine, respectively), sodium chloride and bidistilled water. Oil phase consisted of emulsifier Isolan PDI (Iso) and the selected oil component, isopropyl myristate (IPM), miglyol 812N (Migl) or paraffinum liquidum (Para), respectively. In case of lipophilic model drug the water phase consisted of sodium chloride, bidistilled water and was buffered to pH 4.5 using sodium acetate (buffer capacity $\beta = 0.01$). Oil phase consisted of lipophilic ibuprofen, emulsifier Isolan PDI (Iso) and the selected oil component, isopropyl myristate (IPM), miglyol 812N (Migl) or paraffinum liquidum (Para), respectively. First, all components of the oil phase were mixed and heated to 80-100°C until complete dissolu tion occurred. Second, water phase was added to the hot oil phase gradually with intense homogenization using a polytron PT 3000 (Kinematica AG Littau, Switzerland) for 5 minutes. Before further use, formulations remained at room temperature for 24h to ensure complete partitioning of the model drug between water and oil phase. Different emulsion formulations with varying amounts of dispersed phase of 30, 50 and 70 weight-% were prepared as formulation E (E30, E50 and E70). If

other total concentrations of caffeine were chosen than the concentrations listed in table 10, the amount of caffeine increase/decreased in as much as the corresponding amount of water decrease/increase, but fraction of the complete water phase remained the same within the whole formulation. If other total concentrations of ibuprofen were selected than the ones listed in table 11, the amount of ibuprofen increase/decrease in as much as the corresponding amount of oil decreased/increased, but fraction of the complete oil phase remained the same within the whole formulation. Pure saturated caffeine oil with or without emulsifier Isolan PDI (Iso) was obtained by adding an excess of caffeine to the homogeneous oily base. These solutions were stirred for 24h at 32℃ and filtrated afterwards. The saturated oils were kept at 32℃ to avoid drug precipitation before further use.

All formulations applied in transport experiments were previously tested for stability. After manufacturing the formulations were filled into falcon tubes, closed to avoid any possible water loss and left at room temperature for 24h (equilibration time) and at 32℃ (water bath) for 48h (duration of the permeation experiment). No phase separation was allowed to occur. w/o-character of the formulations was confirmed with conductivity measurements before and after stability testing. Therefore, a conductometer 660 and a conductivity-measuring cell 60323110 with a cell constant of 0.8cm^{-1} (Metrohm AG, Herisau, Switzerland) were used.

Table 9. Composition of the study formulations (in weight-%) for benzyltrimethyammonium chloride BTA-Cl

BENZYLTRIMETHYLAMMONIUM CHLORIDE	E70[1]	E30[1]
Benzyltrimethylammonium chloride[2]	1	0.5
Sodium Chloride	0.5	0.5
Water	68.5	29
Oil (IPM, Migl or Para)	27	65
Isolan PDI	3	5
TOTAL	**100**	**100**

[1] w/o-emulsion with 70% and 30% of dispersed phase, respectively
[2] for paraffinum liquidum only E70 was examined

Table 10. Composition of the study formulations (in weight-%) for caffeine

CAFFEINE	E70[1]	E50[1]	E30[1]	Oil	Oil with Isolan PDI[2]
Caffeine[3,4,5,6]	0.2	0.2	0.2	Saturated	saturated
Sodium Chloride	0.5	0.5	0.5		
Water	69.3	49.3	29.3		
Oil (IPM, Migl or Para)	27	45	65	100	92.86
Isolan PDI	3	5	5	-	7.14
TOTAL	**100**	**100**	**100**	**100**	

[1] w/o-emulsion with 70%, 50% and 30% of dispersed phase, respectively
[2] ratio of Isolan PDI to oil is in the same order of magnitude than the examined emulsions E70, E50 and E30, respectively
[3] for IPM a total drug concentration of 0.08% and 0.25% for E70, 0.08% for E50 and E30 was chosen
[4] for Migl a total drug concentration of 1% and 0.2% for E70, 0,2% for E50 and 0.2% and 0.5% for E30 was chosen
[5] for Para a total drug concentration of 0.2% for E70, E50 and E30 was chosen
[6] for saturation procedure see 4.2.2.1

Table 11. Composition of the study formulation (in weight-%) for ibuprofen

IBUPROFEN	E70[1]	E50[1]	E30[1]	Oil	Oil with Isolan PDI[2]
Sodium Chloride	0.5	0.5	0.5	-	-
Sodium Acetate Buffer pH 4.5	69.5	49.5	29.3	-	-
Ibuprofen[3]	1	1	0.5	1	1
Oil (IPM, Migl or Para)	26	44	64.5	99	91.84
Isolan PDI	3	5	5	-	7.12
TOTAL	**100**	**100**	**100**	**100**	**100**

[1] w/o-emulsion with 70%, 50% and 30% of dispersed phase, respectively
[2] ratio of Isolan PDI to oil is in the same order of magnitude than the examined emulsions E70, E50 and E30, respectively
[3] for paraffinum liquidum also a total concentration of 0.5% and 1% for E50 was chosen

4.2.2.2 Ultracentrifugation and Chemical Analysis of the Formulations

Ultracentrifugation was applied to fractionate intact structures of the formulations as previously demonstrated as a good methodology to investigate multi-phase dermatological formulations by separation of the comprising dispersed phase structures [3]. The study formulations were fractionated using an ultracentrifuge type Centricon T-1075 and a rotor TFT 7013 (Kontron Instruments, Mailand, Italy). Quick-seal centrifuge tubes, 5/8X3 (Beckmann Instruments, Palo Alto, USA) were used. All emulsion formulations were centrifuged for 2 hours at 222000-450000 g. The operation time was optimized to achieve total breaking of the emulsions. Following this treatment, the received fractions were carefully isolated by a syringe or spattle and analyzed for model drug content (caffeine or ibuprofen).

All model drugs were assayed by HPLC (Hewlett Packard, series 1050, Waldborn, Germany). For BTA-Cl a reversed phase RP-18 column (CC125/2 LiChrospher 100-5 RP-18) was used. The mobile phase consisted of 15% acetonitrile and 85% phosphate buffer (pH 3.5; buffer capacity β = 0.05) containing 5 mM 1-octanesulfonic acid sodium salt. For caffeine, a reversed phase RP-8 column was used (CC 125/2 Nucleosil 100-5 C8 ec). The mobile phase consisted of 10% acetonitrile and 90% phosphate buffer (pH 7.4; buffer capacity β = 0.01). For ibuprofen a reversed phase RP-18 column (CC125/2 LiChrospher 100-5 RP-18) was used. The mobile phase consisted of 55% acetonitrile and 45% phosphate buffer (pH 2.1; buffer capacity β = 0.02). Detection was performed UV-spectrophotometrically at 210nm, 275nm and 214 nm for BTA-Cl, caffeine and ibuprofen, respectively. The flow rate was 0.25 ml/min and injection volume was 10 µl in all cases. For the determination of drug content in the formulation or the oil fraction after ultracentrifugation, the samples were prepared by extraction with an at least 100-fold amount of phosphate buffer pH 7.4 (buffer β = 0.05) in the case of caffeine or methanol in the case of ibuprofen. Diluted samples were sonificated for 5 minutes and subsequently centrifuged at 15800 g for 10 minutes. Clear samples were obtained and injected.

4.2.2.3 Determination of Drug Distribution Coefficient Oil / Water

The drug distribution of model drug between oil (IPM, Migl or Para) and water or buffer pH 4.5 (buffer capacity β = 0.01), respectively, was determined by shake-flask method. Briefly, in case of caffeine 1 weight-% of drug was dissolved in water with 0.5% sodium chloride to simulate the water phase of the formulations. This solution was transferred into a separation funnel together with equal amount of selected oil (IPM, Migl or Para). In case of ibuprofen, 1 weigth-% of model drug was dissolved in the selected oil (IPM, Migl or Para) and transferred into a separation funnel together with equal amount of sodium acetate buffer pH 4.5 (buffer capacity β = 0.01). This mixture was extensively shaken for 10 minutes. The two phases were allowed to separate over night before drug content in each phase was quantitatively determined. Therefore, the phases were separated and diluted with an at least 100-fold amount of water or methanol (for conditions see 4.2.2.2). Validity of the procedure was proven by equally determining drug distribution out of saturated caffeine oil into water. Partition coefficient $K_{L/W}$ was calculated as the ratio of drug concentration in the lipophilic oil phase C_L over drug concentration in the hydrophilic phase C_W.

$$K_{L/W} = \frac{C_L(Drug)}{C_W(Drug)} \quad \text{[equation I]}$$

4.2.2.4 Determination of Drug Distribution Coefficient Stratum Corneum / Oil

Stratum corneum sheets were prepared from porcine skin by trypsin treatment. In particular, full-thickness skin samples were cut to a thickness of 200 µm with a pneumatic dermatome (Zimmer, Dover, OH, USA) and then spread, stratum corneum side up, on filter paper soaked with 0.5% crude trypsin in phosphate buffered saline (pH 7.4, buffer capacity β = 0.005) inside a petri dish. After incubation at 37°C for 75 minutes stratum corneum was carefully peeled off from the underlying epidermal cells and washed three times with bidistilled water to prevent further degradation. The isolated stratum corneum sheets were incubated with phosphate buffered trypsin inhibitor solution from soy bean (pH 7.4, buffer capacity β = 0.005) at 37°C for further 120 minutes. The stratum corneum sheets were washed again with bidistilled water three times and then stored on wire mesh as support in a desiccator over silica gel for at least 2 days until mass remained constant, but not longer than two weeks before use [157].

Originally, the idea was to determine drug distribution between (continuous) oil (phase) and dried stratum corneum. Some experiments were performed, but failed as it was not possible to remove adherent oil entirely for stratum corneum pieces without loosing drug substance or destroying the skin sheets. Thus, an indirect method had to be used. Therefore, first drug distribution between stratum corneum and water was determined and corrected for drug partition coefficient between stratum corneum and oil with the help of drug distribution coefficient between oil and water $K_{L/W}$ previously determined by shake-flask method (4.2.2.3):

$$K_{SC/L} = \frac{C_{SC}}{C_W} \cdot \frac{C_W}{C_L} = \frac{C_{SC}}{C_L} \quad \text{[equation II]}$$

Dried stratum corneum pieces (mass between 1.5 and 3 mg) were weighted, transferred into an Eppendorf tube and additionally an exactly determined amount of caffeine in water or ibuprofen in sodium acetate buffered solution (pH 4.5, buffer capacity β = 0.01) was added (1ml). Entire contact of the solution with the area of stratum corneum sheets was obligatory. The Eppendorf cups were incubated at 32°C for 25h until equilibration. Different concentrations of caffeine or ibuprofen solutions, respectively, were examined. The concentration range was 1.1 – 147.9 µg/g for caffeine and 14.56 – 128.19 µg/g (saturated) for ibuprofen. Every single concentration was examined on at least three different stratum corneum sheets. To determine possible adsorption of small amounts of the drug by the Eppendorf tubes themselves, experiments were carried out by incubating caffeine or ibuprofen solutions under the same conditions as in the distribution coefficient measurements, but without stratum corneum (blank solutions). No influence of the test

conditions on drug concentrations could be detected (data not shown). After incubation solutions were removed, centrifuged for 10 minutes at 15800 g (Eppendorf centrifuge 5415C, Dr. Vaudaux AG, Schönenbuch, Germany) and analyzed using HPLC (for conditions see 4.2.2.2). The difference in drug amount present in drug solutions with stratum corneum and drug amount present in solutions of blank cups after 25h of incubation represented the amount of penetrated drug into the stratum corneum. For calculation of drug concentration within stratum corneum a density of 1000 mg/g was assumed. According to Parry, the stratum corneum/water partition coefficient was determined over the slope of regression line which was obtained by plotting the concentration of caffeine in supernatant of the solutions incubated with stratum corneum pieces (mg/g) against caffeine concentration in stratum corneum (mg/g) [82, 152, 154] . 25 hours of incubation time were chosen because equilibrium was established between the drug amount penetrated into the stratum corneum pieces and the drug amount still present in the solutions. The time needed to reach equilibrium was investigated by collecting samples at varying incubation times (data not shown).

4.2.2.5 Determination of Continuous Phase Drug Concentration (Occlusive Conditions)

Continuous phase drug concentration was determined by two different approaches. The experimental approach used ultracentrifugation experiments in order to break the formulation into its two distinct phases. Subsequently, the oil phase was analysed quantitatively for its containing model drug concentration. The theoretical approach applied the formula derived in 4.3.1.3 for calculation of continuous phase drug concentration taking into consideration the drug distribution coefficient between the selected oil component (IPM, Migl or Para) and the corresponding water phase $K_{L/W}$. For a multi-phase formulation with mass fraction of the continuous phase Φ_L and mass fraction of the dispersed phase Φ_W this continuous phase drug concentration C_L is given by:

$$C_L = \frac{C_{tot}}{\phi_W / K_{L/W} + \phi_L} \quad \text{[equation III]}$$

where C_{tot} denotes the total drug concentration of the formulation. Mass concentrations (mg/g) and mass fractions (weight-%/100) were considered [2, 3].

4.2.2.6 Permeation Experiments

In all cases, drug permeation was studied in Franz-type diffusion cells with a diffusion surface area between 2.99 – 4.05 cm^2 at 32°C across excised full thickness pig ear skin . The ears of domestic pigs were obtained from a local slaughter house directly post-mortem. The skin was separated from the cartilage tissue with a scalpel, stored in a freezer at -75°C and used within 4 weeks. To overcome the effect of individual skin variability, every single formulation was examined in at least three permeation experiments. For a single permeation experiment skin of the same pig was used. The receiver medium for the permeation experiments with a volume of 8.5 to 9 ml consisted of an aqueous phosphate buffer solution (pH 7.4, buffer capacity β = 0.05) and 0.1% sodium azide. Skin integrity was tested by measurements of transepidermal water loss (TEWL) (Tewameter TM 210, Courage & Khazaka electronic GmbH, Cologne, Germany) after 4 hours of equilibration. Then, a practically infinite dose of 700 mg/cm^2 formulation was applied onto the skin to ensure that donor compartment would not become poor of model drug over the entire duration of the transport experiment. For occlusive conditions the donor compartment was covered with a rubber stopper. At predetermined time intervals, samples of the receiver compartment were collected and replaced with fresh buffer. The entire duration of an experiment was 48 hours. The samples were analyzed for model drug concentration by HPLC (for conditions see 4.2.2.2) without further treatment except centrifugation for 10 minutes at 15800 g (Eppendorf centrifuge 5415C, Dr. Vaudaux AG, Schönenbuch, Germany).

For caffeine and ibuprofen three different series of permeation experiments were performed in which the applied oil was the discriminating factor. A series consisted of the formulations E70, E50, E30 and pure oil with emulsifier, respectively.

Table 12. Overview on the permeation series performed

Drug	Oil	SERIES
Caffeine	Isopropyl myristate	A
	Miglyol 812N	B
	Paraffinum liquidum	C
Ibuprofen	Isopropyl myristate	D
	Miglyol 812N	E
	Paraffinum liquidum	F

In order to compare the different formulations of one series against each other, the composition of the vehicles was arranged in a way that the ratio of emulsifier content over oil content was in the same order of magnitude, i.e. E70 comprising 3%, E50 and E30

comprising 5% and pure oil comprising 7% of emulsifier. For more detailed discussion, see Part III. Table 12 summarizes the series performed.

4.2.2.7 Data Analysis of the Permeation Experiments

4.2.2.7.1 Theory (P_{app}, P_m, P_{dbl})

For interpretation of permeation data, it is postulated that continuous phase drug concentration of a multi-phasic formulation governs permeation kinetics alone [2, 3]. This concentration is given by equation III (see 4.2.2.5). Assuming the conditions of a perfect sink (receiver concentration negligible), an infinite donor reservoir, assuring a constant continuous phase drug concentration C_L, rate limiting membrane (skin) diffusion with the diffusion coefficient D and drug distribution between the skin and the continuous phase of the formulation with the distribution coefficient $K_{SC/L}$, drug flux J may be described by Fick's first diffusion law. Drug flux J is defined as the amount of drug permeated through the skin per unit time and unit area and is given by:

$$J = P \cdot C_L \qquad \text{with} \qquad P = \frac{D \cdot K_{SC/L}}{h}$$

where P is the permeability coefficient (cm/s) and h as thickness of the diffusion rate limiting membrane.

Combining the formulas leads to equation IV:

$$J = \frac{C_L \cdot D \cdot K_{SC/L}}{h} \quad \text{[equation IV]}$$

In general, apparent permeability coefficient P_{app} is dependent on several processes proceeding in parallel, whereas the slowest is decisive and determinant. Concerning the w/o-emulsions examined, it is mainly the diffusivity of the drug within the vehicle, reflected by the permeability coefficient of the diffusion boundary layer, P_{dbl}, and the permeability through the membrane/skin, reflected by the permeability coefficient P_m, that determine apparent permeability coefficient P_{app}. Hence, for a reasonable discussion of the permeation results, it is obligatory to have an idea about P_{dbl} and P_m. Equation V, derived in 4.3.2.1, delineates the relation among these parameters.

$$\frac{1}{P_{app}} = \frac{1}{P_{dbl}} + \frac{1}{{}^{C_L}\!/_{C_{tot}}\, P_m} \quad \text{[equation V]}$$

4.2.2.7.2 Easy Fit®

Based on the permeation data derived from the transport experiments across pig ear skin, namely apparent permeability coefficient P_{app} and continuous phase drug concentration over total drug concentration C_L / C_{tot}, the corresponding values for permeability coefficient of diffusion boundary layer P_{dbl} and permeability coefficient of the membrane P_m can be computed for every single transport series using non linear regression (EasyFit® software; Prof. Schittkowski, University of Bayreuth).

For analysis the model type EXPLICIT and the numerical method SQP-Gauss-Newtonian were applied. Scaling was -1 in case of caffeine and +1 in case of ibuprofen.

4.3 Results

4.3.1 Characterization of the Formulations

4.3.1.1 Oil / Water Drug Distribution Coefficient [$K_{L/W}$]

Drug distribution coefficients between oil and water phase were determined for caffeine and ibuprofen in order to calculate continuous phase drug concentration of the formulations applied according to equation III (see 4.3.1.3). Table 13 shows the values obtained by shake-flask method (see 4.2.2.3).

Table 13. Drug partition coefficient between oil and water phase $K_{L/W}$

	Caffeine[1]	Ibuprofen[2]
Isopropyl myristate	0.040 ± 0.005	883.40 ± 11.84
Miglyol 812N	0.129 ± 0.006	841.35 ± 44.04
Paraffinum liquidum	0.00265 ± 0.0001	122.65 ± 1.24

All values denote mean ± standard deviation. n = 4-12
[1] water phase consisted of pure water with 0.5% sodium chloride
[2] water phase consisted of a sodium acetate puffer (pH 4.5, buffer capacity β = 0.01)

The values depicted in table 13 clearly demonstrate the hydrophilic nature of caffeine (values below unity; pK_a = 0.6) and the lipophilic nature of ibuprofen (values above unity, pK_a = 5.2) if, in the case of ibuprofen, water phase was buffered to pH 4.5. Furthermore, it is obvious that, for both drugs, the oil components isopropyl myristate and miglyol 812N differed greatly from paraffinum liquidum. This can be attributed to differences in the chemical structure and polarity. Isopropyl myristate and miglyol 812N possess ester bonds, whereas paraffinum liquidum consists only of saturated carbon chains.

4.3.1.2 Stratum Corneum / Oil Drug Distribution Coefficient [$K_{SC/L}$]

In order to gain a deeper understanding of the different drug permeation behaviour as a function of physicochemical properties of the formulation, drug distribution coefficients $K_{SC/L}$ between stratum corneum (SC) and, according to the proposed concept, continuous oil phase of the formulation (L) were determined (see equation II and 4.2.2.4).

Table 14. Drug distribution coefficient $K_{SC/L}$ between stratum corneum SC and continuous oil phase L[1]

	Caffeine[2]	Ibuprofen[3]
Isopropyl myristate	73.75	0.101
Miglyol 812N	22.87	0.106
Paraffinum liquidum	1113.21	0.724

1 all values denote mean. n = 3
[2] $K_{SC/W}$(caffeine) = 2.95 for pure water with 0.5% sodium chloride selected as water phase
[3] $K_{SC/W}$(ibuprofen) = 88.8 for sodium acetate buffer pH 4.5 (buffer capacity β = 0.01) selected as water phase

For both drugs distribution into the stratum corneum was favoured from paraffinum liquidum. For caffeine drug distribution coefficients between stratum corneum and oil differed only 3-fold between isopropyl myristate and miglyol, but almost 50-fold between paraffinum liquidum and miglyol 812N. For ibuprofen drug distribution coefficients $K_{SC/L}$ were almost the same for isopropyl myristate and miglyol, but 7 times smaller than for paraffinum liquidum. Distribution into stratum corneum is favoured for caffeine compared to ibuprofen. This is due to different solubility properties in the lipophilic vehicle and, consequently, varying escaping tendencies into the partly hydrophilic and partly lipophilic stratum corneum. Nevertheless, as determination was performed indirectly (see 4.2.2.4), this method masked the direct effect oil might have on skin such as changes in skin structure or extraction of lipids. Comparison of the distribution coefficient between stratum corneum and water $K_{SC/W}$ for caffeine with values available in literature underlined the accuracy of the performed measurement. $K_{SC/W}$ determined for caffeine was 2.95. Hansen et al. found values between 2.92 and 4.70, depending on the equilibration time selected [158]. Dias et al. studied the topical delivery of caffeine from some commercial formulations and computed values of 0.05 and 1.79 for $K_{SC/W}$ depending on path of diffusion through stratum corneum [151].

4.3.1.3 Calculated and Experimentally Determined Continuous Phase Drug Concentration C_L

Total drug concentration C_{tot} is connected to the drug concentration in continuous phase C_L with phase fraction Φ_L and to the drug concentration in the dispersed phase C_W with phase fraction Φ_W by equation VI:

$$C_{tot} = \phi_W \cdot C_W + \phi_L \cdot C_L \quad \text{[equation VI]}$$

$$\text{with } \phi_W + \phi_L = 1; \quad \phi_W = \frac{V_W}{V_{tot}} \text{ and } \phi_L = \frac{V_L}{V_{tot}}$$

where, V_{tot} denotes total volume of the formulation, V_L volume of the continuous phase and V_W volume of the dispersed phase.

Drug distribution coefficient between oil and water phase $K_{L/W}$ is defined by equation I (see 4.2.2.3):

$$K_{L/W} = \frac{C_L}{C_W} \quad \text{[equation I]}$$

Connecting both equations and solving for C_L leads to the following equation III:

$$C_L = \frac{C_{tot}}{\phi_W / K_{L/W} + \phi_L} \quad \text{[equation III]}$$

Continuous phase drug concentrations for caffeine and ibuprofen were calculated based on equation III and drug partition coefficient $K_{L/W}$ (see 4.3.1.1) for all formulations studied in permeation experiments in order to find a correlation between apparent permeability coefficient P_{app} and continuous phase over total drug concentration C_L/C_{tot}. Besides, calculated values for C_L were compared to the values derived experimentally by ultracentrifugation and subsequent quantification of drug in the oil phase (see figure 15).

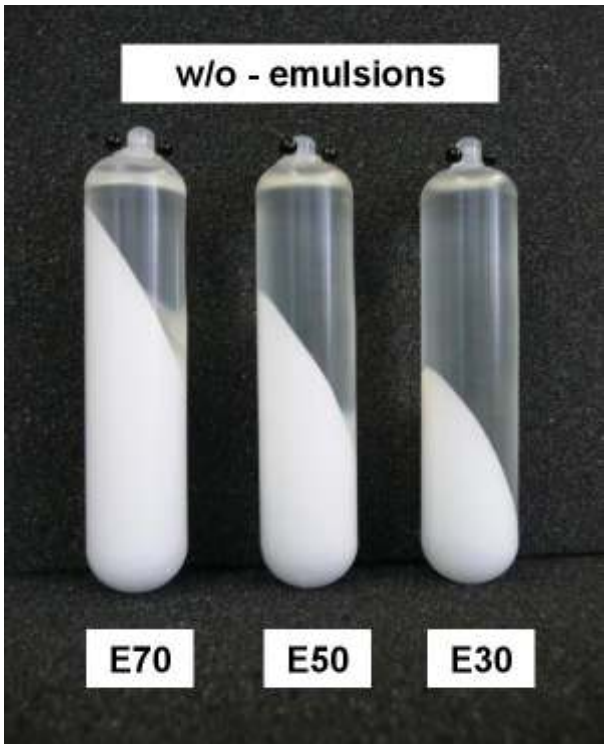


Figure 15. Distinct phases within w/o-emulsions of varying phase fractions after ultracentrifugation (top part: oil fraction, bottom part: complex mixture of water and further emulsion ingredients; E70, E50 and E30 denote emulsion with 70%, 50% and 30% of dispersed water phase)

Data are shown in table 15 for caffeine and in table 16 for ibuprofen.

Table 15. Calculated and measured values of continuous phase drug concentration for caffeine in w/o-emulsions of varying phase fractions (units µg/g)

	E70[1,2]		E50[1,2]		E30[1,2]	
	Calculated	Measured[3]	Calculated	Measured[3]	Calculated	Measured[3]
Isopropyl myristate	44.94	111.78±12.14	61.54	151.01±5.42	97.56	211.45±6.01
Miglyol 812N	349.26	479.98±13.62	457.04	613.88±20.2	661.03	806.32±3.13
Paraffinum liquidum	7.58	20.28±1.95	10.58	29.42±1.60	17.92	34.98±0.27

[1] w/o-emulsion with 70%, 50% or 30% of dispersed phase, respectively.
[2] total drug concentration was 2000 µg/g for all formulations on the basis of miglyol 812N or paraffinum liquidum and 800 µg/g for all formulations on the basis of isopropyl myristate
[3] all values denote mean ± standard deviation. n = 3 – 6

Table 16. Calculated and measured values of continuous phase drug concentration for ibuprofen in w/o-emulsions of varying phase fractions (units mg/g)

	E70[1,2]		E50[1,2]		E30[1,2]	
	Calculated	Measured[3]	Calculated	Measured[3]	Calculated	Measured[3]
Isopropyl myristate	33.25	31.5±1.81	19.98	19.73±0.41	7.14	7.56±0.91
Miglyol 812N	33.24	34.58±1.56	19.98	19.5±0.45	7.14	7.65±0.13
Paraffinum liquidum	32.71	n.m.[4]	19.84	n.m.[4]	14.24[5]	11.95±0.84

[1] w/o-emulsion with 70%, 50% or 30% of dispersed phase, respectively.
[2] for E70 and E50 a total concentration of 10000 µg/g caffeine, for E30 a total concentration of 5000 µg/g was selected
[3] all values denote mean ± standard deviation. n = 3 - 6
[4] not measured.
[5] a total concentration of 10000 µg/g was selected.

Calculated and measured values agreed very well for all formulations and all oils examined in case of ibuprofen. Additionally, the values clearly indicate that the studied emulsions with varying phase fractions (E70, E50 and E30) possessed different continuous phase drug concentrations. Ibuprofen concentrations within an emulsion of defined phase fraction but formulated on the basis of different oils did not show any influence on the resulting drug oil concentration.

However, for caffeine, the agreement between calculated and measured continuous phase drug concentration was not as good as compared to ibuprofen. Best values were achieved for caffeine in E70, E50 and E30 manufactured on the basis of miglyol 812N. In general, the concentrations examined were up to 1500-fold smaller (unit µg/g instead of mg/g). The emulsions E70, E50 and E30 varied in their continuous phase drug concentration for all oils studied. Contrary to the results obtained for ibuprofen, emulsions with the same phase fraction also possessed different continuous phase drug concentrations, depending on the oil selected. Based on the determination of saturation concentration of caffeine in oil with or without Isolan PDI, it can be excluded that the drug distribution and consequently the drug

content was influenced by the presence of any free emulsifier in the oil phase (see Part III, 5.3.1.3).

Generally, total drug concentration and partitioning of caffeine into the continuous phase of the formulation was lower if compared to ibuprofen and, thus, achieved continuous phase drug concentrations were smaller. The solubility of caffeine in oily vehicles was limited; hence, in terms of manufacturing the formulations, it had to be assured that no saturation of the continuous phase occurred. Neither for caffeine, nor for ibuprofen saturation of the continuous oil phase was achieved (for saturation concentration see Part III, 5.3.1.3).

Equation III uses mass fractions of continuous and dispersed phase, Φ_L and Φ_W, respectively, for calculation of continuous phase drug concentration C_L. To test how much continuous phase drug concentration C_L is affected by the emulsifier content, emulsions with varying amounts of Isolan PDI were manufactured and subjected to ultracentrifugation experiments. Measured continuous phase drug concentrations were compared to calculated values, assuming that Isolan PDI was either part of continuous phase or part of dispersed phase. The values obtained are depicted in table 17.

Table 17. Isolan PDI and its influence on continuous phase drug concentration by ultracentrifugation experiments of ibuprofen formulations (units mg/g)

	E30 Miglyol[1]			E70 Miglyol[1]		
Isolan PDI	**Measured[2]**	**Calculated[3]**	**Calculated[4]**	**Measured[2]**	**Calculated[3]**	**Calculated[4]**
3%	15.80±0.18	14.28	14.92	34.07±2.44	33.24	36.92
5%	17.86±2.14	14.28	13.33	38.76±0.94	33.24	39.86
10%	15.32±0.45	14.28	12.50	33.75±1.48	33.24	49.76

[1] a total drug concentration of 10000 µg/g was selected for w/o-emulsion containing 30% and 70% of dispersed phase, respectively
[2] all values denote mean ± standard deviation. n = 3
[3] assumption that continuous phase is formed by the oil component and emulsifier Isolan PDI.
[4] assumption that continuous phase is formed only by the oil component.

The values clearly show that best agreement was achieved if emulsifier is accounted for continuous phase. Furthermore, for E30 and E70, the continuous phase drug concentration did not differ strongly between formulations that were manufactured with 3%, 5% or 10% of emulsifier if calculation is based on the assumption that external phase is composed of oil and emulsifier. This is consistent with determined saturation concentrations of ibuprofen in miglyol 812N with varying amounts of Isolan PDI (see part III, 5.3.1.3) which did not influence saturation concentration to any great extent.

To summarize, the good agreement between calculated and measured values strongly indicates the validity of the equation III derived in 4.3.1.3.

In terms of correlating apparent permeability coefficient P_{app} of the different formulations (see 4.3.2.3 and 4.3.2.4) with the continuous phase drug concentration C_L, calculated values were used (see equation III, 4.2.2.5). As the formulations differed in their total concentrations, the fraction continuous phase drug concentration over total drug concentration C_L/C_{tot} was introduced (see table 18 and 19).

Table 18. Continuous phase caffeine concentration over total concentration C_L/C_{tot} of all formulations used in permeation experiments (total concentration in weight-%)

Vehicle base	Formulation	Total Concentration	C_L/C_{tot}
Isopropyl myristate	E70	0.08%	0.06
	E50	0.08%	0.08
	E30	0.08%	0.12
	Oil + 7% Isolan PDI	Saturated	1.00
Miglyol 812N	E70	0.25%	0.18
	E70	0.2%	0.18
	E50	0.2 %	0.23
	E30	0.2%	0.33
	Oil + 7% Isolan PDI	Saturated	1.00
Paraffinum liquidum	E70	0.2%	$1.43 \cdot 10^{-3}$
	E50	0.2%	$2.00 \cdot 10^{-3}$
	E30	0.2%	$3.33 \cdot 10^{-3}$
	Oil + 7% Isolan PDI	Saturated	1.00

Table 19. Continuous phase ibuprofen concentration over total concentration C_L/C_{tot} for all formulations examined in permeation experiments (total concentration in weight-%)

Vehicle base	Formulation	Total Concentration	C_L/C_{tot}
Isopropyl myristate	E70	1%	3.32
	E50	1%	2.00
	E30	0.5%	1.43
	Oil + 7% Isolan PDI	1%	1.00
Miglyol 812N	E70	1%	3.32
	E50	1%	2.00
	E30	0.5%	1.43
	Oil + 7% Isolan PDI	1%	1.00
Paraffinum liquidum	E70	1%	3.27
	E50	0.5%	1.98
	E30	0.5%	1.42
	Oil + 7% Isolan PDI	1%	1.00

4.3.2 Permeation Experiments

4.3.2.1 Equations of Transport Kinetics

It is postulated that a drug has to diffuse through the diffusion boundary layer of the vehicle, until it reaches the interface vehicle-skin. The relatively fast partitioning of drug from continuous phase into stratum corneum is followed by diffusion through the skin.

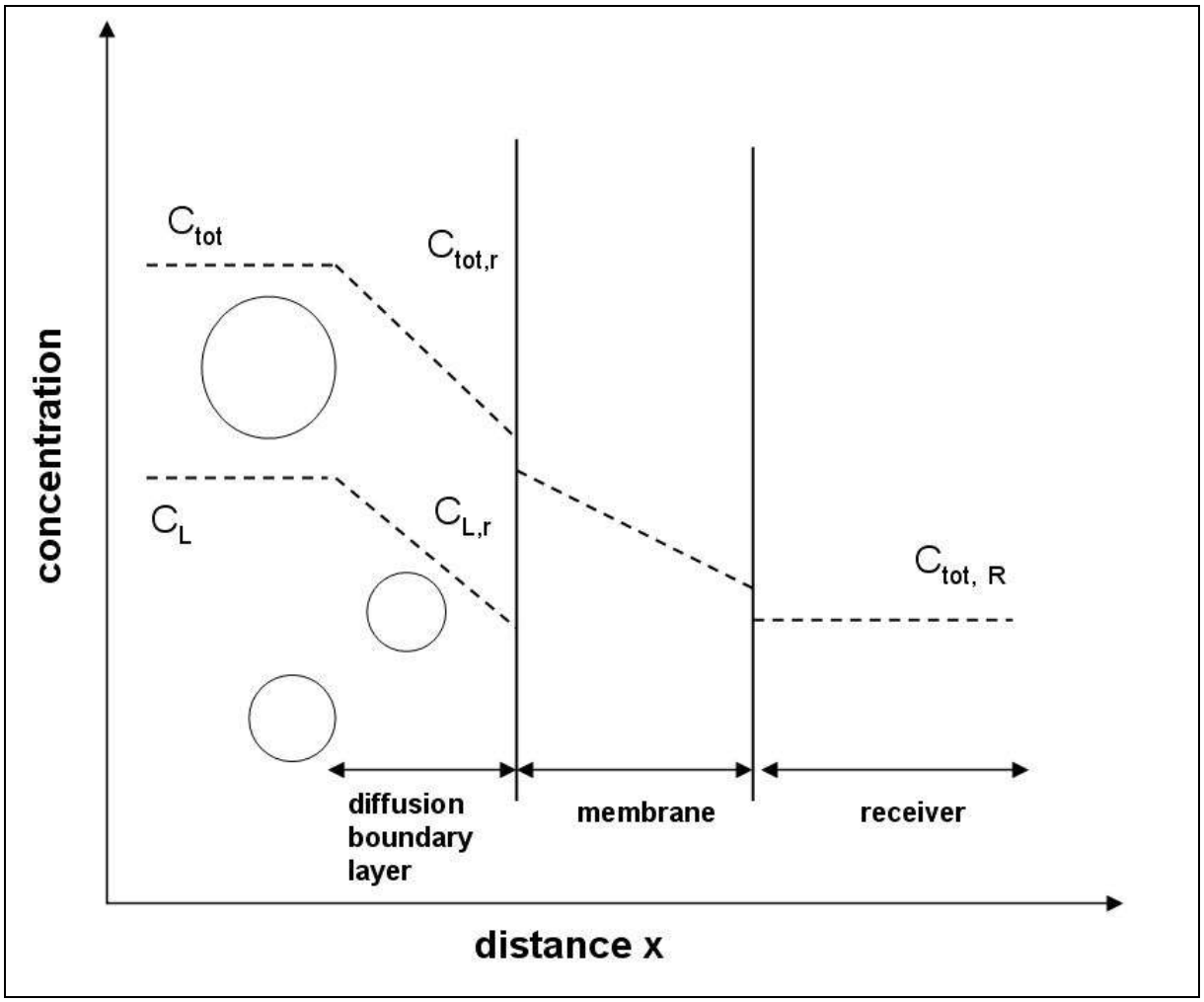


Figure 16. Drug diffusion through a fictitious diffusion boundary layer and skin membrane - adapted from [2]: C_{tot} denotes total drug concentration, C_L denotes continuous phase drug concentration, subscript r denotes concentration directly present on the membrane surface, subscript R denotes concentration present in the receiver compartment

C_{tot} denotes total drug concentration of the formulation, C_L is the continuous phase drug concentration. The subscript r is equivalent to the corresponding concentration directly on the skin membrane surface. The subscript R is equivalent to the corresponding concentration in the receiver phase. For steady-state conditions, it is assumed that the flux through diffusion boundary layer J_{dbl} is equal to the flux through the skin membrane J_m.

$$J_{dbl} = J_m \quad \text{and accordingly}$$

$$P_{dbl}(C_{tot} - C_{tot,r}) = P_m C_{L,r} \quad \text{[equation VII]}$$

where, P_{dbl} denotes permeability coefficient of diffusion boundary layer and P_m denotes permeability coefficient of the skin membrane. P_{dbl} reflects the diffusivity of the drug in the vehicle. $C_{tot,r}$ is the total drug concentration and $C_{L,r}$ the continuous phase drug concentration directly on the surface of the membrane r. $C_{tot,R}$ is assumed to be zero (sink conditions).

If equation VI for the calculation of total drug concentration (see 4.3.1.3) is considered and drug partition coefficient $K_{W/L}$ is introduced as drug concentration in the water phase over drug concentration in the oil phase

$$K_{W/L} = {}^{C_W}\!/_{C_L} \quad \text{[equation VIII]}$$

the total drug concentration $C_{tot,r}$ at the membrane surface r can be replaced by the term

$$C_{tot,r} = C_{L,r}[\phi_W(K_{W/L} - 1) + 1] \quad \text{[equation IX]}$$

leading to equation X

$$P_{dbl}C_{tot} - P_{dbl}(C_{L,r}(\phi_W(K_{W/L} - 1)) + 1) = P_m C_{L,r} \quad \text{[equation X]}$$

Simplifying and dissolving this equation for $C_{L,r}$ gives

$$C_{L,r} = \frac{P_{dbl}C_{tot}}{P_m + P_{dbl}(\phi_W(K_{W/L} - 1) + 1)} \quad \text{[equation XI]}$$

Inserting the term for $C_{L,r}$ into $J = P_m C_{L,r}$ (equation VII) gives

$$J = \frac{P_m P_{dbl} C_{tot}}{P_m + P_{dbl} \cdot C_{L,r}[\phi_W(K_{W/L} - 1) + 1]} \quad \text{[equation XII].}$$

As apparent permeability coefficient is defined as the quotient of flux J over the total drug concentration C_{tot}, this can be applied to equation XII. Replacing $C_{L,r}[\Phi_W(K_{W/L}-1)+1]$ by C_{tot}/C_L (equation IX) gives equation V. This formula shows the correlation between apparent

permeability coefficient P_{app} with the permeability coefficient of the diffusion boundary layer P_{dbl} and the permeability coefficient of the membrane P_m, respectively.

$$\frac{1}{P_{app}} = \frac{1}{P_{dbl}} + \frac{1}{C_L/C_{tot}\, P_m} \quad \text{[equation V]}$$

Depending on the contribution of P_{dbl} and $(C_L/C_{tot})P_m$ to the value of P_{app} three different cases can be distinguished:

a) $P_{dbl} >> C_L/C_{tot}\, P_m$

b) $P_{dbl} << C_L/C_{tot}\, P_m$

c) $P_{dbl} \approx C_L/C_{tot}\, P_m$

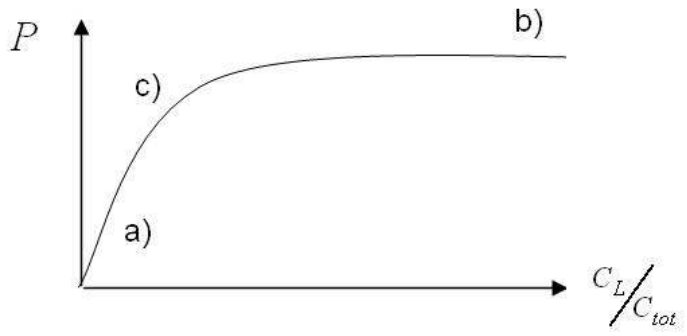


Figure 17. Correlation between apparent permeability P_{app} and continuous phase drug concentration over total drug concentration C_L/C_{tot} in terms of P_{dbl} and P_m contribution

The interplay is demonstrated in Figure 17. If diffusion through the membrane is rate limiting for overall permeation, the correlation between apparent permeability coefficient P_{app} and continuous phase drug concentration over total concentration C_L/C_{tot} will be linear. A line parallel to the x-axis will appear if permeation within the vehicle is slower than permeation through the skin. If the values of P_{dbl} and $(C_L/C_{tot})P_m$ are comparable, the correlation will result in a curve.

It has to be assumed that permeability coefficient of the diffusion boundary layer P_{dbl} may be different for emulsions comprising a drug that is mainly present in the dispersed phase (caffeine) and a drug that is mainly present in the continuous phase (ibuprofen). Additionally, P_{dbl} may also differ for emulsions comprising the same oil component/oil phase, but varying phase fractions due to arising changes in the microstructure, i.e. droplet size and density of droplet packing. Considering these differences, macroscopic permeation through an emulsion is described as effective permeability coefficient P_e. Following the Maxwell-

Rayleigh-Lorentz-Clausius-Mosotti-Wagner-Wiener relation, this P_e may be calculated by the following equation:

$$\frac{P_e - P_L}{P_e + 2P_L} = \left(\frac{P_W - P_L}{P_W + 2P_L} \right) V_W \quad \text{[equation XIII]}$$

where, the subscripts e, W and L denote effective, dispersed and continuous. P denotes permeability coefficient and V_W is the volume fraction of the internal water phase [159-161].

Based on equation XIII effective diffusion coefficient D_e can be calculated, if the following assumptions are set:

(1) Diffusion coefficients for caffeine and ibuprofen are identical.

(2) Dispersed particles do not interact with each other.

(3) Drug diffusion coefficient in the oil phase is estimated based on drug diffusion coefficient in the water phase ($D_W \approx 7 \cdot 10^{-6}$ cm^2/sec at 32°C, viscosity 1 mPas) and viscosity of the oil phase (see table 20)

Table 20. Measured oil viscosity and estimation of the diffusion coefficients D_L for the different oils – based on diffusion coefficient D for water ($D_W \approx 7 \cdot 10^{-6}$ cm^2/sec at 32°C assuming a viscosity of 1 mPas)

	Viscosity at 32°C [mPas] [1]	Estimated D_L
Isopropyl myristate	7	$9.8 \cdot 10^{-7}$
Miglyol 812N	25	$2.8 \cdot 10^{-7}$
Paraffinum liquidum	77	$9.1 \cdot 10^{-8}$

[1] for viscosity measurement see Part III, 5.3.1.1

(4) According to Higuchi [159], it holds:

$$D_e = \frac{P_e}{K_e} \quad \text{[equation XIV]}$$

and

$$K_e = K_{W/L} V_W + V_L \quad \text{[equation XV]}$$

where, $K_{W/L}$ denotes drug partition coefficient between water and oil phase, V_W and V_L denote volume fractions of internal (subscript W) and continuous phase (subscript L), respectively.

Taken together, effective diffusion coefficient D_e can be calculated based on equation XVI:

$$\frac{D_e K_e - D_L}{D_e K_e + 2D_L} = \frac{K_{W/L} D_W - D_L}{K_{W/L} D_W + 2D_L} \cdot V_W \qquad \text{[equation XVI]}$$

From effective diffusion coefficient D_e effective permeability coefficient P_e can be calculated using equation XIV.

4.3.2.2 Transdermal Permeation of Caffeine

4.3.2.2.1 Apparent permeability coefficient P_{app} (Experimental Data)

For caffeine the partition coefficients between oil and water phase were always below unity, indicating that most of the drug is entrapped within the water droplets of the w/o-emulsion. Therefore, also the ratio of continuous phase drug concentration over total concentration C_L/C_{tot} possessed values below unity. However, as confirmed by ultracentrifugation experiments and calculation, continuous phase drug concentration was not zero and differed among the formulations studied. Accordingly, different values for P_{app} were received (see table 21).

Table 21. Apparent permeability coefficient P_{app} (cm/sec) and continuous phase caffeine concentration over total concentration C_L/C_{tot}

Isopropyl myristate	C_{tot} (µg/g)	c_L / c_{tot}	P_{app} ± SEM[1] [cm/sec]
E70[2]	800	0.06	$4.62 \cdot 10^{-7} \pm 3.25 \cdot 10^{-8}$
E50[2]	800	0.08	$7.98 \cdot 10^{-7} \pm 4.98 \cdot 10^{-8}$
E30[2]	800	0.12	$1.15 \cdot 10^{-6} \pm 1.11 \cdot 10^{-7}$
Oil + 7% Isolan PDI	Saturated	1.0	$3.05 \cdot 10^{-6} \pm 4.45 \cdot 10^{-7}$
Miglyol 812N			
E70[2]	2500	0.17	$2.60 \cdot 10^{-7} \pm 1.57 \cdot 10^{-8}$
E70[2]	2000	0.18	$2.65 \cdot 10^{-7} \pm 2.09 \cdot 10^{-8}$
E50[2]	2000	0.23	$2.41 \cdot 10^{-7} \pm 1.77 \cdot 10^{-8}$
E30[2]	2000	0.33	$3.47 \cdot 10^{-7} \pm 3.21 \cdot 10^{-8}$
Oil + 7% Isolan PDI	Saturated	1.0	$6.32 \cdot 10^{-7} \pm 1.46 \cdot 10^{-7}$
Paraffinum Liquidum			
E70[2]	2000	$1.43 \cdot 10^{-3}$	$1.77 \cdot 10^{-7} \pm 3.28 \cdot 10^{-8}$
E50[2]	2000	$2.00 \cdot 10^{-3}$	$1.60 \cdot 10^{-7} \pm 2.37 \cdot 10^{-8}$
E30[2]	2000	$3.33 \cdot 10^{-3}$	$2.21 \cdot 10^{-7} \pm 4.75 \cdot 10^{-8}$
Oil	Saturated	1.0	$1.86 \cdot 10^{-6} \pm 2.05 \cdot 10^{-7}$

[1] all values denote mean ± standard error of mean. n = 3 - 9
[2] w/o-emulsion with 70%, 50% or 30% dispersed phase, respectively

The correlation between P_{app} and C_L/C_{tot} is illustrated graphically in figure 18. Depending on the oil selected different curve progressions are observed. For isopropyl myristate correlation was non-linear, whereas for miglyol 812N a nearly linear correlation was observed. For paraffinum liquidum no clear statement was possible. The single data points are not evenly distributed along the x-axis and it turned out that combination of caffeine with paraffinum liquidum was not suitable due to the low drug distribution coefficient $K_{L/W}$ between oil and water phase (see 4.3.1.1).

Permeation of caffeine was strongly dependent on the oil base selected. Highest permeation was achieved for isopropyl myristate, followed by paraffinum liquidum and miglyol 812N.

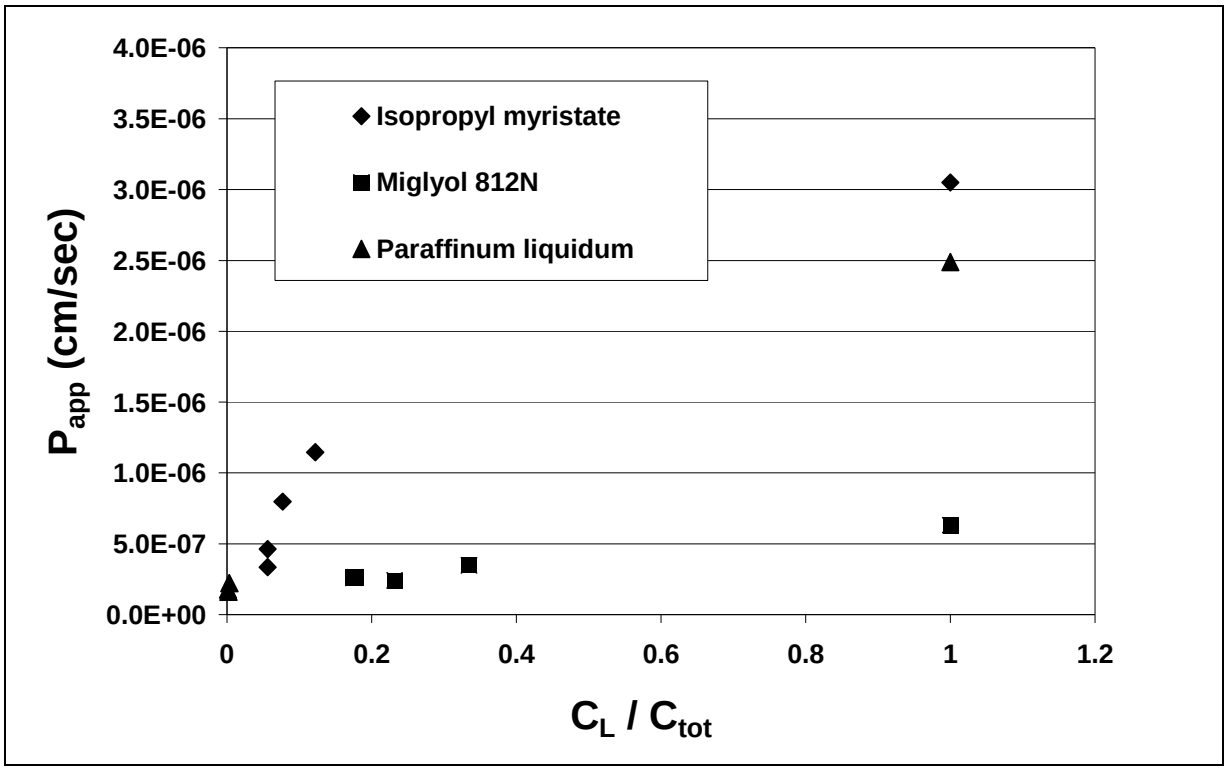


Figure 18. Correlation of apparent permeability coefficient P_{app} and continuous phase caffeine concentration over total concentration C_L/C_{tot} for lipophilic vehicles of varying phase fractions on the basis of different oils (n = 3 - 9; error bars not shown)

4.3.2.2.2 Permeability coefficients of the vehicle P_{dbl} and the skin P_m (non-linear regression)

To delineate the contribution of permeability through diffusion boundary layer P_{dbl} and permeability through skin P_m to overall apparent permeability P_{app} of each transport series (isopropyl myristate, miglyol 812N or paraffinum liquidum, see table 12), the individual values were calculated. For this purpose equation V was fitted to the data of P_{app} versus C_L/C_{tot}. EasyFit® curves are shown in figure 19 and demonstrate a good agreement of the curve with experimental data for all studied series. The deduced values for P_{dbl} and P_m are shown in table 22.

Concerning the permeability coefficient of the skin P_m large differences between the oils were obtained. Largest P_m was obtained for the paraffinum liquidum series. It was 9-fold higher compared to the IPM series and 56-fold higher compared to the miglyol series. Concerning the permeability coefficient of the diffusion boundary layer P_{dbl}, the differences between the oils were smaller than for P_m. IPM series had an almost 2.4-fold higher value of P_{dbl} compared to paraffinum liquidum series and an almost 4.5-fold higher value of P_{dbl} compared to miglyol series. It is noted that P_{dbl} values obtained from the fit are average values of all four formulations of a transport series.

For a deeper understanding of the received P_{dbl} values, effective permeability P_e in the w/o-emulsion formulations was considered.

Table 22. Representative values for permeability coefficients of the diffusion boundary layer P_{dbl} and the skin P_m derived by easy fit® software for the caffeine transport series (n = 3 - 9) with isopropyl myristate (IPM), miglyol 812N (Migl) and paraffinum liquidum (Para)

Oil	Formulation	C_L/C_{tot} [1]	P_{app} [2] [cm/sec]	P_{dbl} [3] [cm/sec]	P_m [4] [cm/sec]
IPM	E70 [5]	0.06	$4.62 \cdot 10^{-7}$	$4.50 \cdot 10^{-6}$	$1.03 \cdot 10^{-5}$
	E50 [5]	0.08	$7.98 \cdot 10^{-7}$		
	E30 [5]	0.12	$1.15 \cdot 10^{-6}$		
	Oil+7% Isolan PDI	1	$3.05 \cdot 10^{-6}$		
Migl	E70 [5]	0.18	$2.65 \cdot 10^{-7}$	$1.01 \cdot 10^{-6}$	$1.61 \cdot 10^{-6}$
	E50 [5]	0.23	$2.41 \cdot 10^{-7}$		
	E30 [5]	0.33	$3.47 \cdot 10^{-7}$		
	Oil+7% Isolan PDI	1.0	$6.32 \cdot 10^{-7}$		
Para	E70 [5]	$1.43 \cdot 10^{-3}$	$1.77 \cdot 10^{-7}$	$1.87 \cdot 10^{-6}$	$9.04 \cdot 10^{-5}$
	E50 [5]	$2.00 \cdot 10^{-3}$	$1.60 \cdot 10^{-7}$		
	E30 [5]	$3.33 \cdot 10^{-3}$	$2.21 \cdot 10^{-7}$		
	Oil	1.0	$1.86 \cdot 10^{-6}$		

[1] continuous phase caffeine concentration over total caffeine concentration
[2] apparent permeability coefficient
[3] permeability coefficient of the diffusion boundary layer
[4] permeability coefficient of the membrane / skin
[5] emulsion E with 70%, 50% or 30% of dispersed phase, respectively

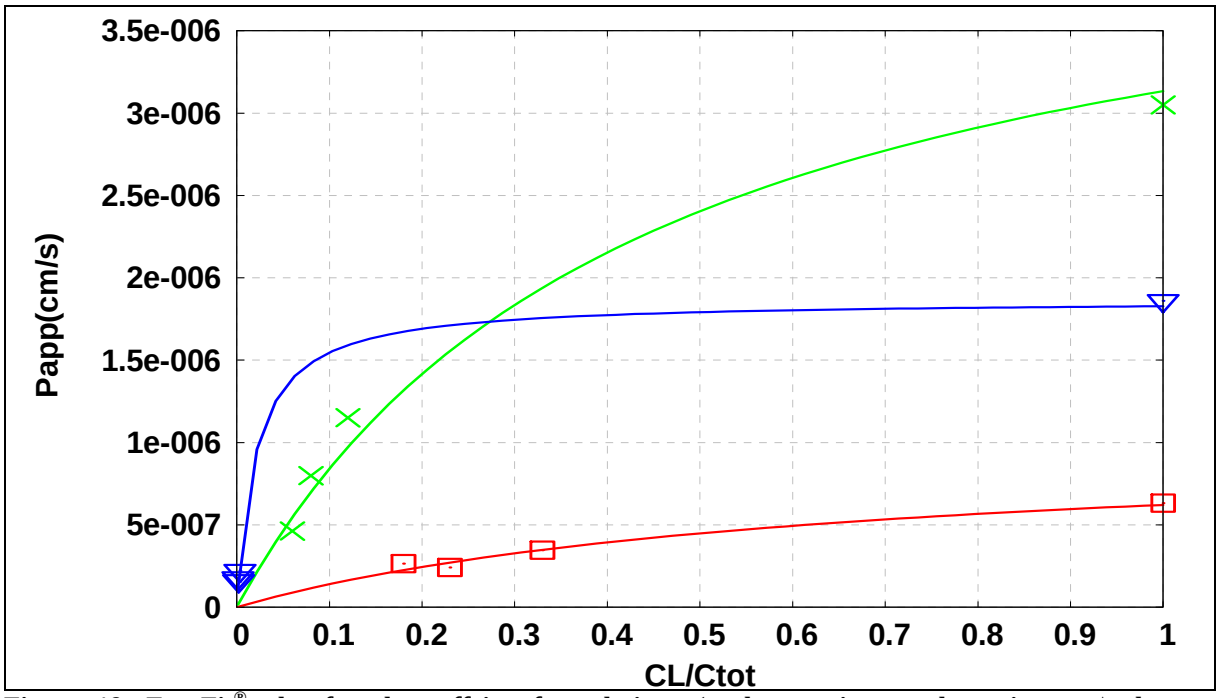


Figure 19. EasyFit® plot for the caffeine formulations (× denotes isopropyl myristate, Δ denotes paraffinum liquidum and □ denotes miglyol 812N)

4.3.2.2.3 Effective Permeability Coefficient P_e of the vehicle (non-linear regression)

Effective permeability coefficient P_e reflects the diffusivity of the drug in the vehicle and was calculated by equation XIV and XVI. For all three oils studied permeability coefficient for the vehicle increased as the phase fraction of dispersed water phase was increased. Additionally, following increasing viscosity (see Part III, 5.3.1.1) of isopropyl myristate, miglyol 812N and paraffinum liquidum, effective permeability coefficient P_e was decreased.

Table 23. Effective permeability coefficient P_e(cm/sec) of caffeine in w/o-emulsions of varying phase fractions and for different oils

	P_e(IPM)[1] [cm/sec]	P_e(Migl)[2] [cm/sec]	P_e(Para)[3] [cm/sec]
E70[4]	$5.40 \cdot 10^{-6}$	$2.13 \cdot 10^{-6}$	$7.21 \cdot 10^{-7}$
E50[4]	$3.73 \cdot 10^{-6}$	$1.07 \cdot 10^{-6}$	$3.64 \cdot 10^{-7}$
E30[4]	$3.46 \cdot 10^{-6}$	$6.16 \cdot 10^{-7}$	$2.08 \cdot 10^{-7}$

[1] isopropyl myristate
[2] miglyol 812N
[3] paraffinum liquidum
[4] w/o-emulsion with 70%, 50% and 30% of dispersed phase, respectively

Effective permeability coefficient P_e of the formulations based on different oils agreed on average very well with the corresponding permeability coefficient P_{dbl} values for isopropyl myristate and miglyol. For paraffinum liquidum an approximately 5-fold difference was observed.

4.3.2.3 Transdermal Permeation of Ibuprofen

4.3.2.3.1 Apparent Permeability Coefficient P_{app} (Experimental Data)

For ibuprofen the drug distribution coefficient between oil and sodium acetate buffer pH 4.5 was always bigger than unity, indicating that the majority of the drug was present in the continuous phase. Therefore also the ratio of continuous phase drug concentration over total concentration possessed values bigger than unity. Ultracentrifugation experiments and calculation revealed that continuous phase drug concentration over total concentration C_L/C_{tot} differed for all formulations studied, depending on the phase fraction and the oil base selected. Accordingly, different values for P_{app} were received (see table 24).

The correlation between P_{app} and C_L/C_{tot} is illustrated in figure 20. Depending on the oil selected different correlation behaviour was observed. Again, ibuprofen showed best permeability for formulations comprising isopropyl myristate. Permeability of formulations comprising paraffinum liquidum was higher compared to miglyol 812N. Considering the range examined, correlation appeared linear at first sight for all three oils, but none of the regression lines hit the point of origin.

Table 24. Correlation between apparent permeability coefficient P_{app} and continuous phase ibuprofen concentration over total concentration C_L/C_{tot}

Isopropyl myristate	C_{tot} (ug/g)	C_L/C_{tot}	P_{app} ± SEM (cm/sec)[1]
E70[2]	10000	3.32	$3.54 \cdot 10^{-7} \pm 4.79 \cdot 10^{-8}$
E50[2]	10000	2.00	$2.82 \cdot 10^{-7} \pm 2.79 \cdot 10^{-8}$
E30[2]	5000	1.43	$2.68 \cdot 10^{-7} \pm 8.41 \cdot 10^{-9}$
Oil + 7% Isolan PDI	10000	1.00	$2.52 \cdot 10^{-7} \pm 1.57 \cdot 10^{-8}$
Miglyol 812N			
E70[2]	10000	3.32	$2.06 \cdot 10^{-7} \pm 2.80 \cdot 10^{-8}$
E50[2]	10000	2.00	$1.39 \cdot 10^{-7} \pm 2.74 \cdot 10^{-8}$
E30[2]	5000	1.43	$1.10 \cdot 10^{-7} \pm 1.04 \cdot 10^{-8}$
Oil + 7% Isolan PDI	10000	1.00	$1.07 \cdot 10^{-7} \pm 1.86 \cdot 10^{-8}$
Paraffinum Liquidum			
E70[2]	10000	3.27	$2.68 \cdot 10^{-7} \pm 3.97 \cdot 10^{-8}$
E50[2]	5000	1.98	$2.24 \cdot 10^{-7} \pm 1.70 \cdot 10^{-8}$
E30[2]	5000	1.42	$1.89 \cdot 10^{-7} \pm 1.90 \cdot 10^{-8}$

[1] all values denote mean ± standard error of mean. n = 5 - 11
[2] emulsion with 70%, 50% and 30% of dispersed phase, respectively

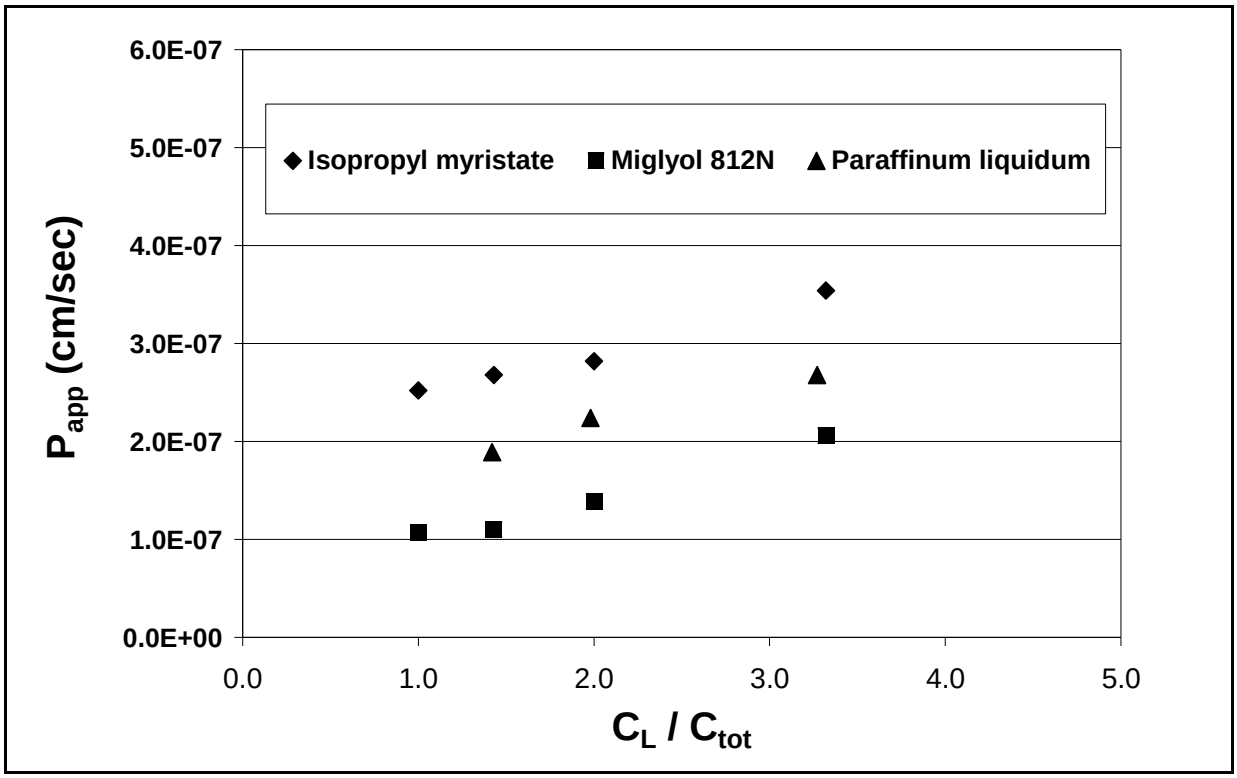


Figure 20. Correlation between apparent permeability coefficient P_{app} and continuous phase ibuprofen concentration over total concentration C_L/C_{tot} of lipophilic vehicles with varying phase fractions on the basis of different oils (n = 5 - 11; error bars not shown)

4.3.2.3.2 Permeability coefficients of the vehicle P_{dbl} and the skin P_m (non-linear regression)

To delineate the contribution of permeability through diffusion boundary layer P_{dbl} and permeability through skin P_m to overall apparent permeability P_{app} of each transport series (isopropyl myristate, miglyol 812N or paraffinum liquidum, see table 12), the individual values were calculated. For this purpose equation V was fitted to the data of P_{app} versus C_L/C_{tot}. EasyFit® curves are shown in figure 21 and demonstrate a good agreement of the curve with experimental data for all studied series. The deduced values for P_{dbl} and P_m are shown in table 25.

Considering a wider range for C_L/C_{tot}, it turned out that correlation for all three transport series / oils studied was non-linear. Permeation was best for isopropyl myristate series. Paraffinum liquidum series showed higher permeability than miglyol 812N series.

Considering permeability coefficient of the skin P_m, values differed at most 5-fold. Contrary to the values received for caffeine transports, permeability coefficient of the diffusion boundary layer P_{dbl} for ibuprofen did not show a difference for all three oil selected. It is noted that P_{dbl} values obtained from a fit are average values of all four formulations of a transport series.

For a deeper understanding of the received P_{dbl} values, effective permeability P_e in the w/o-emulsion formulation was considered.

Table 25. Representative values for permeability coefficient of the diffusion boundary layer P_{dbl} and the skin P_m derived by easy fit® software for the ibuprofen transport series (n = 5 - 11) with isopropyl myristate (IPM), miglyol 812N (Migl) and paraffinum liquidum (Para)

Oil	Formulation	C_L / C_{tot}	P_{app} (cm/sec)[1]	P_{dbl} (cm/sec)	P_m (cm/sec)
IPM	E70[2]	3.32	$3.54 \cdot 10^{-7}$	$4.18 \cdot 10^{-7}$	$5.53 \cdot 10^{-7}$
	E50[2]	2.00	$2.82 \cdot 10^{-7}$		
	E30[2]	1.43	$2.68 \cdot 10^{-6}$		
	Oil+7% Isolan PDI	1.00	$2.52 \cdot 10^{-6}$		
Migl	E70[2]	3.32	$2.06 \cdot 10^{-7}$	$4.20 \cdot 10^{-7}$	$1.16 \cdot 10^{-7}$
	E50[2]	2.00	$1.39 \cdot 10^{-7}$		
	E30[2]	1.43	$1.10 \cdot 10^{-7}$		
	Oil+7% Isolan PDI	1.00	$1.07 \cdot 10^{-7}$		
Para	E70[2]	3.27	$2.68 \cdot 10^{-7}$	$3.93 \cdot 10^{-7}$	$2.59 \cdot 10^{-7}$
	E50[2]	1.98	$2.24 \cdot 10^{-7}$		
	E30[2]	1.42	$1.89 \cdot 10^{-7}$		

[1] mean value. Standard error not shown. n = 5 – 11
[2] emulsion with 70%, 50% and 30% of dispersed phase, respectively

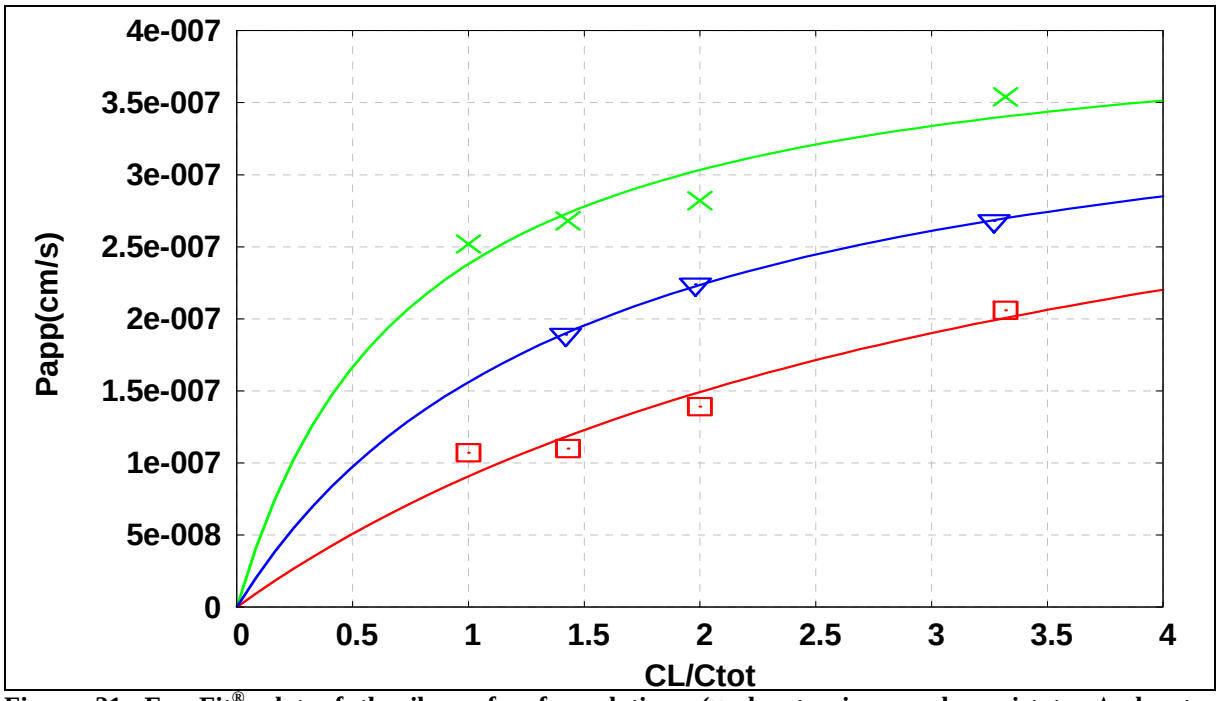


Figure 21. EasyFit® plot of the ibuprofen formulations (× denotes isopropyl myristate, Δ denotes paraffinum liquidum and □ denotes miglyol 812N)

4.3.2.3.3 Effective Permeability Coefficient P_e within the vehicle (non-linear regression)

Effective permeability coefficient P_e reflects the diffusivity of a drug in a vehicle and was calculated by equation XIV and XVI. For all three oils studied permeability coefficient for the diffusion boundary layer P_{dbl} decreased as the phase fraction of dispersed water phase was increased. Additionally, following increasing viscosity (see Part III, 5.3.1.1) of isopropyl myristate, miglyol 812N and paraffinum liquidum, effective permeability coefficient P_e was decreased in all cases.

Table 26. Effective permeability coefficient P_e(cm/sec) of ibuprofen in emulsions with varying phase fractions and for of different oils

	P_e(IPM)[1] [cm/sec]	P_e(Migl)[2] [cm/sec]	P_e(Para)[3] [cm/sec]
E70[4]	$2.25 \cdot 10^{-7}$	$6.3 \cdot 10^{-8}$	$6.65 \cdot 10^{-8}$
E50[4]	$3.99 \cdot 10^{-7}$	$1.19 \cdot 10^{-7}$	$7.21 \cdot 10^{-8}$
E30[4]	$6.03 \cdot 10^{-7}$	$1.75 \cdot 10^{-7}$	$7.84 \cdot 10^{-8}$

[1] isopropyl myristate
[2] miglyol 812N
[3] paraffinum liquidum
[4] emulsion with 70%, 50% and 30% of dispersed phase, respectively

Permeability coefficient of the diffusion boundary layer P_{dbl} is a representative value for all emulsion of a transport series, i.e. emulsions of different phase fractions and different droplet sizes (see Part III, 5.3.1.2). Effective permeability coefficient P_e, however, is a specific value for the oil and the mass fractions selected. Comparison of the permeabilities P_{dbl} and P_e of the corresponding transport series yielded a good correlation only for the isopropyl myristate series.

4.3.2.4 Transdermal Permeation of Benzyltrimethylammonium Chloride

For benzyltrimethylammonium chloride (BTA-Cl) the drug distribution coefficient between oil and water phase is supposed to be zero, as salts do not possess solubility in oil. According to the proposed concept that continuous drug phase concentration governs skin permeation, no permeation should occurr as drug substance remaines entrapped in water droplets dispersed in oil. In all cases studied, occlusive application of BTA-Cl formulations with an infinite dose of 0.7 g/cm^2 did not yield a steady-state flux within 48h or no permeation was observed at all. The comparatively low amounts that permeated through the skin can be attributed to possible damages of the skin, permeation through shunt routes or instabilities of the formulations occurring directly at the emulsion – stratum corneum surface within 48h. Furthermore, it is possible that ion pair complexes were formed with anions resulting from the skin or the vehicle that enabled distribution and consequently permeation. Figure 22 is a representative and averaged permeation pattern of the studied formulations.

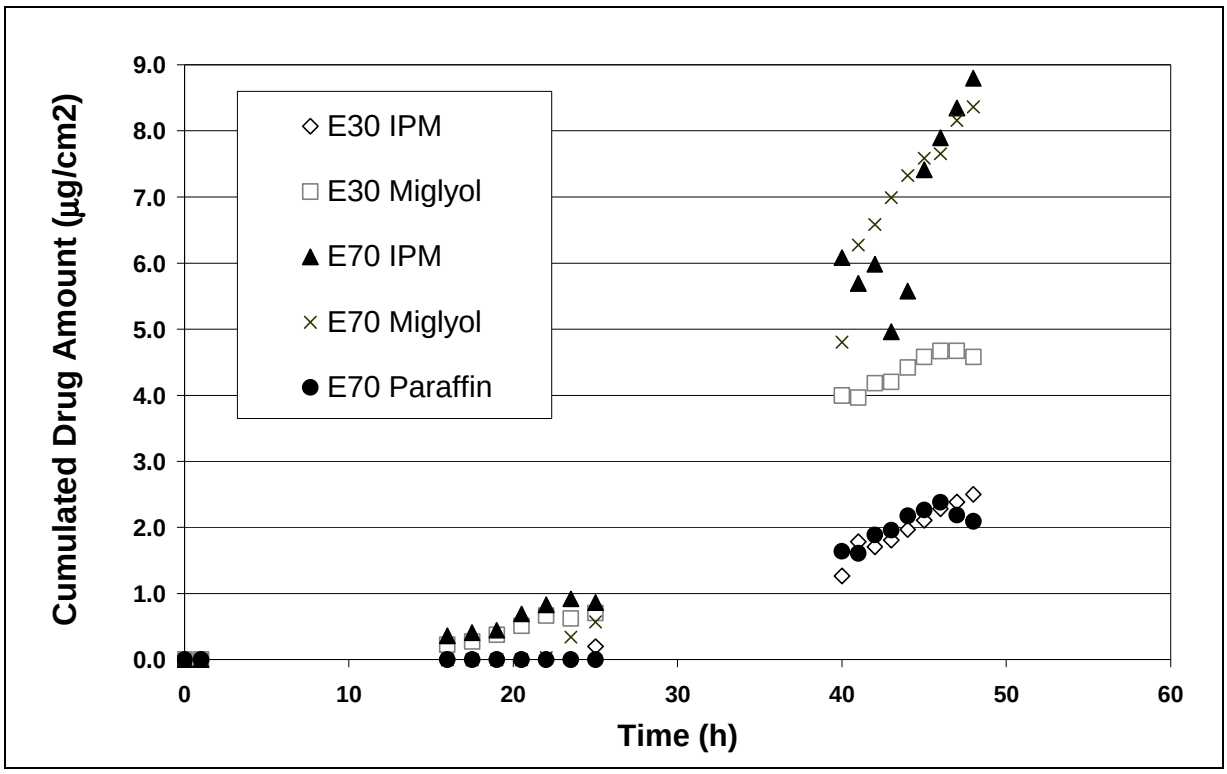


Figure 22. Averaged permeation pattern of Benzyltrimethylammonium Chloride (1% and 0.5% of total drug concentration for E70 and E30, respectively; error bars not shown; n = 4 - 11)

4.4 Discussion

The efficiency, tolerability and applicability of topical agents are directly related to employed vehicles. Thus, to achieve optimum topical therapy, a solid knowledge of the vehicles, their composition, and their physical and dermato-pharmacological actions is important [36].

The overall topical delivery process is extremely complex in that competitive effects proceeding in parallel or consecutively are not easily separated since components of the vehicle themselves may dramatically alter the permeability of the skin to the drug. Clearly, the relative importance of each of these determinants is a function of the physicochemical properties of the drug, its vehicle and the membrane, i.e. the skin [6].

Transdermal permeation of a hydrophilic and a lipophilic model drug (caffeine and ibuprofen, respectively) out of w/o-emulsions of varying phase fractions (E70, E50 and E30) and differing oil components (isopropyl myristate IPM, miglyol 812N Migl, paraffinum liquidum Para) was studied. Additionally, these formulations also differed in their microstructure showing decreasing droplet sizes and denser droplet packing as the fraction of the dispersed water phase was increased. Microscopic pictures obtained by freeze fraction scanning electron microscopy (see Part III, 5.3.1.2) revealed that this observation was consistent for all formulations studied. The formulations were designed specifically to quantitatively understand the mechanisms that determine absorption processes across the skin. For that purpose, continuous phase drug concentration of the formulation was considered, according to a proposed concept, which postulates that this concentration is the driving force governing permeation kinetics [2, 3].

Continuous phase drug concentration was determined experimentally by ultracentrifugation experiments and theoretically by calculation based on equation III taking into consideration total drug concentration, phase fractions and drug partitioning between the corresponding oil and water phase. Drug distribution coefficients between oil and water phase did not only disclose the hydrophilic and lipophilic nature of the model drugs studied, but additionally showed that distribution was different depending on the oil selected. Concerning the drug distribution coefficient for caffeine and ibuprofen, the values for IPM and Migl were closer to each other than the values of Para.

For ibuprofen formulations, the calculated and measured continuous phase drug concentration correlated impressively well. The continuous phase ibuprofen concentrations differed within one transport series (E70, E50, E30 and oil) according to the phase fraction,

but were similar for w/o-emulsions of the same phase fraction, although different oil components were selected. This is because continuous phase drug concentration for a w/o-emulsion is calculated based on formula III. For a lipophilic drug, the term volume fraction of dispersed phase over drug partition coefficient between oil and water phase $\Phi_W / K_{L/W}$ can be neglected compared to the volume fraction of the continuous phase Φ_L due to the high values of partition coefficient $K_{L/W}$ for lipophilic model compounds.

For caffeine the total drug concentration applied in formulations was smaller compared to ibuprofen in order to prevent any possible saturation or drug precipitation within the continuous oil phase (for saturation concentration, see Part III, 5.3.1.3 and [3]). Accordingly, the continuous phase drug concentration was up to 1500-fold smaller. The agreement between calculated and measured values was not as good as for ibuprofen. The reason for this is presently not clear, might be related though to experimental error due to the much smaller drug concentration. Continuous phase drug concentration differed within the transport series (E70, E50, E30 and oil), but also had different values for w/o-emulsions of the same phase fraction, but different oil components. This is because equation III was applied for calculation of caffeine concentration in the oil phase. For hydrophilic drug components possessing a drug distribution coefficient $K_{L/W}$ below unity, the term $\Phi_W / K_{L/W}$ outweighs Φ_L.

To summarize, validity of equation III for calculation of continuous phase drug concentration in w/o-emulsions under occlusive conditions is given for hydrophilic and lipophilic model drugs (see table 15 and 16) and, therefore can be used with other lipophilic vehicle systems.

Varying amounts of emulsifier Isolan PDI in emulsions comprising the same phase fractions did not affect continuous phase drug concentration examined with ibuprofen for which emulsifier in the oil phase could, in theory (equation III), have a measurable effect. Further studies revealed that emulsifier did also not affect saturation concentration of the drugs in the oils (see Part III, 5.3.1.3).

Transdermal absorption processes with lipophilic vehicle systems were studied with transport experiments across full-thickness pig ear skin. Correlation of apparent permeability coefficient P_{app} for steady-state conditions with continuous phase drug concentration over total concentration C_L/C_{tot} was examined (see figure 19 for caffeine and 21 for ibuprofen).

To consider possible effects Isolan PDI could exert with respect to transdermal permeation, its influence was studied more in detail (discussed in an additional publication, see part III). No effect of Isolan PDI was found. With respect to the transdermal permeation of the

formulations discussed here, w/o-emulsions were designed in a way that ratio of emulsifier content over oil content ranged in the same order of magnitude. Consequently, for reference formulation pure oil was mixed with a corresponding amount of emulsifier so as to be comparable to the emulsion applied.

In the situation of transdermal drug delivery from a multi-phasic dermatological formulation, two diffusion-related processes take place: drug diffusion within the skin and drug diffusion within the formulation to the skin. Apparent permeability coefficient P_{app} was determined by transport experiments across full-thickness pig ear skin using Franz-type diffusion cells and is therefore dependent on permeability coefficient of the diffusion boundary layer (vehicle) P_{dbl} and of the membrane (skin) P_m. Dependent on the contribution of these two determinants to overall measured apparent permeability coefficient P_{app}, correlation between P_{app} and C_L/C_{tot} resulted in a non-linear relationship, except for caffeine/Migl transport series (see 4.3.2.1).

By plotting apparent permeabilities P_{app} of all caffeine formulations studied (series IPM, Migl, Para) against corresponding continuous phase drug concentration over total concentration C_L/C_{tot}, obtained correlation differed for all three oils selected. A linear correlation for miglyol based formulations and a nonlinear correlation for IPM based formulation was observed. A clear statement for paraffinum liquidum based formulations was not possible. The single data points are not evenly distributed along the x-axis and it turned out that combination of caffeine with paraffinum liquidum was not suitable due to the low drug distribution coefficient $K_{L/W}$ between oil and water phase (see 4.3.1.1). Depending on the oil selected, apparent permeability coefficients P_{app} were different. Highest permeability was found for caffeine/IPM series, followed for caffeine/Para series and caffeine/Migl series.

Considering apparent permeabilities P_{app} of all examined ibuprofen formulations and the corresponding continuous phase drug concentration over total concentration C_L/C_{tot}, correlation pattern was similar, but not identical. In the examined range of values correlation appeared linear at first sight for all three oils, but none of the regression lines hit the point of origin. As already observed for the hydrophilic drug caffeine, ibuprofen possessed highest apparent permeability coefficients P_{app} in formulations comprising IPM, followed by Para and Migl based formulations, thus, the ranking of drug permeabilities with respect to the oils was the same.

To delineate the contribution of permeability through diffusion boundary layer P_{dbl} and permeability through the skin P_m to overall apparent permeability P_{app} of each transport

series, the individual values were calculated. Equation V was fitted to the data of P_{app} versus C_L/C_{tot}.

Taking into account caffeine distribution coefficient between stratum corneum and oil (see 4.3.1.2), the values, determined indirectly without any direct interaction of the oil with the stratum corneum, could only explain the permeability coefficient P_m of Migl and Para. Contrary, IPM possessed a low drug distribution coefficient $K_{SC/L}$ of all three oils, but showed highest permeation. Permeability coefficient of the skin P_m differed 6.4-fold between IPM and Migl series, 8.8-fold between Para and IPM series, and 56-fold between Para and Migl series. Considering the ratios of P_m values for the caffeine series and the ratio of the corresponding distribution coefficients of caffeine partitioning between stratum corneum and oil, there is a good correlation for the Para and Migl series (ratios almost identical), whereas ratios differed 2-fold for IPM and Migl series and 1.7-fold for Para and IPM series (see table 27).

Taking into account ibuprofen distribution coefficient between stratum corneum and oil (see 4.3.1.2), the values, determined indirectly without any direct interaction of the oil with the stratum corneum, could only explain the ranking of permeation for Migl and Para. Ibuprofen distribution between stratum corneum and oil was similar for IPM and Migl, but IPM series showed highest permeation. Drug distribution coefficient between stratum corneum and oil was 7-fold smaller for IPM than for Para, but permeability coefficient of the skin P_m differed 2-fold between IPM and Para.

For Migl and Para, there was an almost linear correlation for permeability coefficient of the skin P_m and caffeine distribution coefficient between stratum corneum and oil $K_{SC/L}$, whereas for ibuprofen only the ranking of the distribution for P_m and $K_{SC/L}$ was similar.

Concerning the oils, it is important to note that according to literature IPM acts as a permeation enhancer. For instance, Dias et al. studied the transdermal permeation of caffeine out of several saturated vehicles. Mineral oil and isopropyl myristate among others enhanced permeation by modulation of the barrier properties. It was concluded that the vehicles permeate into the skin lipids and alter the solubility properties in a favourable way. The degree to which the vehicles can achieve this depends on their uptake into the skin and their solubility of caffeine in the modified environment [112]. Other researcher claim that IPM places itself within lipid bilayers present in stratum corneum (intercalation), thus, the high ordered structure is disturbed, bilayers get more liquid and diffusion velocity is increased [36, 118, 122, 123]. Potentially, IPM also extracts lipophilic components of the skin and therefore disturbs skin barrier function [138]. As determination of the partition coefficient for caffeine

between stratum corneum and IPM was performed indirectly, direct effects IPM might exert on stratum corneum were masked and, thus, lower values than effectively present were obtained. For ibuprofen transport series with IPM, the received drug distribution values for $K_{SC/L}$ did also not correlated with permeability coefficient P_m. Consequently, the permeation enhancement of ibuprofen is not limited to hydrophilic drug, but also valid for lipophilic drugs.

Table 27. Permeability coefficient of the membrane P_m for caffeine and ibuprofen taking into account drug distribution between stratum corneum and oil phase $K_{SC/L}$

	Isopropyl myristate [cm/sec]	Miglyol 812N [cm/sec]	Paraffinum liquidum [cm/sec]
CAFFEINE series	**A**	**B**	**C**
P_m	$1.03 \cdot 10^{-5}$	$1.61 \cdot 10^{-6}$	$9.04 \cdot 10^{-5}$
$K_{SC/L}$	73.75	22.87	1113.21
IBUPROFEN series	**D**	**E**	**F**
P_m	$5.53 \cdot 10^{-7}$	$1.16 \cdot 10^{-7}$	$2.59 \cdot 10^{-7}$
$K_{SC/L}$	0.101	0.106	0.724

The deduced permeability coefficient of the diffusion boundary layer P_{dbl} is a representative values for lipophilic emulsions of varying phase fractions and varying droplet sizes and does not consider the microstructure present in each formulation. Consequently, the theoretically calculated effective permeability coefficient P_e was introduced taking into account different phase fractions and drug distribution coefficients between oil and water phase.

Concerning the hydrophilic drug caffeine in all series studied (IPM, Migl, Para), in most cases permeability coefficient of diffusion boundary layer P_{dbl} were in the same order of magnitude as effective permeability coefficient P_e (see table 28). P_{dbl} values differed 4.5-fold between IPM and Migl series, 2.5-fold between IPM and Para series and 1.8-fold between Para and Migl series. For IPM and Migl series, effective permeability coefficient P_e showed a good agreement with estimated permeability coefficients of the diffusion boundary layer P_{dbl}. Effective permeability coefficient P_e of the formulation increased as phase fraction of the dispersed water phase is increased. Additionally, within one formulation of defined phase fraction, effective permeability coefficient increased as viscosity of the formulation or the oil was decreased.

Caffeine incorporated in w/o-emulsions of varying phase fractions is mainly entrapped inside water droplets. As fraction of internal phase is increased, droplet size decreases and packing

becomes denser. It is likely that these changes in microstructure facilitate diffusion of a hydrophilic drug within w/o-emulsions.

Concerning the lipophilic drug ibuprofen in all series studied, permeability coefficients of the diffusion boundary layer P_{dbl} were almost identical for all three series studied (see table 28). In most cases computed values for P_e are in the same order of magnitude as permeability coefficient of the diffusion boundary layer P_{dbl}. Best agreement was found for IPM series.

Ibuprofen, in contrary, is mainly present in the continuous oil phase. If the fraction of the internal phase is increased, resulting in a denser droplet packing and reduced droplet size, diffusion of ibuprofen slows down. The path of diffusion is becoming more and more tortuous and ibuprofen has to circumvent more and more droplet "obstacles".

The tendency of effective permeability coefficient P_e was different compared to caffeine with respect to the phase fractions, but not with respect to viscosity. The smaller the dispersed phase fraction of water in oil was, the higher the calculated effective permeability coefficient P_e became. Within one formulation of defined phase fraction, effective permeability coefficient P_e increased as the viscosity of the formulation or oil, respectively, was decreased.

Table 28. Effective permeability coefficient $P_e \cdot 10^6$ and permeability coefficient of the diffusion boundary layer $P_{dbl} \cdot 10^6$ [units cm/sec]

	CAFFEINE			IBUPROFEN		
	Isopropyl myristate	**Miglyol 812N**	**Paraffinum liquidum**	**Isopropyl myristate**	**Miglyol 812N**	**Paraffinum liquidum**
E70[1]	5.40	2.13	0.72	0.23	0.06	0.067
E50[1]	3.73	1.07	0.36	0.40	0.12	0.072
E30[1]	3.46	0.62	0.21	0.60	0.18	0.078
P_{dbl}	4.50	1.01	1.87	0.42	0.42	0.39

[1] emulsions with 70%, 50% and 30% of dispersed phase, respectively

Previous work that focused on multi-phasic hydrophilic formulations figured out that diffusion within the vehicle was at least two orders of magnitude faster than diffusion within stratum corneum [3]. This finally indicated that diffusion in the skin dominated permeation kinetics, confirming a published work where diclofenac permeation from different phospholipids-based formulations was investigated [162]. In contrary, Clément et al. studied the effect of formulation parameters on the in vitro release of caffeine from concentrated w/o-emulsions obtaining four different emulsifiers across a polysulfone membrane. The concentration of the emulsifier did not have a significant effect on the release of caffeine, but in contrast, diffusion of caffeine from w/o-emulsions was found to be highly dependent on the internal phase

volume. The flux of caffeine increased with the percentage of the dispersed water phase. The droplet diameter decreased and also apparent viscosity increased with the percentage of dispersed phase. Results further suggested that the shape factor of dispersed phase may also have an influence on the release of caffeine from concentrated emulsions [45, 163]. Izquierdo et al. examined the influence of emulsion droplet size on skin penetration of tetracaine using 3 macro-emulsions with droplet-sizes > 1 µm and 3 nano-emulsions with droplet sizes < 100 nm, but could not confirm the general anticipated increase in (transdermal) drug delivery with reduced emulsion droplet size [164-166]. Thus, it is likely that besides droplet size also drug properties and drug partitioning have to be considered. The above citations show that literature is conflicting with respect to phase fraction and particle size. Our findings could confirm the work of Clément et al..

Based on the data derived for P_{dbl} and P_m of all series studied, caffeine possessed an up to 10-fold higher permeability coefficient P_{dbl} of the diffusion boundary layer and an up to 350-fold higher permeability coefficient P_m of the skin compared to ibuprofen. The drug distribution coefficients between stratum corneum und oil phase were up to 1500-fold higher for caffeine than for ibuprofen which is consistent with the data for permeability coefficients P_m (see table 27).

Additionally, w/o-emulsions containing benzyltrimethylammonium chloride as a model drug were examined to confirm the postulated concept. Formulations comprising benzyltrimethylammonium chloride did not establish a steady state flux within 48h, if they showed any permeation at all. This observation is consistent with the assumption that no salt was present in the continuous oil phase of the formulations and, accordingly, no driving concentration gradient existed. Benzyltrimethylammonium chloride remained entrapped in the water droplets. If permeated drug was detected, however, steady-state conditions were not established and transported drug amount was very low (see figure 22). This was most probably due to transport through shunt routes, emulsion instabilities and ion pairing complexes occurring within 48h.

To conclude, a correlation between apparent permeability coefficient P_{app} and continuous phase drug concentration over total concentration C_L/C_{tot} was established taking into account permeability coefficient of the diffusion boundary layer P_{dbl} and permeability coefficient of the skin P_m. These values are in general comparable indicating that depending on the ratio of C_L/C_{tot} both can be rate controlling. Deduces values of P_{dbl} and P_m were reasonably consistent with independent theoretical calculation (in case of P_{dbl}), independent experimental and literature data (in case of P_m). This supports validity of the established

correlation. The proposed concept can be used to predict drug delivery rates of lipophilic vehicle systems under occlusive conditions.

Part III

5 Influence of Emulsifier Content, Thickener, Dose and Evaporation on Transdermal Drug Skin Permeation with Lipophilic Vehicles

Abstract

A concept for the interpretation of drug permeation was proposed that considered continuous phase drug concentration as the driving force for transdermal permeation of w/o-emulsions if occlusive conditions are assumed (see Part II). In order to implement this concept to the situation of non-occlusive application, changes vehicle undergo in the course of their application like evaporation of volatile components were studied. The extent of water loss and consequently the degree of occurring compositional changes is dependent on the applied dose. Continuous phase drug concentration can further be influenced by the presence of free emulsifier or an interaction with additives such as thickener Aerosil 200.

The purpose of this study was to elucidate the effect of these (formulation) parameters on transdermal absorption of a hydrophilic and a lipophilic model drug (caffeine or ibuprofen) incorporated in w/o-emulsions.

The studied w/o-emulsions consisted of an oil phase into which water phase was dispersed in mass fractions of 70%, 50% and 30% (E70, E50 and E30, respectively). Oil phase consisted of an oil component (isopropyl myristate, miglyol 812N or paraffinum liquidum) and of polymeric emulsifier Isolan PDI. Water phase contained sodium chloride and was buffered to pH 4.5 for all emulsions containing ibuprofen. Transport experiments were carried out in Franz-type diffusion cells across pig ear skin at 32℃ under occlusive and non-occlusive conditions with the infinite dosing of 0.3 g/cm^2 and 0.7 g/cm^2.

Freeze fracture scanning electron microscopy revealed a decreasing droplet size and denser droplet packing as phase fraction of dispersed water phase was increased. Additionally, viscosity increased with the amount of dispersed water phase.

A reduction of applied dose did not affect apparent permeability coefficient, but extent of water loss, as confirmed by studies on skin and in beaker. For implementing continuous phase drug concentration concept to non-occlusive conditions, a formula was derived that considered observed water loss and permeated drug amount in order to calculate the calculate the resulting drug concentration in the continuous formulation phase over time. An

increase of the drug concentration in the continuous oil phase was estimated which, however, did not lead to a measurable increase of the apparent permeability coefficient.

Free emulsifier present in the continuous oil phase neither affected saturation concentration nor transdermal absorption of the model drugs. Thickener Aerosil strongly decreased transdermal permeation of caffeine, but did not show any interaction with ibuprofen.

The performed characterisations demonstrate the possible influence of formulations parameters on regulation of drug skin permeation. Continuous phase drug concentration concept for lipophilic vehicles is expanded to the situation of non-occlusive application, but confirmation requires further investigations.

5.1 Introduction

In clinical practice, a drug is barely applied to the skin in form of a pure chemical, but instead, is incorporated into a carrier system, the vehicle, to guarantee efficient topical or systemic therapy. Convincing and suitable applicability, compatibility, adequate stability and above all efficacy with respect to duration and strength of the desired pharmacological action are requirements directly related to the employed vehicle. Thus, development and optimization of these dermatological formulations is a challenging task [1, 36].

Nowadays, it is widely acknowledged that transdermal permeation is regulated by the formulation of the drug product. This regulation may take place not only based on physico-chemical principles such as diffusion and partitioning of the active ingredient, but also by an interaction with the absorptive epithelium, i.e. the epidermis, affecting the permeability of the drug. Hence, a solid knowledge of the composition, including its physico-chemical properties and present microstructures is crucial in terms of achieving optimal topical delivery.

However, if a finite dose of dermatological formulation is applied onto the skin, the physico-chemical and thermodynamic conditions of the freshly applied emulsion change radically. Initial structural matrix and quantitative composition will most likely change during and after mechanical agitation associated with the application of the product (e.g. rubbing) and/or evaporation of ingredients (phase inversion). Medications are typically applied to the skin as a thin layer under non-occlusive conditions and are intended to deliver the active ingredient for hours. A change in the composition due to evaporation likely elicits alterations of the phase structure of the system and of the concentration and distribution of the active ingredient in it. These alterations can in turn affect delivery performance. Consequently, the transformation of the formulation while on the skin surface may be a crucial factor determining drug delivery. Possible evaporation of the volatile components of some vehicles, for instance o/w-emulsions, can result in an appreciable increase in solute drug concentration, first leading to saturation condition and then to a supersaturation or drug precipitation. Yet, it is still the beginning to research the complicating effects of vehicle metamorphosis on the entire clinical picture. On the contrary, if an infinite dose is applied, the formulation does not involve at the skin surface and the thermodynamic parameters are not altered [1, 3, 5].

A comprehensive and uniform theory that considers this multifaceted complexity present in dermatological formulations in order to quantitative understand the mechanisms of transdermal absorption is consequently a valuable contribution to research and development.

Previous work of our group proposed the continuous phase drug concentration concept as a model to delineate regulation of skin permeation considering drug distribution among distinct phases of multi-phasic hydrophilic formulations. Validity of this concept was confirmed for occlusive and non-occlusive conditions even though vehicles underwent considerable changes in their composition and microstructure due to evaporation of volatile ingredients [2, 3]. In a separate manuscript this concept was applied to w/o-emulsions of varying phase fractions and oil components and confirmed for occlusive conditions. Concerning lipophilic vehicle systems, resulting apparent permeability coefficients were not only dependent on drug permeability through the skin, but also on drug diffusivity within the vehicle itself. To date, drug diffusion inside the vehicle and its overall contribution to transdermal permeation has received too little attention. Permeability through the vehicle is mainly dependent on vehicle and drug properties, but also on phase fractions intermixed with each other and on arising microstructures (Part II). Additionally, drug concentration in the external oil phase can be influenced by the presence of free emulsifier, interaction with additives like thickeners or changes vehicle undergo in the course of their application like evaporation of volatile components. The extent of water loss and consequently the degree of occurring compositional changes is furthermore dependent on the applied dose .

Thus, the goal of the present work was to investigate the dependence of drug skin permeation of w/o-emulsions on (formulation) parameters like emulsifier and thickener content, dose and evaporation. The studied vehicles consisted of an oil phase into which water phase was dispersed in phase fractions of 70%, 50% and 30%, respectively (E70, E50 and E30). Oil phase consisted of a single oil component (isopropyl myristate, miglyol 812N or paraffinum liquidum) and polymeric emulsifier Isolan PDI. Water phase contained sodium chloride and was buffered to pH 4.5 in case of ibuprofen. Pure oil with and without emulsifier were selected as reference formulations. These formulations were designed specifically to elucidate the effect of differing continuous phase drug concentrations, microstructures and oil bases on transdermal delivery (see Part II). Caffeine (hydrophilic) and ibuprofen (lipophilic) were applied as model drugs, owning different solubility and permeation properties. Permeation experiments were carried out in vitro using Franz-type diffusion cells and pig ear skin as a model for human skin [134].

The influence of a reduced though still infinite dose (0.3 g/cm^2 instead of 0.7 g/cm^2) on apparent permeability coefficient was studied under occlusive conditions. Additionally, the reduced dose appeared handy and practical in terms of studying evaporation and transdermal permeation in parallel for non-occlusive conditions. Evaporation was examined in beaker and on skin mounted in Franz-type diffusion cells. Different experimental setup

allowed investigating any possible water uptake resulting from upward diffusion of the receiver compartment. Furthermore evaporation was studied in dependence of time and phase fraction present. Taking into account continuous phase drug concentration concept as a model to delineate transdermal drug delivery (see Part II), a formula is derived that considers observed water loss in the course of non-occlusive application and drug amount permeated in order to calculate new arising drug concentration in the external oil phase.

Isopropyl myristate and miglyol 812N were mixed with Isolan PDI in varying ratios being in the same order of magnitude or even similar to the situation present in the study formulation in order to examine the influence of free available emulsifier on the model drugs in terms of saturation concentration, continuous phase drug concentration and absorption process. The effect of thickener Aerosil 200 on macroscopic viscosity and apparent permeability coefficient for both model drugs was additionally investigated.

Besides, particle size of the examined formulations was approximated by freeze fracture scanning electron microscopy in order to gain a deeper understanding for the present microstructures and, thus, the entire absorption process. The understanding was completed through viscosity measurements of the examined formulations.

5.2 Materials and Methods

5.2.1 Materials

Caffeine was a gift from Boehringer Ingelheim (Ingelheim, Germany) and ibuprofen was obtained from Glatt GmbH (Binzen, Germany). Miglyol 812N® (Migl), medium chain triglycerides, was a gift from Hüls AG (Witten, Germany) and the polymeric emulsifier Isolan PDI (INCI: Diisostearoyl Polyglycerly-3 dimer dilinoleate) was donated from Goldschmidt GmbH (Essen, Germany). Isopropyl myristate (IPM), paraffinum liquidum (Para), sodium chloride, sodium dihydrogenphosphate dihydrate and phosphoric acid 85% were purchased from Haenseler AG (Herisau, Switzerland). Sodium azide and sodium acetate were purchased from Fluka Chemie GmbH (Buchs, Switzerland). Methanol, CombiTitrant5, a one-component reagent for the volumetric Karl Fischer titration and CombiSolventOil, the basic solution for Karl Fischer titration, consisting of methanol and toluol, were obtained from Merck KGaA (Darmstadt, Germany). Acetonitrile (HPLC, gradient grade) was ordered from Biosolve (Valkeensward, Netherlands). Aerosil 200, used as a stabilizer and thickener for oleogels, was obtained from Degussa (Düsseldorf, Germany). All other chemicals and reagents used in this study were of analytical grade. Bidistilled water was used in all cases.

5.2.2 Methods

5.2.2.1 Manufacturing of the Formulations

The study formulations (for composition see table 29 and 30) were prepared by first mixing water and oil phase separately. In case of hydrophilic model drug water phase consisted of caffeine, sodium chloride and bidistilled water. Oil phase consisted of emulsifier Isolan PDI (Iso) and the selected oil component, i.e. isopropyl myristate (IPM), miglyol 812N (Migl) or paraffinum liquidum (Para), respectively. In case of lipophilic model drug water phase consisted of sodium acetate buffer (pH 4.5, buffer capacity $\beta = 0.01$) and sodium chloride. Oil phase consisted of ibuprofen as model drug, emulsifier Isolan PDI (Iso) and the selected oil component, i.e. isopropyl myristate (IPM), miglyol 812N (Migl) or paraffinum liquidum (Para), respectively. First, all components of the oil phase were mixed and heated to 80-100°C until complete dissolution occurred. Second, water phase was added to the hot oil phase gradually with intense homogenization using a polytron PT 3000 (Kinematica AG Littau, Switzerland) for 5 minutes. Before further use, formulations remained at room temperature for 24h to ensure complete partitioning of the model drug between water and oil phase. Different emulsion formulations comprising varying amounts of dispersed phase (30, 50 and 70 weight-%, respectively) were prepared as formulation E (E30, E50 and E70). If other total

concentrations of caffeine were chosen than the listed concentrations in table 29, the amount of caffeine increased/decreased in as much as the corresponding amount of water decreased/increased, but fraction of the complete water phase remained the same within the whole formulations. If other total concentrations of ibuprofen were selected that the concentrations listed in table 30, the amount of ibuprofen increased/decreased in as much as the corresponding amount of oil decreased/increased, but fraction of the complete oil phase remained the same within the whole formulation. If other emulsifier concentrations were selected (3%, 5% or 10%), the concentration of oil also changed, but fraction of the lipophilic phase remained the same within the whole formulation. If thickener Aerosil 200 was added to the oil formulations in order to form organogels, the added percentage referred to the ratio of emulsifier Isolan PDI to oil component. Pure saturated caffeine oil with or without emulsifier Isolan PDI was obtained by adding an excess of caffeine to the homogeneous oily base. These solutions were stirred for 24h at 32°C and filtrated afterwards. The saturated oils were kept at 32°C to avoid drug precipitations before further use.

Table 29. Composition of the study formulations (in weight-%) for caffeine

	E70[1]	**E50[1]**	**E30[1]**	**Oil[2]**	**Oil + 4.5% Isolan PDI**	**Oil + 7.7% Isolan PDI**	**Oil + 11.1% Isolan PDI**	**Oil + 20.0% Isolan PDI**
Caffeine[3,4,5]	0.2	0.2	0.2	Saturated	Saturated	Saturated	Saturated	Saturated
Sodium Chloride	0.5	0.5	0.5	-	-	-	-	-
Water	69.3	49.3	29.3	-	-	-	-	-
Isolan PDI	3	5	5	-	4.29	7.14	10	16.67
Oil[6,7,8,9]	27	45	65	100	95.71	92.86	90	83.33
TOTAL	100	100	100	100	100	100	100	100
$\left[{}^{IsolanPDI}/_{Oil}\right]$ **in %**	11.1	11.1	7.69	0	4.48	7.7	11.1	20

[1] emulsion with 70%, 50% and 30% of dispersed phase, respectively
[2] for isopropyl myristate and paraffinum liquidum oil formulations an addition of 5% Aerosil 200 was examined, for miglyol oil formulations an addition of 3%, 5% and 10% Aerosil 200 was studied (viscosity, apparent permeability)
[3] for isopropyl myristate a total concentration of 1%, 0.25% and 0.08% for E70, 0.08% for E50 and 0.5%, 0.3% and 0.08% for E30 were studied
[4] for miglyol a total concentration of 1%, 0.2% and 0.25% for E70, 0.2% for E50 and 0.3% and 0.5% for E30 were studied
[5] for paraffinum liquidum a total concentration of 0.2% for E70, E50 and E30 were studied
[6] isopropyl myristate (IPM), miglyol 812N (Migl) or paraffinum liquidum (Para)
[7] for isopropyl myristate oil formulations the influence of 7.7%, 11.1% and 20% Isolan PDI was studied
[8] for miglyol oil formulations the influence of 4.5%, 7.7%, 11.1% and 20.0% Isolan PDI was studied
[9] for paraffinum liquidum the influence of Isolan PDI in oil formulations could not be studied (solubility problem)

Table 30. Composition of the study formulations (in weight-%) for ibuprofen

	E70[1,2]	E50[1]	E30[1,3]	Oil[4,5]	Oil + 4.5% Isolan PDI	Oil + 7.7% Isolan PDI	Oil + 11.1% Isolan PDI	Oil + 20.0% Isolan PDI
Sodium Chloride	0.5	0.5	0.5	-	-	-	-	-
Buffer pH 4.5[6]	69.5	49.5	29.5	-	-	-	-	-
Ibuprofen[7]	1	0.5	0.5	1	1	1	1	1
Isolan PDI	26	44.5	64.5	-	4.28	7.12	10.24	17.08
Oil[8]	3	5	5	99	94.72	91.84	88.76	81.92
TOTAL	100	100	100	100	100	100	100	100
$\left[{}^{IsolanPDI}/_{Oil}\right]$ **in %**	11.5	11.24	7.75	0	4.5	7.75	11.5	20.84

[1] emulsion with 70%, 50% and 30% of dispersed phase, respectively
[2] for E70 the influence of 3%, 5% and 10% Isolan PDI on viscosity and apparent permeability were studied with miglyol as oil component
[3] for E30 the influence of 3%, 5% and 10% Isolan PDI on viscosity and apparent permeability were studied with miglyol as oil component, all formulations additionally contained 3% Aerosil 200 as thickener component
[4] for miglyol oil formulations the influence of additional 5% and 7% Aerosil 200 on viscosity and apparent permeability were studied
[5] for isopropyl myristate and paraffinum liquidum oil formulations the influence of 5% Aerosil 200 on apparent permeability was examined
[6] sodium acetate puffer pH 4.5 (buffer capacity β = 0.01)
[7] the influence of 4.5%, 7.7%, 11.1% and 20.0% Isolan PDI for miglyol oil formulations and 7.7% and 20% Isolan PDI for isopropyl myristate oil formulations were studied
[8] isopropyl myristate (IPM), miglyol 812N (Migl) or paraffinum liquidum (Para)

All formulations employed for transport experiments were previously tested for stability. After manufacturing, the formulations were filled into falcon tubes, closed to avoid any possible water loss and left at room temperature for 24h (equilibration time) and at 32°C (water bath) for 48h (duration of the permeation experiment). No phase separation was allowed to occur. w/o-character of the formulations was confirmed by conductivity measurements before and after stability testing. Therefore, a conductometer 660 and a conductivity-measuring cell 60323110 with a cell constant of 0.8cm^{-1} (Metrohm AG, Herisau, Switzerland) were used.

5.2.2.2 Chemical Analysis of the Formulations

Water was assayed by Karl Fischer titration using a KF 701 Titrino (Metrohm AG, Herisau, Switzerland) and a one-component reagent CombiTitrant5. The titrations were performed in CombiSolventOil, a mixture of methanol and toluol to guarantee complete dissolution of the formulation. The titer was exactly determined with double distilled water prior to every analytical run. For water content of 1000 ppm a relative standard deviation between 0.5-1 % has to be assumed. For water content > 1000 ppm a relative standard deviation of < 0.5% has to be assumed.

All model drugs were assayed by HPLC (Hewlett Packard, series 1050, Waldborn, Germany). For caffeine, a reversed phase RP-8 column was used (CC 125/2 Nucleosil 100-5

C8 ec). The mobile phase consisted of 10% acetonitrile and 90% phosphate buffer (pH 7.4, buffer capacity β = 0.01). For ibuprofen a reversed phase RP-18 column (CC125/2 LiChrospher 100-5 RP-18) was used. The mobile phase consisted of 55% acetonitrile and 45% phosphate buffer (pH 2.1, buffer capacity β = 0.02). Detection was performed UV-spectrophotometrically at 275nm and 214 nm for caffeine and ibuprofen, respectively. The flow rate was 0.25 ml/min and the injection volume 10 µl in all cases. For the determination of drug content in the formulation or the oil fraction after ultracentrifugation, samples were prepared by extraction with an at least 100-fold amount of buffer pH 7.4 in the case of caffeine or methanol in the case of ibuprofen. Diluted samples were sonificated for 5 minutes and subsequently centrifuged at 15800 g for 10 minutes. Clear samples were obtained and injected.

5.2.2.3 Viscosity Measurement

Viscosity and flow behaviour of emulsions, oils and organogels with varying amounts of thickener Aerosil 200 and emulsifier Isolan PDI was measured with a rheometer at 32°C (Rotovisco RV 12, Haake, Elektro-Wiget GmbH, Arth, Switzerland). A suitable measuring cylinder and head were selected and filled with formulation. After 1 hour of temperature equilibration, measurement was performed and viscosity was determined for a shear rate of $\gamma = 100\ s^{-1}$ so that different formulations possessing non-newtonian flow behaviour could be compared against each other. For experimental setup, an increase in shear stress was defined for the first 8 minutes and a decrease in shear stress for the following 8 minutes. Maximum shear stress was selected depending on the formulations examined.

5.2.2.4 Freeze Fracture Scanning Electron Microscopy

Freeze fracture scanning electron microscopy was performed in order to estimate the particle size of the dispersed phase droplets within formulations of different phase fractions (70%, 50% and 30% of dispersed phase, respectively) and on the basis of different oils (isopropyl myristate, miglyol 812N and paraffinum liquidum). For specimen preparation a small droplet of the formulation is placed on a specimen table and rapidly frozen in liquid propane / liquid nitrogen. The samples are rapidly cryo-transferred into a freeze-fracturing device, where the fracture is performed at low temperature under high vacuum. The sample will cleave along a fracture plain with the least cohesion. The fracture plane is finally coated with a metal-carbon film before scanning electron microscopic inspection.

5.2.2.5 Saturation Concentration

The solubility of caffeine in water, phosphate buffer (pH 7.4, buffer capacity β = 0.05), isopropyl myristate (IPM), miglyol 812N (Migl) and paraffinum liquidum (Para) with and without emulsifier Isolan PDI was determined as well as the solubility of ibuprofen in sodium acetate buffer (pH 4.5, buffer capacity β = 0.01), phosphate buffer (pH 7.4, buffer capacity β = 0.05), isopropyl myristate (IPM), miglyol 812N (Migl) and paraffinum liquidum (Para) with and without emulsifier Isolan PDI (Iso). Therefore an excess of drug substance was equilibrated with each vehicle. The solutions were left under magnetic stirring overnight at 32°C. Upon equilibration, excess of drug was allowed to sediment / filtered off and all samples were diluted and assayed for model drug as described in 5.2.2.2. Every solubility analysis was performed in replicates of three or more.

5.2.2.6 Evaporation Analysis

In order to figure out if evaporation takes place within w/o-formulations, beakers with predetermined surfaces where placed in water bath (32°C, 48h) and filled with study formulations using a dose of 0.7 g/cm^2 and 0.3 g/cm^2, respectively. Caffeine emulsions that comprised 70%, 50% and 30% of dispersed water phase (E70, E50 and E30, respectively) in miglyol 812N (0.2% total concentration) were selected as study formulation. Ibuprofen emulsions that comprised 70% and 30% of dispersed sodium acetate buffer pH 4.5 in miglyol 812N (1% and 0.5% total concentration for E70 and E30, respectively) were additionally examined. At predetermined time points (0h, 16h, 25h, 40h, 48h) formulations were removed from the beaker and analyzed for water content with Karl Fischer titration. Every single formulation was examined at least three times.

In order to compare evaporation pattern, further evaporation studies were performed using pig ear skin in Franz-type diffusion cells (32°C, 4 8h). The same formulations were examined, but this time only a dose of 0.3 g/cm^2 under open and occlusive conditions was applied. Again, at predetermined time points water content was assayed by Karl Fischer titration. Every single formulation was examined at least three times on skin sheets from three different donors.

5.2.2.7 Permeation Experiments

In all cases, drug permeation was studied in Franz-type diffusion cells with a diffusion surface area between 2.99 - 4.05 cm^2 at 32°C across excised full thickness pig ear skin. The ears of domestic pigs were obtained from a local slaughter house directly post-mortem. The

skin was separated from the cartilage tissue with a scalpel, stored in a freezer at -75°C and used within 4 weeks. To overcome the effect of individual skin variability, every single formulation was examined in at least three permeation experiments. For a single permeation experiment skin of the same pig was used. The receiver medium for the permeation experiments with a volume of 8.5 to 9 ml consisted of an aqueous phosphate buffer solution (pH 7.4, buffer capacity β = 0.05) and 0.1% sodium azide. Skin integrity was tested by measurements of transepidermal water loss (TEWL) (Tewameter TM 210, Courage & Khazaka electronic GmbH, Cologne, Germany) after 4 hours of equilibration. Then, a practically infinite dose of 0.3 g/cm^2 or 0.7 mg/cm^2 formulation, respectively, was applied onto the skin. A dose of 0.3 g/cm^2 was selected in terms of studying evaporation profiles and dosing effects, a dose of 0.7 g/cm^2 was selected in terms of studying occlusive conditions and dosing effects. For occlusive conditions the donor compartment was covered with a rubber stopper. At predetermined time intervals, samples of the receiver compartment were collected and replaced with fresh buffer. The entire duration of one experiment was 48 hours. The samples were analyzed for model drug concentration by HPLC (for conditions see 4.2.2.2) without further treatment except centrifugation for 10 minutes at 15800 g (Eppendorf centrifuge 5415C, Dr. Vaudaux AG, Schönenbuch, Germany).

5.2.2.8 Data Analysis of the Permeation Experiments

For interpretation of permeation data, it is postulated that continuous phase drug concentration C_L of a multi-phase formulation governs permeation kinetics alone. This concentration is given by equation III (see Part II)

$$C_L = \frac{C_{tot}}{\phi_W / K_{L/W} + \phi_L} \quad \text{[equation III]}$$

where C_{tot} denotes overall drug concentration, Φ_W and Φ_L phase fractions of dispersed and continuous phase, respectively, and $K_{L/W}$ drug partition coefficient between oil and water phase. Assuming the conditions of a perfect sink (receiver concentration negligible), an infinite donor reservoir, assuring a constant continuous phase drug concentration C_L, rate limiting membrane (skin) diffusion with the diffusion coefficient D and drug distribution between the skin and the continuous phase of the formulation with the distribution coefficient $K_{SC/L}$, drug flux J may be described by Fick's first law of diffusion. Drug flux J is defined as the amount of drug permeated through the skin per unit time and unit area and is given by:

$$J = P \cdot C_L$$

with P as the permeability coefficient (cm/s) and h as thickness of the diffusion rate limiting membrane.

$$P = \frac{D \cdot K_{SC/L}}{h}$$

Combining both formulas lead to equation IV (see Part II):

$$J = \frac{C_L \cdot D \cdot K_{SC/L}}{h} \quad \text{[equation IV]}$$

Due to possible evaporation of volatile formulation components following a non-occlusive application, the composition of the vehicle may change. A model is developed to interpret the permeation data that considers the situation within the formulations, i.e. partitioning of the drug and phase ratio of dispersed to continuous phase of a multi-phase formulation subjected to non-occlusive conditions (see 5.3.2.4.1).

5.3 Results

5.3.1 Characterization of the Formulations

5.3.1.1 Viscosity Measurement

To guarantee a long-life stability of emulsions, the addition of thickeners or the alteration of emulsifier content can be advisable. Hence, various vehicles with varying amounts of Aerosil 200 and Isolan PDI were manufactured. The altered viscosity was measured and correlations were established (Table 31). For non-newtonian flow behaviour viscosity η at D = 100 1/s is shown.

Table 31. Measured viscosity of isopropyl myristate, miglyol, paraffinum liquidum and emulsions comprising 30% and 70% of dispersed water in miglyol (E30 and E70) in dependence of emulsifier content and thickener content

	Isolan PDI (%)	Aerosil 200 (%)	η measured at D = 100 s^{-1} (average)	SD[1]	Flow behaviour
IPM[2]	-	-	6.71	5.44	Newtonian
Migl[3]	-	-	22.78	9.51	Newtonian
Para[4]	-	-	72.35	7.97	Newtonian
Migl	-	5	423.97	33.51	Newtonian
Migl	-	7	847.93	18.59	Newtonian
E30 Migl	5	-	67.98	3.10	Non-Newtonian
E30 Migl	3	3	279.96	22.50	Non-Newtonian
E30 Migl	5	3	343.47	47.80	Non-Newtonian
E30 Migl	10	3	252.23	9.30	Non-Newtonian
E70 Migl	3		1075.48	90.65	Non-Newtonian
E70 Migl	5		1179.33	183.86	Non-Newtonian
E70 Migl	10		2397.65	155.33	Non-Newtonian
E70 IPM	3	-	233.52	55.60	Non-Newtonian

[1] standard deviation (n = 3 – 4)
[2] isopropyl myristate
[3] miglyol 812N
[4] paraffinum liquidum

As illustrated in table 31 oils with newtonian flow behaviour differed strongly in their viscosity. Isopropyl myristate possessed the smallest viscosity, whereas the viscosities were approximately 3-fold and 10-fold higher for miglyol 812N and paraffinum liquidum, respectively. Macroscopically, this difference could also be observed for formulations E70, E50 and E30 manufactured with these oils. Formulations on the basis of isopropyl myristate possessed lotion like appearance, while emulsions on the basis of miglyol and paraffinum liquidum had cream or paste like appearance. Within one series comprising the same oil component (E70, E50, E30 and oil), viscosity always increased as the amount of dispersed

phase was raised. Emulsifier content only exerted an influence on an emulsion comprising 70% of dispersed water phase.

If Aerosil 200 was added to miglyol 812N in order to produce organogels, the observed viscosity increased with the amount of thickener added (see data table 31). For E70 miglyol 812N with increasing amounts of added emulsifier an increase of viscosity was observed, but not for E30 Miglyol 812N.

Isolan PDI possesses long polymeric carbon chains that, within w/o-emulsions, project into continuous oil phase if interface is enriched with emulsifier. Within E70, packing of the droplets is denser than in E50 and E30, respectively (see 5.3.1.2). The close alignment of the droplets facilitates possible interaction of the polymeric carbon chains with the consequence of an increased viscosity and subsequently a better stability.

5.3.1.2 Estimation of Particle Size by Freeze Fraction Scanning Electron Microscopy

Freeze fracture scanning electron microscopy was performed to reveal the microstructure present within the studied formulations and especially to gain an idea about the particle sizes. Freeze fracture scanning electron microscopy was performed for E70 using isopropyl myristate, miglyol 812N and paraffinum liquidum as oil components. Concerning E50 and E30, only miglyol 812N and paraffinum liquidum as oil components were analyzed as probes with isopropyl myristate did not form a droplet on the specimen table and therefore could not be subjected to freeze fracture. Figure 23 shows the microscopic pictures obtained with a roughly approximation of particle sizes (see table 32).

Table 32. Estimation of particle size based on freeze fracture scanning electron microscopy pictures (diameter in µm)

	Isopropyl myristate	Miglyol 812N	Paraffinum liquidum
E70[1]	2	1.5	1
E50[1]	n.m.[2]	2	1.5
E30[1]	n.m.[2]	3	1.5

[1] emulsion with 70%, 50% and 30% of dispersed phase, respectively
[2] not measurable

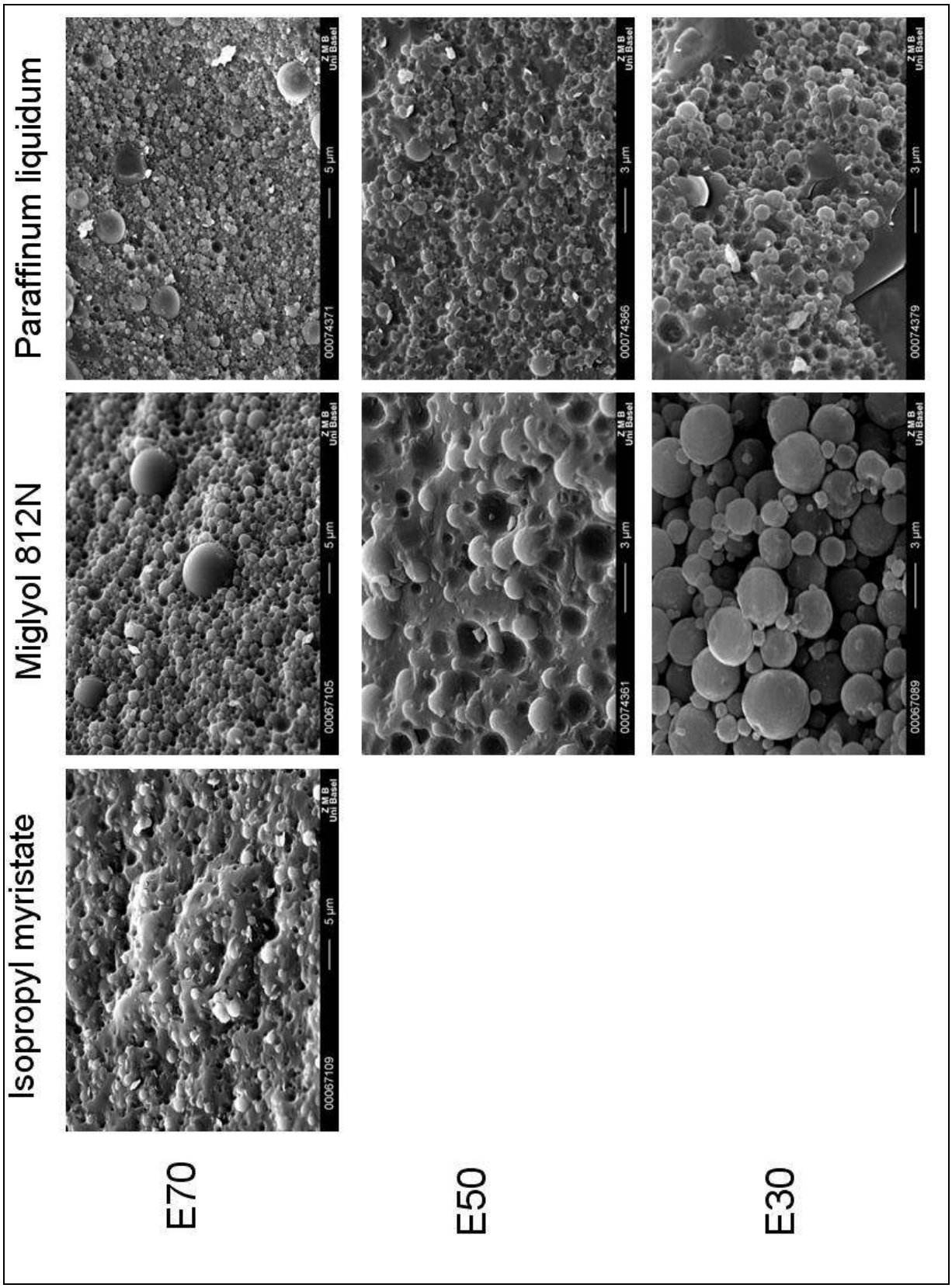


Figure 23. Microscopic pictures of the study formulations by freeze fracture scanning electron microscopy (see also appendix)

5.3.1.3 Saturation Concentration

The values obtained for saturation concentration determination are depicted in table 33 for caffeine and table 34 for ibuprofen, respectively.

Table 33. Saturation concentration of caffeine in various vehicles determined at 32°C

Vehicle	Emulsifier (%)	Saturation concentration ± SD(µg/g) [1]
Isopropyl myristate	0	1248.06 ± 26.23
Isopropyl myristate	4	1482.13 ± 15.00
Isopropyl myristate	7	1482.72 ± 127.19
Isopropyl myristate	10	1393.46 ± 21.01
Isopropyl myristate	16	1607.77 ± 31.66
Miglyol 812 N	0	2476 ± 127.14
Miglyol 812 N	4	2707.11 ± 84.76
Miglyol 812 N	7	2659.07 ± 144.78
Miglyol 812 N	10	2514.18 ± 235.22
Miglyol 812 N	16	2776.18 ± 257.36
Paraffinum liquidum	0	120.14 ± 5.28
Water	0	30789.47 ± 633.10
Phosphate buffer pH 7.4 (acceptor solution)	0	25628.10 ± 260.36

[1] all values denote mean ± standard deviation. n = 3 - 15

Concerning saturation concentration of caffeine in various vehicles, it is obvious that its solubility is much higher in hydrophilic solutes (water, phosphate buffer pH 7.4) than in lipophilic solutes. Saturation concentration of caffeine in oily vehicles showed highest solubility in miglyol 812N, followed by isopropyl myristate and paraffinum liquidum. For isopropyl myristate and miglyol 812N saturation concentration of caffeine was not affected by the presence of varying amounts of emulsifier Isolan PDI (table 33).

Table 34. Saturation concentration of ibuprofen in various vehicles at 32°C

Vehicle	Emulsifier (%)	Saturation concentration ± SD[1](mg/g)
Isopropyl myristate	0	163.72 ± 7.80
Isopropyl myristate	4	153.23 ± 1.87
Isopropyl myristate	7	159.55 ± 7.17
Isopropyl myristate	20	147.19 ± 4.41
Isopropyl myristate	52	152.14 ± 2.42
Miglyol 812 N	0	142.03 ± 2.66
Miglyol 812 N	4	149.94 ± 1.35
Miglyol 812 N	7	188.09 ± 5.97
Miglyol 812 N	11	142.90 ± 12.19
Paraffinum liquidum	0	25.50 ± 1.19
Sodium acetate buffer pH 4.5	0	0.127±0.0003
Phosphate buffer pH 7.4 (acceptor solution)	0	n.m.[2]

[1] all values denotes mean ± standard deviation. n = 3 - 4
[2] not measured

Saturation concentration of ibuprofen possessed highest values for isopropyl myristate and was similar to saturation concentration in miglyol 812N. Nevertheless, these concentrations were approximately 6 times greater than the concentrations observed for paraffinum liquidum. Concerning solubility in hydrophilic solutes, e.g. sodium acetate buffer pH 4.5 representing the dispersed phase of all ibuprofen formulations, saturation was quickly achieved (127.19 µg/g). Isolan PDI did not strongly affect saturation concentration neither in case of isopropyl myristate nor in case of miglyol 812N (table 34). The influence of emulsifier was not studied for paraffinum liquidum as solubility of Isolan PDI was very low.

5.3.1.4 Evaporation as a function of Time and Dose

Evaporation of w/o-emulsions was studied as a function of time and dose with miglyol 812N as a representative oil component. Beakers of defined surfaces were filled with the formulation and remained at 32°C (water bath heating) for 48 hours under non-occlusive conditions. Figure 24 describes the water content as a function of time and dose for E70 and E30 received from these examinations. Concerning a dose of 0.7 g/cm^2 and 0.3 g/cm^2, water loss was observed, although it is more pronounced for E70 than for E30. The percentage of water loss related to original water content is 16.27% and 18.55% for E70 and E30, respectively, if a dose of 0.7g/cm^2 was applied. Reduction of the dose and, hence, vehicle layer thickness resulted in an approximately 2-fold greater water loss related to original water content (%): 44.06% for E70 and 33.48% for E30, respectively. Besides, figure 24 nicely describes continuing water loss over 48h which is equal to the duration of performed transport experiments. Similar results were obtained if ibuprofen formulations using miglyol 812N as oil component were studied instead of caffeine (datas not shown).

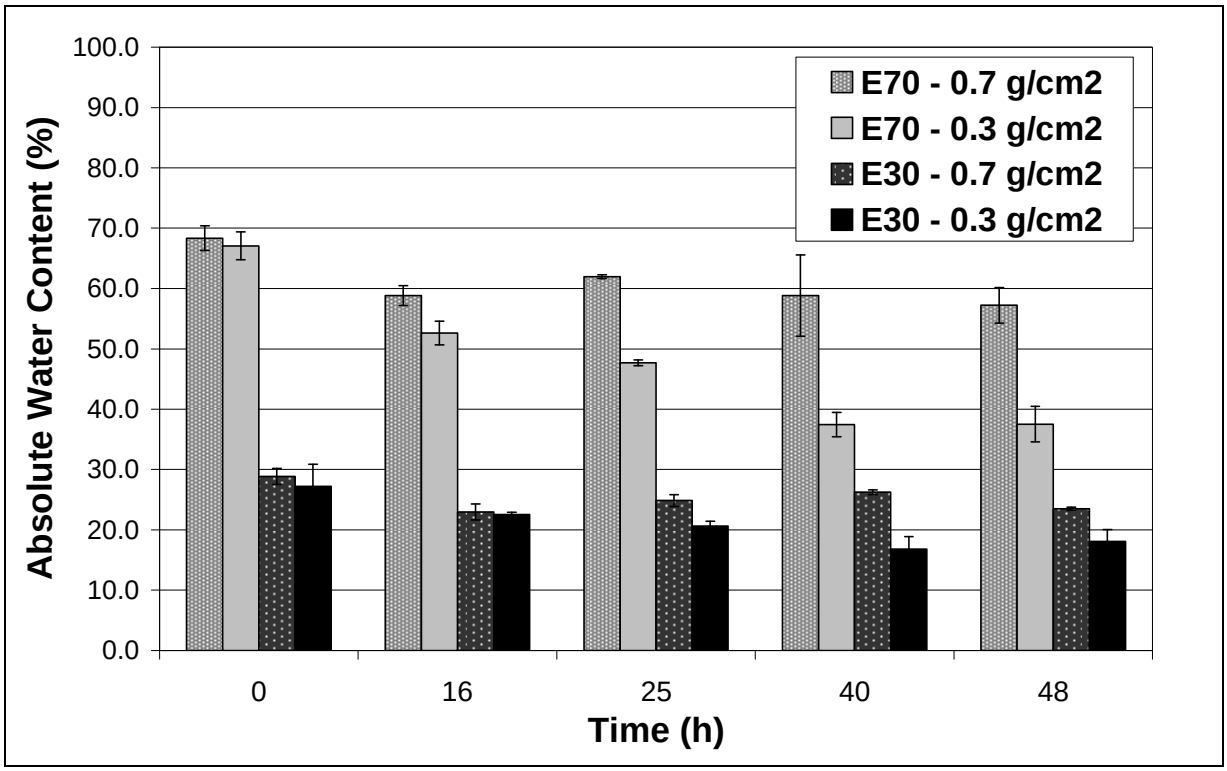


Figure 24. Evaporation in dependence of time and dosage for caffeine emulsions comprising 70% and 30% of dispersed water phase in miglyol oil (E70, E30) studied in beaker under non-occlusive conditions. Error bars denote standard deviation (n = 3 - 5).

Due to the results obtained in figure 24, a dose of 0.3 g/cm^2 was selected for all further evaporation studies. Evaporative changes within dermatological vehicles may differ, dependent on whether water loss of a formulation is studied in beaker or on skin mounted in Franz-type diffusion cells. In the transport cells, water resulting from an upward diffusion out of the receiver compartment can be emulsified by the formulations applied onto the skin. Figure 25 compares the changes formulations undergo in dependence of experimental setup, i.e. beaker or skin, using a dose of 0.3 g/cm^2. For all three formulation types examined, E70, E50 or E30, there was a clear difference between occlusive and open conditions. Water loss in dependence of time was the same for experiments performed in beaker or on skin (t-test, two-sided, p=0.95). If water is lost within a formulation due to evaporative processes, this water is not replaced by water uptake from an upward diffusion process. Additionally, possible alteration oil vehicles may undergo was studied. Miglyol 812N was applied onto skin mounted into diffusion cells with a dose of 0.1 g/cm^2. The vehicle contained 7.75% of emulsifier and, hence, was similar to continuous phase of formulation E30 comprising 30% dispersed water phase and 5% Isolan PDI (ratio emulsifier content over oil content). The mixture of oil and emulsifier facilitates possible emulsification of water received from upward diffusion from the receiver compartment. However, neither under

occlusive nor under open conditions, a significant water uptake was observed (datas not shown). This result is in good agreement with the conclusion drawn from figure 25.

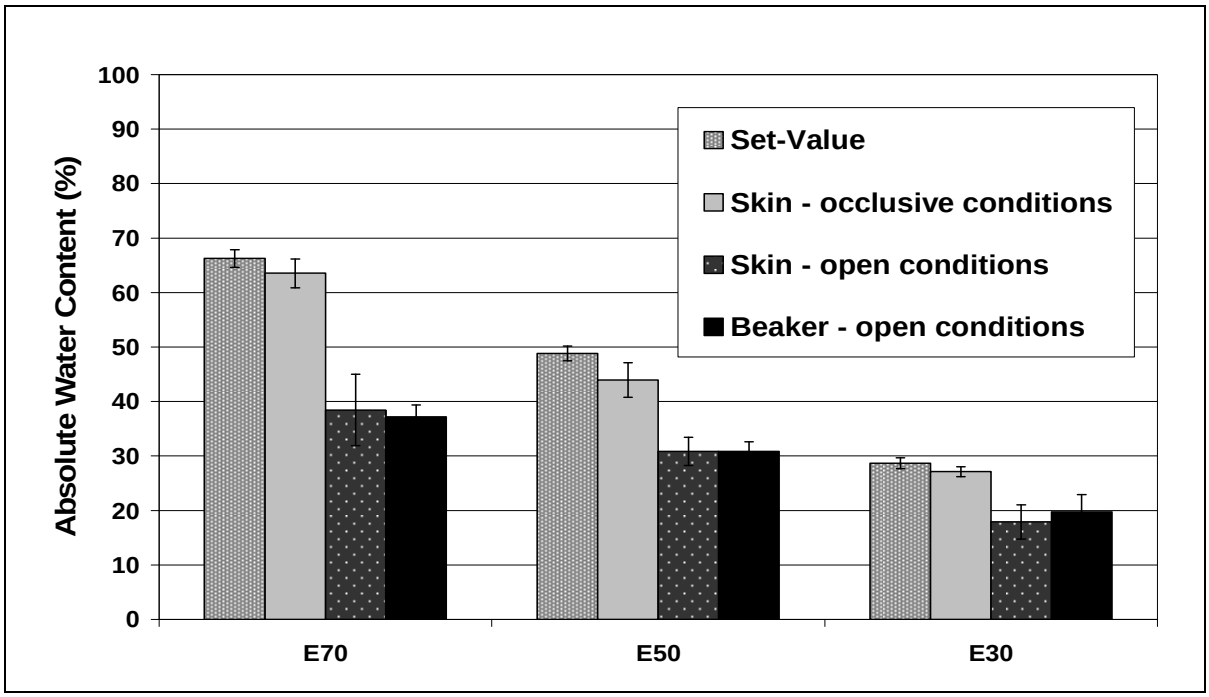


Figure 25. Absolute water content (%) after 48h studied in dependence of experimental setup for caffeine emulsions comprising 70%, 50% and 30% of dispersed water phase in miglyol oil (E70, E50, E30). Dose: 0.3 g/cm^2. Error bars denote standard deviation (n = 3 - 6).

Knowledge about alteration of water content within dermatological vehicles is necessary in terms of determining continuous phase drug concentration C_L^t taking into consideration evaporative processes and drug permeation behaviour over time (see 5.3.2.4). Table 35 and 36 depict the absolute water loss of various formulations within 48h applied under open conditions:

Table 35. Water loss (%)of dermatological caffeine vehicles (total drug concentration 0.2%) applied non-occlusively onto skin (dose 0.3 g/cm^2, application time 48h) compared to original value

Original formulation	E70[1]	E50[1]	E30[1]
Water loss [%][2]	41.95	36.86	37.54
New formulation	E38	E31	E18

[1] emulsions comprising 70%, 50% and 30% of dispersed phase, respectively, in miglyol oil
[2] percentage related to original water content

Table 36. Water loss (%) of dermatological ibuprofen vehicles (total drug concentration 1% for E70 and 0.5% for E30, respectively) applied non-occlusively (dose 0.3 g/cm^2, application time 48h) compared to original value

Original formulation	E70[1]	E50	E30[1]
Water loss [%][2]	45.16	n.m.[2]	39.55
New formulation	E36	n.m.[3]	E18

[1] emulsions comprising 70%, 50% and 30% of dispersed phase, respectively, in miglyol oil
[2] percentage related to original water content
[3] not measured

Independently of the amount of water dispersed in oil, the percentage of water loss related to original water content was approximately 40% within 48h. Obviously, kinetic of evaporation is similar for w/o-emulsions comprising different droplet sizes and droplet arrangements.

5.3.2 Permeation Experiments

5.3.2.1 Influence of Thickener Aerosil 200 on Transdermal Permeation

The influence of thickener Aerosil 200 on apparent permeability coefficient P_{app} was studied for caffeine and ibuprofen in pure oil formulations. Results obtained are shown in table 37 and 38.

Aerosil 200 did not exert any statistically significant effect on apparent permeability coefficient P_{app} regarding ibuprofen in isopropyl myristate, miglyol 812N or paraffinum liquidum vehicles as confirmed by a two-sided t-test (p=0.95). For paraffinum liquidum P_{app} was 1.5-fold bigger if transport experiments were performed without Aerosil 200. Concerning the oils alone, apparent permeability coefficient P_{app} of ibuprofen was in the same order of magnitude for isopropyl myristate and paraffinum liquidum and approximately two times greater than miglyol 812N.

Table 37. Aerosil 200 and its influence on apparent permeability coefficient P_{app} of ibuprofen in oil formulations

	Aerosil 200 [%]	P_{app} ± SEM (cm/sec)[1]
IPM[2]	0	$3.08 \cdot 10^{-7} \pm 2.47 \cdot 10^{-8}$
IPM[2]	5	$2.70 \cdot 10^{-7} \pm 1.30 \cdot 10^{-8}$
MigI[3]	0	$1.30 \cdot 10^{-7} \pm 1.13 \cdot 10^{-8}$
MigI[3]	5	$1.18 \cdot 10^{-7} \pm 1.70 \cdot 10^{-8}$
MigI[3]	7	$1.30 \cdot 10^{-7} \pm 1.14 \cdot 10^{-8}$
Para[4]	0	$2.96 \cdot 10^{-7} \pm 2.36 \cdot 10^{-8}$
Para[4]	5	$1.96 \cdot 10^{-7} \pm 1.51 \cdot 10^{-8}$

[1] all values denote mean ± standard error. n = 5 – 22
[2] isopropyl myristate
[3] miglyol 812N
[4] paraffinum liquidum

Table 38. Aerosil 200 and its influence on apparent permeability coefficient P_{app} of caffeine

	Aerosil 200 [%]	P_{app} ± SEM[1] (cm/sec)
IPM[2]	0	$3.81 \cdot 10^{-6} \pm 5.91 \cdot 10^{-7}$
IPM[2]	5	$3.89 \cdot 10^{-7} \pm 3.27 \cdot 10^{-8}$
MigI[3]	0	$8.26 \cdot 10^{-7} \pm 2.58 \cdot 10^{-7}$
MigI[3]	5	$1.26 \cdot 10^{-7} \pm 1.07 \cdot 10^{-8}$
Para[4]	0	$1.86 \cdot 10^{-6} \pm 2.05 \cdot 10^{-7}$
Para[4]	5	No permeation measured

[1] all values denote mean ± standard error. n = 5 – 21
[2] isopropyl myristate
[3] miglyol 812N
[4] paraffinum liquidum

Concerning P_{app} of caffeine, Aerosil 200 exerted an effect on transdermal permeation. The thickener led to a 10-fold reduction of P_{app} in case of isopropyl myristate and an 8-fold reduction in case of miglyol 812N. Caffeine dissolved in paraffinum liquidum possessed a comparably high permeability, but after addition of Aerosil 200, no permeation could be observed at all. Concerning the oils alone, apparent permeability coefficients of isopropyl myristate was 2-times greater than for paraffinum liquidum and 4.5-fold greater than for miglyol 812N.

Additionally, influence of Aerosil 200 was studied in two w/o - formulations with a dispersed phase content of 30% (E30), as these vehicles showed a tendency for instability and, thus, increased viscosity would have been favoured. However, Aerosil 200 reduced P_{app} here as well, although reduction was at most 2-fold (see table 39).

Table 39. Aerosil 200 and its influence on apparent permeability coefficient P_{app} of caffeine in E30[1]

	Aerosil 200 [%]	P_{app} ± SEM[2] (cm/sec)
E30 IPM[3]	0	$1.11 \cdot 10^{-6} \pm 1.57 \cdot 10^{-7}$
E30 IPM[3]	3	$5.89 \cdot 10^{-7} \pm 3.50 \cdot 10^{-8}$
E30 Migl[4]	0	$3.47 \cdot 10^{-7} \pm 3.21 \cdot 10^{-8}$
E30 Migl[4]	3	$2.42 \cdot 10^{-7} \pm 1.66 \cdot 10^{-8}$

[1] emulsion with 30% of dispersed phase
[2] all values denote mean ± standard error. n= 4 – 7
[3] isopropyl myristate
[4] miglyol 812N

5.3.2.2 Influence of Emulsifier Content on Transdermal Permeation

5.3.2.2.1 Caffeine and Isolan PDI

In the w/o-emulsions studied, Isolan PDI enriches itself at the interfaces between continuous oil phase and dispersed water droplets. As it is not clear if free emulsifier is also present in the continuous oil phase, its possible influence on drug permeation was studied. For that purpose, varying amounts of emulsifier were dissolved in either isopropyl myristate (IPM) or miglyol 812N (Migl). The amounts selected were in the same order of magnitude or similar to the ratio of Isolan PDI content over oil content of the studied formulations (see table 29). Neither in case of IPM nor in case of Migl, a statistically significant difference was observed for the vehicles studied (t-test, 2-sided, p=0.95). P_{app} values are shown in table 40. As Paraffinum liquidum possessed only small solubility for Isolan PDI, studies could not be carried out with an oil-emulsifier mixture.

Table 40. Isolan PDI and its influence on apparent permeability coefficient P_{app} of caffeine studied in oil formulations comprising varying amounts of emulsifier Isolan PDI

	Isolan PDI [%]	P_{app} ± SEM[1]
IPM[2]	0	$3.81 \cdot 10^{-6} \pm 5.91 \cdot 10^{-7}$
IPM[2]	7.7	$3.05 \cdot 10^{-6} \pm 4.45 \cdot 10^{-7}$
IPM[2]	11.1	$2.83 \cdot 10^{-6} \pm 4.97 \cdot 10^{-7}$
Migl[3]	0	$8.26 \cdot 10^{-7} \pm 2.58 \cdot 10^{-7}$
Migl[3]	4.3	$6.97 \cdot 10^{-7} \pm 7.55 \cdot 10^{-8}$
Migl[3]	7.7	$6.32 \cdot 10^{-7} \pm 1.46 \cdot 10^{-7}$
Migl[3]	11.1	$8.07 \cdot 10^{-7} \pm 2.02 \cdot 10^{-7}$

[1] all values denote mean ± standard error. n = 4 – 5
[2] isopropyl myristate
[3] miglyol 812N

5.3.2.2.2 Ibuprofen and Isolan PDI

Varying amounts of emulsifier were dissolved in either isopropyl myristate (IPM) or miglyol 812N (Migl). Again, the ratio of Isolan PDI content over oil content was in the same order of magnitude as for the studied formulations (see table 30). Neither in case of IPM nor in case of Migl, a statistically significant difference was seen for the vehicles studied (t-test, 2-sided, p=0.95). P_{app} values are shown in table 41. As Paraffinum liquidum possesses only small solubility for Isolan PDI, studies could not be carried out with this oil component.

Table 41. Isolan PDI and its influence of apparent permeability coefficient P_{app} of ibuprofen in oil and emulsion comprising varying amounts of Isolan PDI

	Isolan PDI [%]	P_{app} ± SEM (cm/sec)[1]
IPM[2]	0	$3.08 \cdot 10^{-7} \pm 3.20 \cdot 10^{-8}$
IPM[2]	7.75	$2.52 \cdot 10^{-7} \pm 1.57 \cdot 10^{-8}$
IPM[2]	20.83	$2.75 \cdot 10^{-7} \pm 1.28 \cdot 10^{-8}$
Migl[3]	0	$1.33 \cdot 10^{-7} \pm 1.29 \cdot 10^{-8}$
Migl[3]	4.5	$1.18 \cdot 10^{-7} \pm 7.79 \cdot 10^{-9}$
Migl[3]	7.75	$1.07 \cdot 10^{-7} \pm 1.86 \cdot 10^{-8}$
Migl[3]	11.5	$1.08 \cdot 10^{-7} \pm 1.95 \cdot 10^{-8}$
Migl[3]	20.83	$9.96 \cdot 10^{-8} \pm 1.53 \cdot 10^{-8}$
E30 Migl[3,4]	3	$1.28 \cdot 10^{-7} \pm 2.79 \cdot 10^{-8}$
E30 Migl[3,4]	5	$1.10 \cdot 10^{-7} \pm 1.04 \cdot 10^{-8}$
E30 Migl[3,4]	10	$8.19 \cdot 10^{-8} \pm 1.66 \cdot 10^{-8}$
E70 Migl[3,5]	3	$2.09 \cdot 10^{-7} \pm 2.80 \cdot 10^{-8}$
E70 Migl[3,5]	5	$2.23 \cdot 10^{-7} \pm 4.28 \cdot 10^{-8}$
E70 Migl[3,5]	10	$1.69 \cdot 10^{-7} \pm 2.80 \cdot 10^{-8}$

[1] all values denote mean ± standard error. n = 4 - 11
[2] isopropyl myristate
[3] miglyol 812N
[4] emulsion with 30% of dispersed water phase
[5] emulsion with 70% of dispersed water phase

5.3.2.3 Influence of Dose on Transdermal Permeation

Transdermal permeation behaviour was studied with two infinite doses of 0.7 g/cm^2 and 0.3 g/cm^2, respectively. The idea was to evaluate whether a reduced vehicle layer thickness might affect apparent permeability coefficient P_{app}. A dose below 0.3 g/cm^2 was not feasible in terms of assuring constant continuous phase drug concentration of the vehicle over the entire duration of a transport experiment, i.e. 48h. Furthermore, this dose was handy with respect to determining water loss in the remaining formulations if non-occlusive conditions were examined (see 5.3.2.4).

Neither for caffeine nor for ibuprofen an alteration in apparent permeability coefficient P_{app} was observed for all examined formulations if dose and, hence, vehicle layer thickness applied onto the skin, was reduced (occlusive conditions, see figure 26 und 27). This was confirmed by a two-sided t-test (p=0.95).

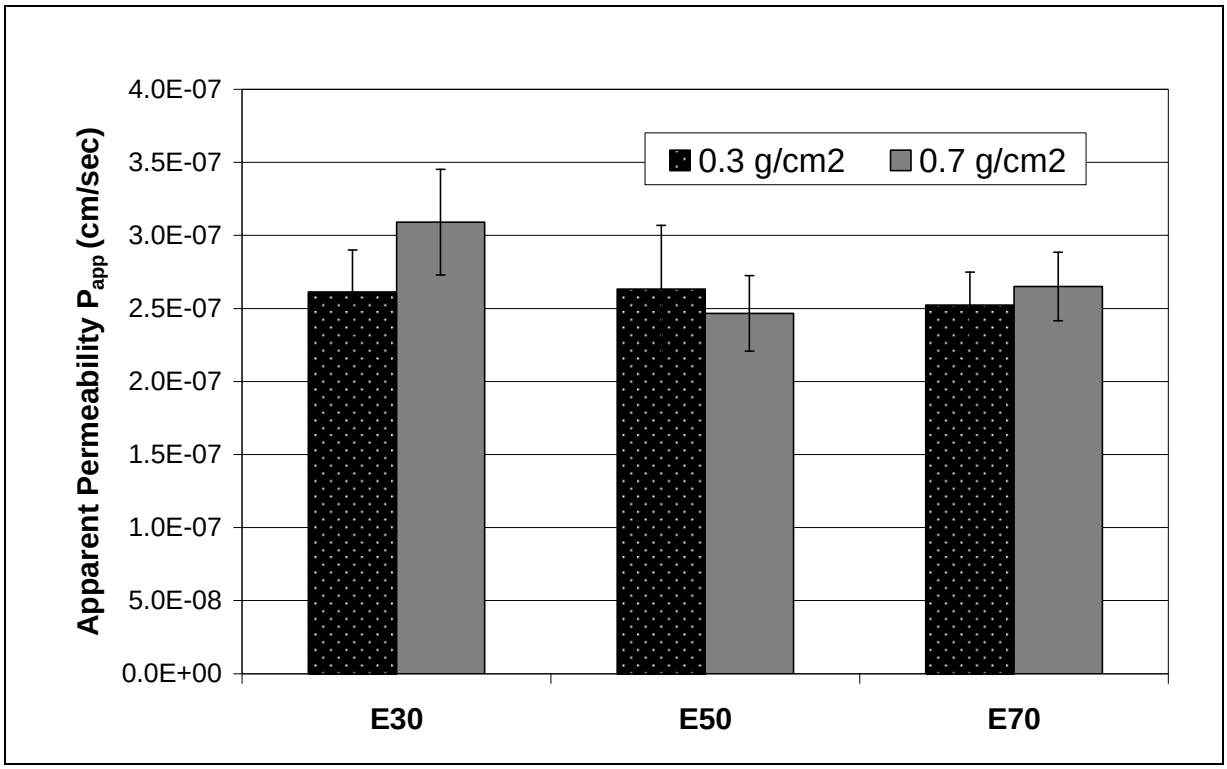


Figure 26. Effect of dose on transdermal permeation of occlusive applied caffeine emulsions comprising 70%, 50% and 30% of dispersed phase in miglyol (E70, E50, E30). Error bars denote standard error of mean (n = 3 - 6).

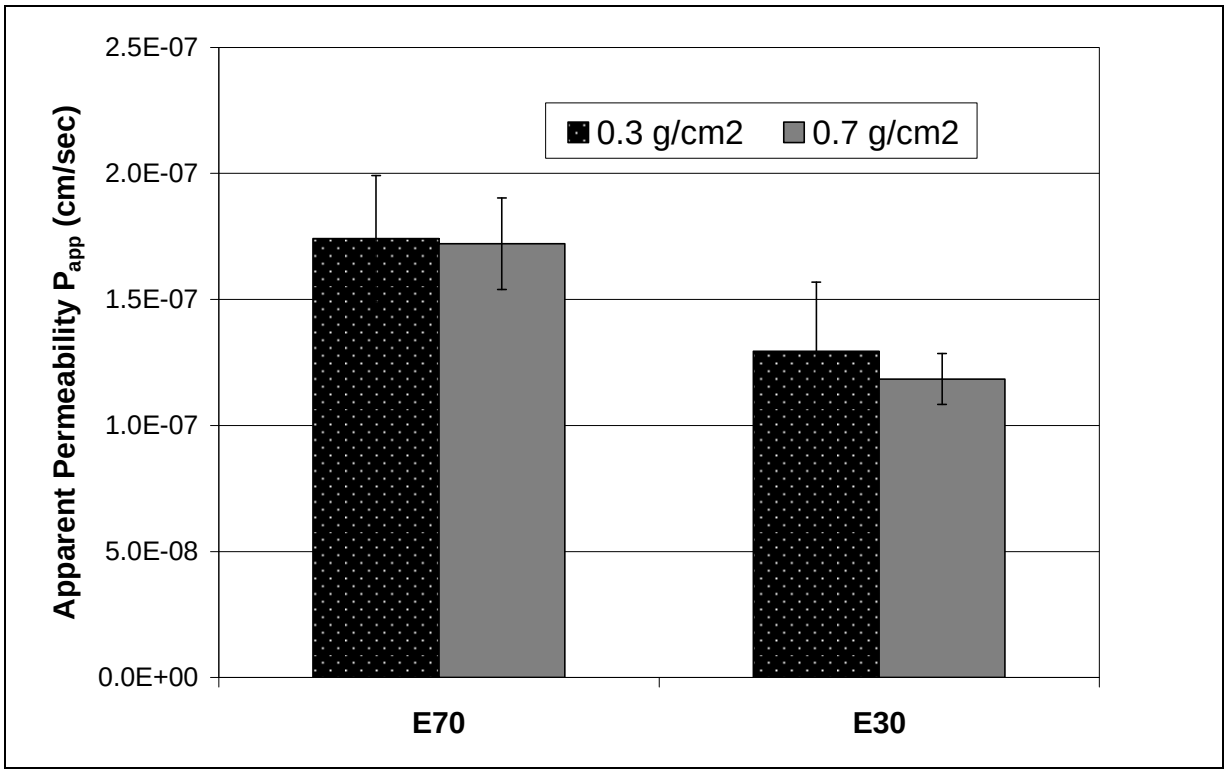


Figure 27. Effect of dose on transdermal permeation of occlusive applied ibuprofen emulsions comprising 70% and 30% of dispersed phase in miglyol. Error bars denote standard error of mean (n = 3 - 9).

5.3.2.4 Influence of Evaporation on Transdermal Permeation

5.3.2.4.1 Transport Kinetic

The influence of dermatological w/o-formulations on in vitro caffeine and ibuprofen permeation was investigated taking especially into consideration alterations these formulations undergo due to evaporation of volatile components following a non-occlusive application. As continuous phase drug concentration is the decisive parameter for skin drug permeation (see Part II), an equation is derived that allows estimation of the change of continuous phase drug concentration in w/o-emulsions caused by the evaporation of volatile ingredients, i.e. water.

The drug concentration within distinct phases of a multi-phase formulation is given by:

$$C_{tot} = \phi_W \cdot C_W + \phi_L \cdot C_W \quad \text{[equation VI]}$$

where C_{tot} is the overall drug concentration in the formulation, C_W and C_L are the concentrations of the dispersed water and the continuous lipid phase, respectively, with Φ_W

and Φ_L as the mass-fraction of the dispersed and continuous phase, respectively. These mass fractions are given by:

$$\phi_L = \frac{V_L}{V_{tot}} \quad \text{and} \quad \phi_W = 1 - \phi_L = \frac{V_W}{V_{tot}}$$

where V_{tot}, V_W and V_L denote the volume of the total formulation, of the dispersed and of the continuous phase, respectively. The distribution of a drug between the dispersed and the continuous phase can be expressed as the partition coefficient $K_{W/L}$ (see Part I, 4.2.2.3):

$$K_{W/L} = \frac{C_W}{C_L} \quad \text{[equation VIII]}$$

By combination of equation VI and VIII the term for calculation of continuous phase drug concentration is derived (see also Part I, 4.3.1.3):

$$C_L = \frac{C_{tot}}{\phi_W (K_{W/L} - 1) + 1} \quad \text{[equation IX]}$$

Equation IV is of general validity. Some additional considerations are necessary to apply them to the situation of non-occlusive application. Over time the fractions Φ_W and Φ_L may change, which, in turn, affects the concentrations C_{tot}, C_W and C_L. Hence, the superscript ^{t} is introduced to denote the arising conditions during evaporation, and the superscript ^{0} will symbolize the situation in the according original formulation for further discussion. Then, during evaporation, the following correlations may be set up:

$$C_{tot} = \frac{M_{tot}}{V_W + V_L} \quad \text{[equation XVII]}$$

with $\phi_W = \frac{V_w}{V_{tot}}$; $\phi_L = \frac{V_L}{V_{tot}}$ and $\phi_W + \phi_L = 1$. It follows:

$$\frac{C_{tot}^t}{C_{tot}^0} = \frac{1 - \phi_w^t}{1 - \phi_W^0} \quad \text{[equation XVIII]}$$

Finally, the combination of equations IX, XVII and XVIII offers a general term that allows calculating continuous phase drug concentration at a specific state during evaporation of a multi-phase w/o-formulation:

$$C_L^t = C_L^0 \cdot \frac{M_{tot}^t}{M_{tot}^0} \cdot \frac{1-\phi_W^t}{1-\phi_W^0} \cdot \frac{\left[\phi_W^0 (K_{W/L} - 1) + 1\right]}{\left[\phi_W^t (K_{W/L} - 1) + 1\right]} \quad \text{[equation XIX]}$$

This formula was applied to the permeation data of caffeine in E70, E50 and E30 (0.2% total drug concentration) and to the permeation data of ibuprofen in E70 and E30 (1% and 0.5% total drug concentration, respectively) both using miglyol 812N as oil component in order to calculate new arising continuous phase drug concentration. Transport experiments were performed non-occlusively with a dosing of 0.3 g/cm^2.

5.3.2.4.2 Ibuprofen

Concerning the permeation of ibuprofen in E70 and E30 (1% and 0.5% total drug concentration, respectively) with miglyol 812N as oil component, following values in view of to equation XIX were obtained (table 42).

Table 42. Alteration of continuous phase ibuprofen concentration (mg/g) due to evaporation and permeation in w/o-formulations (dose: 0.3 g/cm^2)

Ibuprofen	**E70**[1,2,3] Average ± SD	**E30**[1,2,3] Average ± SD
$\frac{M_{tot}^t}{M_{tot}^0}$	0.92 ± 0.02	0.94 ± 0.02
C_L^0	33089.33 ± 45.56	6954.59 ± 129.62
C_L^t	40806.59 ± 822.71	6950.47 ± 275.82
Change of C_L	**+ 23.32 %**	**- 0.06 %**

[1] emulsion with 70% and 30% dispersed phase in miglyol 812N oil, respectively
[2] total concentration of ibuprofen was 1% for E70 and 0.5% for E30, respectively
[3] al values denote mean ± standard deviation. n = 4 - 5

In total, only 8% of ibuprofen for E70 and 6% for E30 permeated into and through the skin within 48h. In the same time, formulations lost 45.16% of water referred to the original water content for E70 and 39.55% for E30, altering them to an E36 and an E18 (see table 36). Accordingly, phase fraction of the continuous phase increased as well as continuous phase drug concentration of ibuprofen which led in part to an increase of continuous phase drug concentration. This consideration is confirmed by the calculated values for C_L^t (see table 42) showing an increase of 23.32% for E70. Apparent permeability coefficient P_{app} did not show

any statistically significant difference for all formulations examined if a dose of 0.3 g/cm^2 for occlusive and non-occlusive conditions was applied (see table 43). This findings were confirmed by a two-sided t-test (p=0.95).

Table 43. Apparent permeability coefficient P_{app} of ibuprofen for open and occlusive conditions

	Open conditions P_{app} ± SEM (cm/sec)[1]	Occlusive conditions P_{app} ± SEM (cm/sec)[1]
E30[2]	$1.28 \cdot 10^{-7} \pm 1.91 \cdot 10^{-8}$	$1.29 \cdot 10^{-7} \pm 2.74 \cdot 10^{-8}$
E70[2]	$1.78 \cdot 10^{-7} \pm 1.80 \cdot 10^{-8}$	$1.74 \cdot 10^{-7} \pm 2.51 \cdot 10^{-8}$

[1] all values denote mean ± standard error. n = 3 - 5
[2] emulsion with 30% and 70% of dispersed phase in miglyol oil

5.3.2.4.3 Caffeine

Concerning the permeation of caffeine in E70, E50 and E30 (0.2% of total drug concentration) with miglyol 812N as oil component, following values were obtained after application of equation XIX (see table 44).

Table 44. Alteration of continuous phase drug concentration due to evaporation and permeation of w/o-formulations (dose: 0.3 g/cm^2)

Caffeine	**E70**[1,2,3] Average ± SD	**E50**[1,2,3] Average ± SD	**E30**[1,2,3] Average ± SD
$\frac{M_{tot}^t}{M_{tot}^0}$	0.90 ± 0.43	0.89 ± 0.05	0.87 ± 0.06
C_L^0	350.61 ± 1.57	456.32 ± 3.14	667.95 ± 5.34
C_L^t	530.21 ± 26.22	765.98 ± 88.61	847.32 ± 55.48
Change of C_L	**+ 51.23%**	**+ 67.86%**	**+26.85%**

[1] emulsion with 70%, 50% and 30% of dispersed phase in miglyol 812N oil
[2] total concentration of caffeine was 0.2% for E70, E50 and E30, respectively
[3] all values denote mean ± standard deviation. n = 5

Water loss of the studied caffeine formulations was 41.95%, 36.86% and 37.54% for E70, E50 and E30, respectively, if referred to the original water content (see table 35). In the course of evaporation continuous oil phase was increased with the consequence that also C_L^0 increased to C_L^t. Continuous phase drug concentration increased 51.23% for E70, 67.86% for E50 and 26.85% for E30. In view of the hypothesis that continuous phase drug concentration governs skin permeation alone, an increased apparent permeability coefficient should be expected (see part II). Observed apparent permeabilities P_{app} for occlusive and non-occlusive conditions did not show any statistically significant difference, if emulsions of the same phase fractions were compared against each other (t-test, two-sided, p=0.95, see table 45). However, overall drug depletion within every caffeine formulation was

underestimated because of drug remaining in the skin. For E70, E50 and E30 depletion was 10%, 11% and 13%, respectively (see table 44), but this depletion was based only on calculation of drug amount in the receiver compartment and did not consider drug amount remaining in the skin.

Table 45. Apparent permeability coefficient of caffeine for open and occlusive conditions

	Open conditions P_{app} ± SEM (cm/sec)[1]	Occlusive conditions P_{app} ± SEM (cm/sec)[1]
E30[2]	$2.74 \cdot 10^{-7} \pm 5.95 \cdot 10^{-8}$	$2.61 \cdot 10^{-7} \pm 2.86 \cdot 10^{-8}$
E50[2]	$2.98 \cdot 10^{-7} \pm 4.47 \cdot 10^{-8}$	$2.63 \cdot 10^{-7} \pm 4.36 \cdot 10^{-8}$
E70[2]	$2.64 \cdot 10^{-7} \pm 4.88 \cdot 10^{-8}$	$2.52 \cdot 10^{-7} \pm 2.25 \cdot 10^{-8}$

[1] All values denote mean ± standard error. n = 4 – 6
[2] emulsion with 30%, 50% and 70% of dispersed phase in miglyol oil

5.4 Discussion

Microscopic pictures obtained by freeze fraction scanning electron microscopy (see 5.3.1.2) revealed that these formulations differed in their microstructure showing a small decrease of droplet size and denser droplet packing as the fraction of the dispersed water phase was increased. This observation was consistent for all formulations studied. The approximated droplet diameters ranged between 0.5 and 4 µm (see figure 23 and table 32). These findings are not astonishing, because already in 1933, Langevin noticed that variation of the proportions of emulsion ingredients influences the size of droplets. Particle size distribution of emulsions is altered by the adjustment of the phase volume ratio, the method of manufacture, temperature and viscosity [42]. It is a general rule that, regarding w/o-emulsions, the larger the water content, the more viscous the systems are [44]. Clément et al. noticed that phase volume ratio in w/o-emulsions plays a role in the variation of apparent viscosity and droplet diameter. An increase in the percentage of disperse phase caused a reduction in droplet diameter and an increase in apparent viscosity. These findings were confirmed by characterization of the formulations used in this study [45].

In addition to decreasing droplet size as the amount of dispersed water was raised, an increase pf viscosity was observed (see table 31). A reduction of the oil content in w/o-emulsions is equivalent to reducing the continuous phase and, by doing so, increasing the interaction between the water droplets in the emulsion. An increase in viscosity is the direct consequence of that. Additionally, the risk of coalescence of water droplets increases as the average distance between the water droplets further decreases [46]. Formulations comprising same phase fractions but different oil components possessed macroscopically different viscosity, following the increasing viscosity of isopropyl myristate, miglyol 812N and paraffinum liquidum.

Thickener Aerosil was able to increase viscosity and therefore also stability. However, it turned out that Aerosil showed strong interaction with hydrophilic model drug caffeine, leading to a tremendous decrease of apparent permeability coefficient P_{app}. Permeation parameters such as diffusivity in a formulation and partitioning from formulation to skin are easily altered by the composition of the formulation, as already stated by Diez-Sales et al. who studied the in vitro percutaneous penetration of acyclovir form solvent systems and carbopol hydrogels in order to figure out the extent of changed viscosity and varying propylene glycol amounts on apparent permeability coefficient[10].

All formulations examined were previously arranged in transport series comprising same ingredients but different ratios these components were intermixed with each other (E70, E50, E30 and oil as reference formulation). In order to implement continuous phase drug concentration concept to the formulations of a transport series (see Part II), particular attention had to be paid to the emulsifier content present in each vehicle.

In this context there are two options how to consider emulsifier content in each vehicle. First, emulsifier content employed in each vehicle is related to the specific surface area of the emulsion droplets present in each emulsion. Second, ratio of emulsifier content over oil content is calculated for each vehicle employed.

Specific surface area of the water droplets present in the w/o-emulsions of varying phase fractions was computed after the following equation XX:

$$\frac{4r^2\Pi}{\frac{4}{3}r^3\Pi}\cdot\phi_W \quad \text{[equation XX]}$$

where the counter denotes droplet surface and the denominator denotes droplet volume with radius r. Φ_W represents the phase fraction of dispersed water phase. Equation XXI was obtained after simplifying and relating to the emulsifier content Φ_{ISO} employed:

$$\frac{\frac{3}{r}\cdot\phi_W}{\phi_{Iso}} \quad \text{[equation XXI]}$$

Particle sizes were estimated based on microscopic pictures obtained by freeze fracture scanning electron microscopy. Table 46 depicts the values obtained after calculation, approximated particle sizes are shown in table 32.

Concerning the specific droplet surface area approach, values should be comparable. Consequently, in order to adjust formulation E70 to the situation present in E50 and E30, this would require that emulsifier content has to be in a range of 9 – 23%, although total oil phase, consisting of oil and emulsifier, comprises only 30%. This approach is not practical as adapted emulsions would then contain emulsifier concentrations far from reality and compatibility. Additionally, specific droplet surface area approach does not reflect real situation existing inside the emulsions, as emulsifiers will not only enrich themselves at the interface between dispersed water globules and continuous oil phase, but also be present in the oil phase. The extent to which the amount of free available emulsifier in the oil phase will be altered due to absorption at the interface oil - water, is much stronger for E70 than for E50

or E30, as it possesses the smallest droplets that are more densely packed and, thus, offers more interface.

The ratio of emulsifier content over oil content (see table 46) was deemed appropriate, as values are in the same order of magnitude. Keeping in mind that emulsifier concentration was originally dispensed empirically following requirements like stability, lipophilic vehicle character and manufacturer's recommendations, small variations from uniformity can be accepted. Pure oil was chosen as reference formulation, but for adjustment to the discussed situation, it was intermixed with 7.7% Isolan PDI for both drugs.

Table 46. Emulsifier approach for the w/o-emulsions studied (oil base: miglyol 812N)

	E70[1]	E50[1]	E30[1]
ϕ_{ISO} [2]	0.03	0.05	0.05
$^{3}/_{r} \cdot \phi_{W}$ [3]	2.8	1.5	0.6
$\frac{^{3}/_{r} \cdot \phi_{W}}{\phi_{Iso}}$ [4]	93	30	12
$\frac{\phi_{Iso}}{\phi_{L}}$ [5]	0.11	0.11	0.077

[1] emulsions with dispersed phases of 70%, 50% or 30%, respectively
[2] Isolan PDI content of the emulsions, considering for instance caffeine
[3] specific surface of the globules
[4] specific surface of the globules related to emulsifier content
[5] Isolan PDI content over oil content of the studied emulsions

Determination of saturation concentration and apparent permeability coefficient across pig ear skin for both drugs was performed in order to profound understanding of the interplay between oil, emulsifier Isolan PDI and model drug. Therefore, varying ratios of Isolan PDI were added to isopropyl myristate and miglyol 812N PDI being in the same order of magnitude or similar to the situation present in the examined w/o-emulsions. The procedure failed for paraffinum liquidum as only small amounts of Isolan PDI were dissolvable in this oil.

Neither in case of caffeine nor in case of ibuprofen presence of Isolan PDI could strongly affect determined saturation concentration. Solubility clearly demonstrated the hydrophilic nature of caffeine and the lipophilic nature of ibuprofen.

For caffeine solubility was best in water/phosphate buffer pH 7.4 and displayed an up to 250-fold difference compared to oil solubility. Miglyol 812N showed highest affinity for caffeine that was 2-fold greater than for isopropyl myristate and 20-fold greater than for paraffinum

liquidum. The order of oil saturation concentration is in good agreement with the order of caffeine partition coefficient oil/water determined in a previous manuscript (see 4.3.1.1, Part II).

For ibuprofen, solubility was highest in isopropyl myristate and miglyol 812N that were similar to each other. Solubility was approximately 6-times smaller in paraffinum liquidum and 1200-times smaller in sodium acetate buffer pH 4.5. The order of the oil saturation concentrations was again in good agreement with the order of ibuprofen partition coefficient oil/water determined before (see 4.3.1.1, Part II).

In a previous manuscript emulsions of varying phase fractions and varying emulsifier contents (3%, 5% and 10%) were subjected to ultracentrifugation experiments with subsequent quantification of continuous phase drug concentration. The determined concentrations revealed that Isolan PDI does not influence drug partitioning and consequently decisive drug concentration in the external formulation phase (see 4.3.1.3, Part II).

Intermixing isopropyl myristate or miglyol 812N with different amounts of Isolan PDI did neither affect transdermal permeation behaviour of caffeine nor ibuprofen. Additionally, ibuprofen emulsions comprising 30% and 70% of dispersed phase in miglyol 812N, respectively, possessed the same apparent permeability coefficient P_{app}, independent whether 3%, 5% or 10% of emulsifier were selected for the studied vehicle of defined phase fraction. These findings were all confirmed by a two-sided t-test (p=0.95) (see 5.3.2.2.1 for caffeine and 5.3.2.2.2 for ibuprofen).

To summarize, it can be assumed that Isolan PDI does not affect continuous phase drug concentration independent of its presence as free available ingredient in the external oil phase or enriched ingredient at the interface water-oil. Any further microstructures present inside the emulsions that are formed due to free Isolan PDI can be excluded as ultracentrifugation experiment only displayed two distinct phases, i.e. dispersed water phase and continuous oil phase [3].

Contrary, literature also reports on emulsifier affecting transdermal permeation. Presence of free emulsifier in the continuous or dispersed phase can change thermodynamic activity and solubility properties of a drug. For instance, Lalor et al. studied transdermal permeation of lipophilic p-aminobenzoate incorporated in o/w-emulsions. The effective activity of any permeant is connected to the state of saturation of the drug in the vehicle. Surfactants led to

a substantial solubilization of the drug in the external phase, thus, lowering the appreciable thermodynamic activity with the net result of decreased transport rate over a membrane. Partitioning of the drug into the internal phase led to an even lower concentration in the external phase and, hence, apparent permeability coefficients for o/w-emulsions were lower than for w/o-emulsions. Besides interaction with the drug, surfactants can also alter skin structure and consequently skin permeability [6, 167].

No statistic significant difference for apparent permeability coefficient P_{app} was evident in terms of testing two infinite doses (0.3 g/cm^2 and 0.7 g/cm^2). Considering caffeine transport experiments using w/o-emulsions with miglyol 812N as oil component, these results are not astonishing. In a previous work, it already turned out that for these systems (dose: 0.7 g/cm^2) permeability coefficient of the skin P_m was rate-limiting compared to permeability coefficient of the vehicle P_{dbl} (see Part II, 4.3.2.3.2, series B). In contrary, for ibuprofen transport experiments using w/o-emulsions with miglyol 812N as oil component, an impact of reduced vehicle layer thickness could have occurred. Regarding a dose of 0.7 g/cm^2, the impact of permeability coefficient of the diffusion boundary layer gained increasing influence on the overall apparent permeability coefficient as demonstrated before (see Part II, 4.3.2.4.2, series D).

In a separate manuscript a concept for the interpretation of transdermal drug permeation using w/o-emulsions of varying phase fractions and oil phases was presented by the authors considering drug distribution among distinct phases and it was postulated that continuous phase drug concentration alone governs drug permeation kinetics. With the derived quantitative model it was possible to explain in vitro skin permeation of benzyltrimethylammonium chloride, caffeine and ibuprofen as model drugs that were applied under occlusive conditions. The proposed concept was further shown to be consistent for multi-phasic hydrophilic formulations comprising different microstructures and phase fractions in previous works [3]. The model was confirmed for hydrophilic and lipophilic drugs applied under occlusive and non-occlusive conditions taking into account changes these vehicles underwent in the course of evaporation of volatile components [2, 3]. Thus, the concept was implemented on the same w/o-emulsions studied before (see Part II), but considering non-occlusive conditions in order to confirm its validity.

Evaporation studies were performed in order to figure out the changes formulations undergo after application. Possible water loss was studied in dependence of phase fraction, time, dose and experimental setup, i.e. pig ear skin mounted in Franz-type diffusion cells and

beaker of known surface. Studies in beaker were necessary to exclude any water uptake from skin surface resulting from an upward diffusion out of the receiver compartment.

Independent of experimental setup employed, water loss was apparent within the duration of a transport experiment (48h), although its extent was more distinct for a dose of 0.3 g/cm^2 than for 0.7 g/cm^2 and hence a reduced vehicle layer thickness. It turned out that evaporation profile and percentage of water loss referred to original water content were the same for all phase fractions studied (approximately 40%). There was no water uptake from the receiver compartment as different experimental setup showed the same results. These finding were further confirmed by miglyol oil that was mixed with 7.7% Isolan PDI and applied in a dose of 0.1 g/cm^2 onto the skin. There was no difference in water content after an incubation time of 48h under occlusive and non-occlusive conditions and consequently no water emulsification.

Considering compositional changes vehicles underwent in the course of evaporation, further transport experiments were carried out across pig ear skin using a dose of 0.3 g/cm^2 under occlusive and non-occlusive conditions (see 5.3.2.4.2 for ibuprofen and 5.3.2.4.3 for caffeine). In general, continuous phase drug concentration of w/o-emulsions is affected by two processes:

(i) Drug permeation into and through the skin leads to a decrease of drug content in the external phase. However, this decrease is balanced by concomitant partitioning of drug between water droplets and oil phase as long as the total amount of permeated drug is low. This assumption is valid for occlusive and non-occlusive application.

(ii) Evaporation leads to a compositional change, decreasing dispersed phase fraction $\Phi_W{}^t$ at a specific point in time t compared to original phase fraction $\Phi_W{}^0$. Due to the loss of water, continuous phase drug concentration increases.

Consequently, as long as sink conditions are considered and no significant drug depletion of the examined vehicle occurs during the time frame studied, evaporation leads to an increase in continuous phase drug concentration. If drug depletion occurs, then continuous phase drug concentration must be calculated with the equation XIX. The assumptions can also be derived if the formula for calculation of new arising continuous phase drug concentration (see 5.3.2.4.1) is considered:

$$C_L^t = C_L^0 \cdot \frac{M_{tot}^t}{M_{tot}^0} \cdot \frac{1-\phi_W^t}{1-\phi_W^0} \cdot \frac{\left[\phi_W^0 (K_{W/L} - 1) + 1\right]}{\left[\phi_W^t (K_{W/L} - 1) + 1\right]} \quad \text{[equation XIX]}$$

It holds:

$$\frac{M_{tot}^{t}}{M_{tot}^{0}} \leq 1 \qquad \text{and} \qquad \frac{1-\phi_{w}^{t}}{1-\phi_{w}^{0}} > 1$$

if non-occlusive conditions are considered. Following evaporation ($\Phi_W^t < \Phi_W^0$) the fragment

$$\frac{\phi_W^0 \left(K_{W/L} - 1\right) + 1}{\phi_W^t \left(K_{W/L} - 1\right) + 1} > 1$$

will always be above unity independent whether a hydrophilic drug with a drug partition coefficient $K_{W/L} > 1$ or a lipophilic drug with $K_{W/L} < 1$ is present and consequently lead to an increase in continuous phase drug concentration. Dependent on which of these terms outweighs, evaporation will lead to an increase of decrease of continuous phase drug concentration.

For caffeine, an increase of 51.23% for E70, 67.86% for E50 and 26.85% for E30 of continuous phase drug concentration was observed, whereas for ibuprofen only for E70 an increase of 23.32% was observed.

However, determined apparent permeability coefficient P_{app} for occlusive and non-occlusive conditions did not reveal any statistically significant difference (t-test, two-sided, p=0.95) although vehicles underwent considerable changes. This was true for caffeine and ibuprofen. Overall drug depletion within every caffeine formulation was so distinct that continuous phase drug concentration did not increase due to considerable depletion. The increase of continuous phase drug concentration calculated with equation XIX underestimated drug depletion because of drug remaining in the skin. The depletion was based only on calculation of drug amount in the receiver compartment and did not consider drug amount in the skin. For caffeine drug depletion was 10%, 11% and 13% for E70, E50 and E30, respectively (see table 44). For ibuprofen the depletion was 6% for E30 and 8% for E70. Thus, to be more precise and make a clear statement, drug content of the vehicle after 48h should be taken into consideration additionally.

Dermatological medications are typically applied as a thin layer and will remain on the skin only for a few hours, which are not exactly the conditions for the studies described in this work. Hence, the discussed alterations of the formulations due to evaporation of volatile components likely take place much faster in clinical situations. Furthermore, the residue may be removed before steady state drug flux is reached, commonly due to mechanical agitation.

The applied experimental setup, however, was deemed appropriate for reaching the specific goals of this study and allowed observing alterations following formulation application in “slow-motion”.

To conclude, evaporation was observed for all examined formulations independent of the phase fraction dispersed and the experimental setup employed, but dependent on dose. Although dosing was the same, the extent of water loss was comparably low if compared to o/w-emulsions studied in a previous work [3].

Anyhow, too little attention has been paid to the issue of evaporative processes within dermatological formulations up to date. Concerning examined lipophilic vehicle systems, it is likely that applying a finite dose (1 – 3 mg/cm^2) instead of an infinite dose examined here due to experimental demand, will result in a more intense water loss and strongly affect transdermal permeation. An optimized experimental setup with respect to dose, drug properties, drug permeability and time schedule would allow studying this process in more detail in order to obtain a more profound understanding. Additionally, drug content in the skin and residual drug content of the formulation after the transport experiment should be considered. Thus, confirmation of the validity of continuous phase drug concentration concept discussed in part II for non-occlusive conditions in w/o-emulsions requires further investigation.

Acknowledgements:

Scanning electron microscopy was performed at the Interdepartmental electron Microscopy (IEM) unit of Biocenter, University of Basel by Marcel Düggeling and Daniel Mathys. We are grateful to Marcel Düggelin and Daniel Matthys for the valuable contribution to this work.

Appendix

6 Appendix

6.1 Characterization of Pig Ear Skin

Transepidermal water loss (TEWL), skin moisture, skin pH and skin temperature were measured 1h after equilibration of pig ear skin mounted inside a Franz-type diffusion cell with receiver solution and directly after a completed transport experiment (52h). The idea was to pursue changes skin undergoes in the course of a transport experiment. For measurements, specific sensors provided by Courage & Khazaka electronic GmbH (Cologne, Germany) were used, i.e. Tewameter TM300 for TEWL measurements, Corneometer CM 825 for skin surface hydration, Skin-pH-Meter PH 905 and Skin-Thermo-Meter TH 500. The values are depicted below (table 47):

Table 47. Characterisation of pig ear skin

	Before transport experiment (1h)[1]	After transport experiment (52h)[1]
TEWL	15.24 ± 3.69	23.90 ± 13.94
Skin surface hydration	22.84 ± 3.03	27.97 ± 10.82
Skin pH	6.11 ± 0.57	5.91 ± 0.54
Skin temperature	24.73 ± 0.13	25.15 ± 0.67

[1] all values denote mean ± standard deviation. n = 3

Skin pH and skin temperature were not changed, but skin surface hydration and transepidermal water loss were slightly increased. As transport experiments were performed under occlusive conditions, these findings are in good agreement with literature that indicates an increase stratum corneum hydration, a swelling of corneocytes and an increased skin temperature. The latter, however, was not observed as experiments were performed in-vitro and not in vivo [91].

6.2 Stability Testing of the Examined Formulations

Prior to examining transdermal delivery rate of the selected dermatological formulations, all vehicles were tested for stability. No phase separation was allowed to occur within 24h after manufacturing at room temperature and additional 48h at 32℃ (water bath heating). For that purpose, emulsions were filled into falcon tubes and closed to avoid any possible water loss. Additionally, lipophilic character was confirmed by conductivity measurements, dilution with water / oil, dyeing with a hydrophilic (indigocarmin) and lipophilic (sudan III) compound before and after stability testing. Conductivity measurements before and after the stability

testing were performed using a conductometer 660 and a conductivity-measuring cell 60323110 with a cell constant of 0.8cm^{-1} (Metrohm AG, Herisau, Switzerland) were used.

Figure 28. Dying of an emulsion comprising 70% dispersed water phase in miglyol 812N (E70) with indigocarmin (hydrophilic) and sudan III (lipophilic)

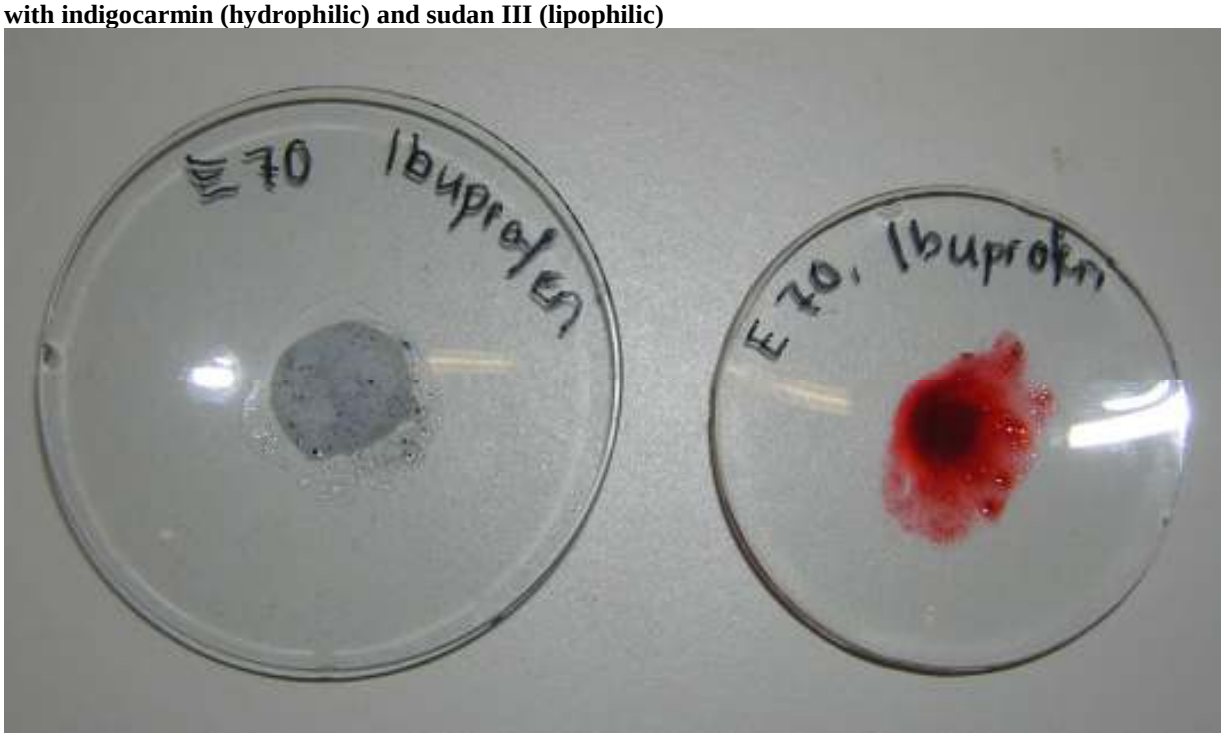
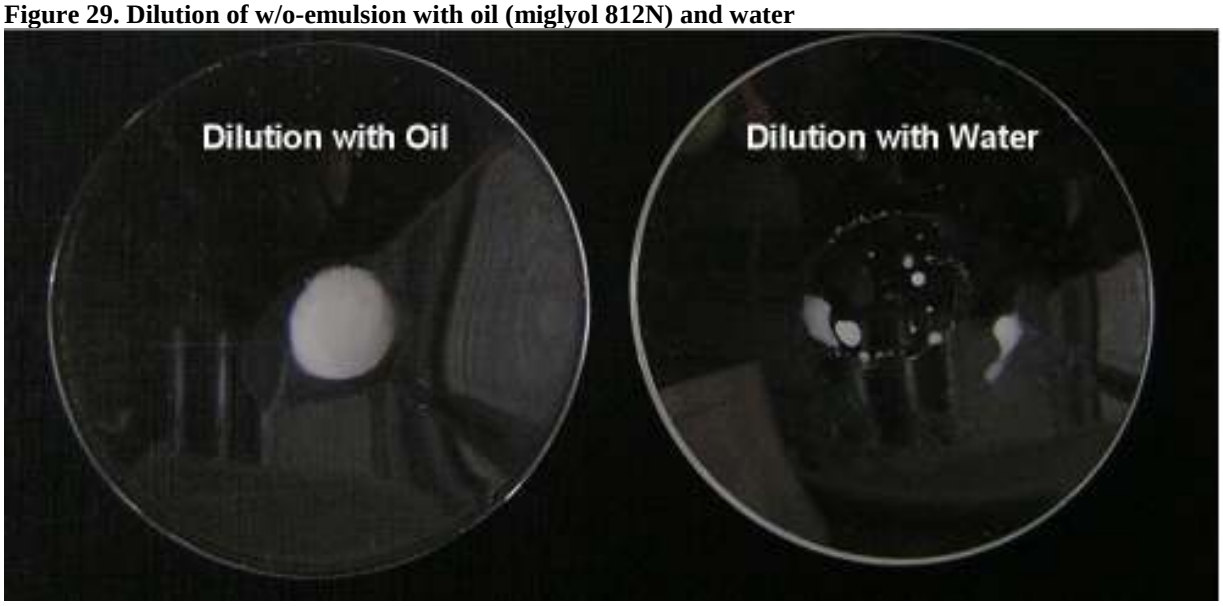


Figure 29. Dilution of w/o-emulsion with oil (miglyol 812N) and water



6.3 Validation of Time for Ultracentrifugal Separation

w/o - emulsions comprising of varying phase fractions of dispersed water phase within continuous oil phase were separated into their distinct phases by ultracentrifugation experiments. Therefore, formulations were filled into quick-seal centrifuge tubes, 5/8X3 (Beckmann Instruments, Palo Alto, USA) and centrifuged at 222000 – 450000 g using an ultracentrifuge type Centricon T-1075 and a rotor TFT 7013 (Kontron Instruments, Mailand, Italy) until complete separation occurred.

Table 48. Staged separation of w/o - emulsions comprising varying phase fractions of dispersed water phase by ultracentrifugation - effect of time(h) and speed (rpm)

Time (h)	Speed (rpm)	Water phase (weight - %) after ultracentrifugation EMULSION E70[1]	Water phase (weight - %) after ultracentrifugation EMULSION E30[1]
1	5000	No separation	No separation
1	10000	No separation	No separation
1	20000	No separation	40.70
1	40000	88.14	37.20
1	68000	79.50	35.36
2	68000	76.89	34.14
4	68000	75.78	33.25
required		**70**	**30**

[1] w/o - emulsion comprising 70% or 30% of dispersed water phase in miglyol 812N, respectively

For the emulsions E70 and E30 best phase separation was achieved if ultracentrifugation was performed for 2h at 220000 – 450000 g (equivalent to 68000 rpm). Even if centrifugation time was expanded to 4h instead of 2h, no improvement was achieved. Thus, all ultracentrifugation experiments in order to quantitatively determine continuous phase drug concentrations were performed at 220000 – 450000 g for 2h.

6.4 Distribution Experiment between Stratum Corneum and Oil

Originally, the idea was to determine drug distribution between (continuous) oil (phase) and dried stratum corneum pieces (see 4.2.2.4). For that purpose, miglyol 812N oil with varying concentrations of ibuprofen (1, 2 and 4 weight-%, respectively) was incubated with dried stratum corneum pieces of known weight. At predetermined time points (20h, 25h, 40h, 48h) samples were withdrawn from the oil and analyzed for their drug content. Depletion was solely due to partitioning into stratum corneum as confirmed by blank control samples. The stratum corneum drug content was quantified indirectly by calculation assuming a density of 1000 mg/g for the skin. Additionally, stratum corneum drug content was directly determined after cleaning the skin sheets (dabbing dry with tissue and washing with water) and subsequent extraction with water solution. However, both approaches failed (no linear regression) as illustrated in the figures below (figure 30): It was not possible to remove adherent oil entirely for stratum corneum pieces without loosing drug substance or destroying the skin sheets. Furthermore, direct quantification of drug content in oily vehicles required dilution steps and therefore it was very likely to implicate experimental failures in this step.

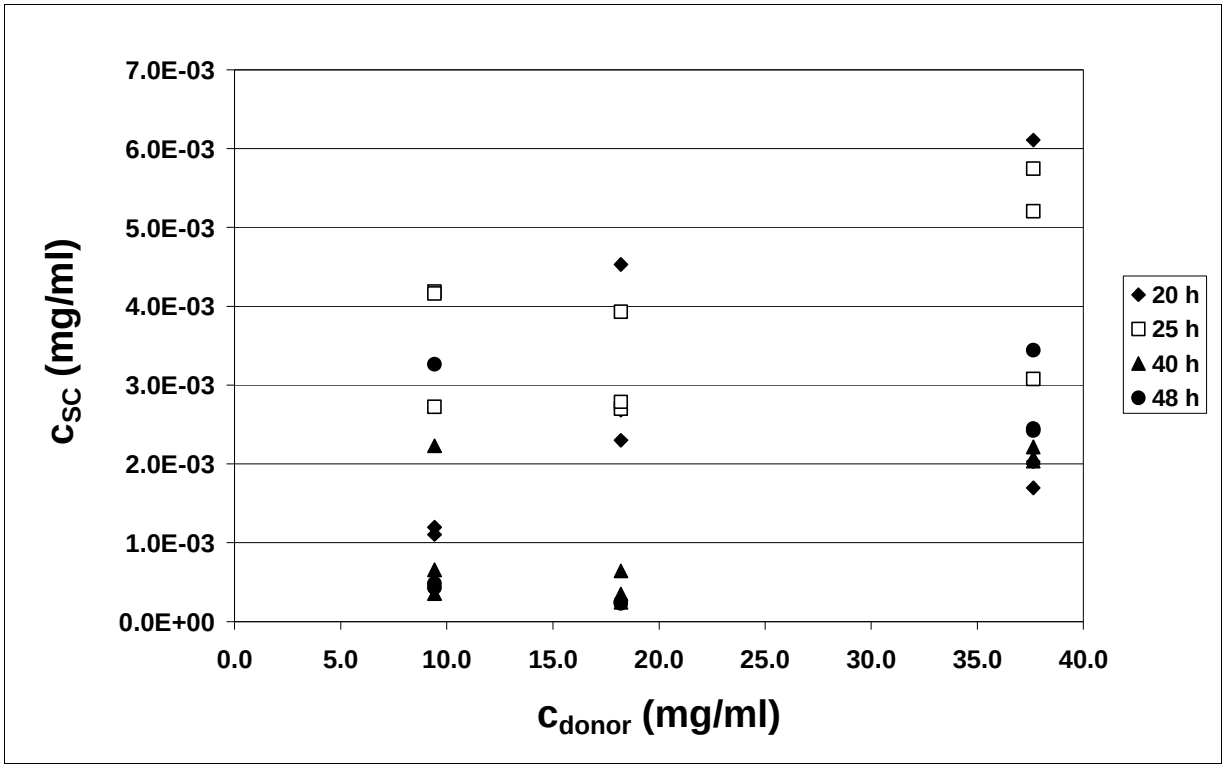


Figure 30. Determination of drug partition coefficient between dried stratum corneum and ibuprofen in miglyol 812N - oil in dependence of incubation time

6.5 Freeze Fracture Scanning Electron Microscopy

6.5.1 W/O-emulsion with Isopropyl myristate

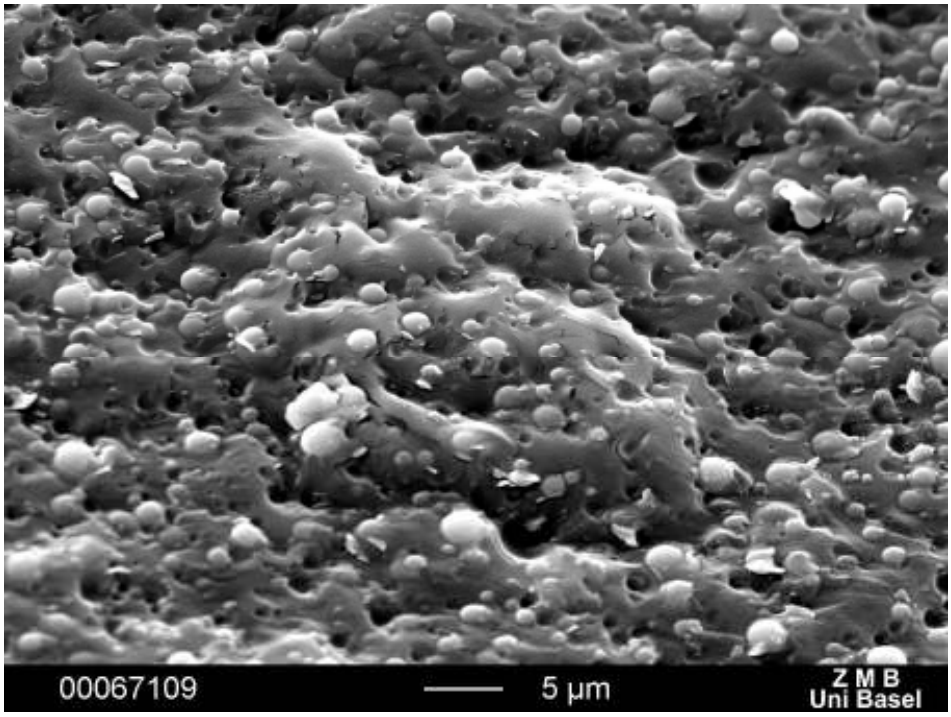


Figure 31. w/o-emulsion with isopropyl myristate (E70): 70% of dispersed water phase

6.5.2 W/O-emulsion with Miglyol 812N

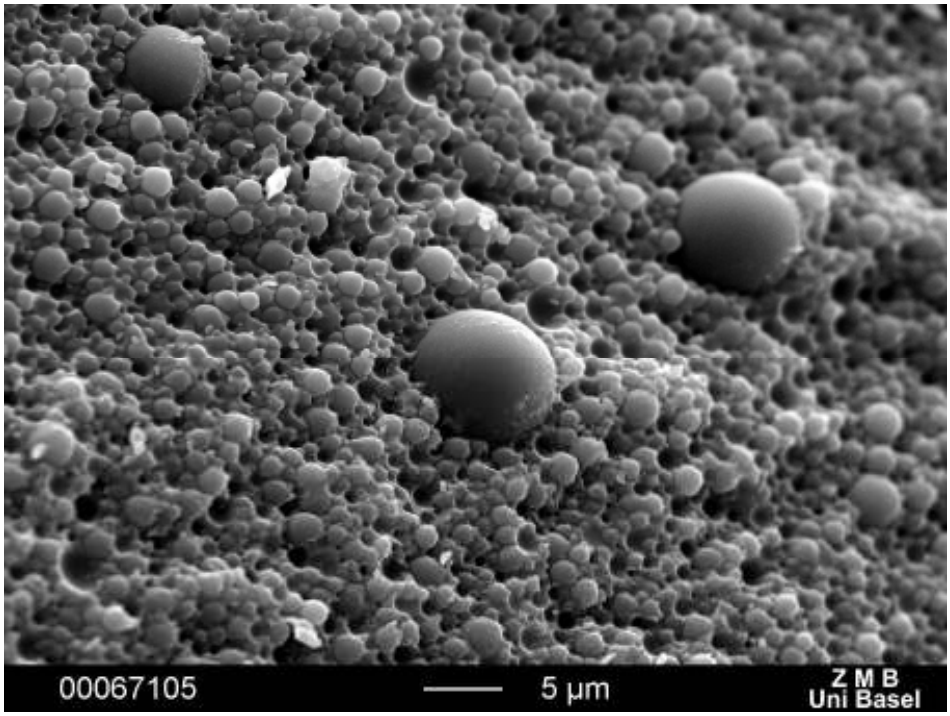


Figure 32. w/o-emulsion with miglyol 812N (E70): 70% of dispersed water phase

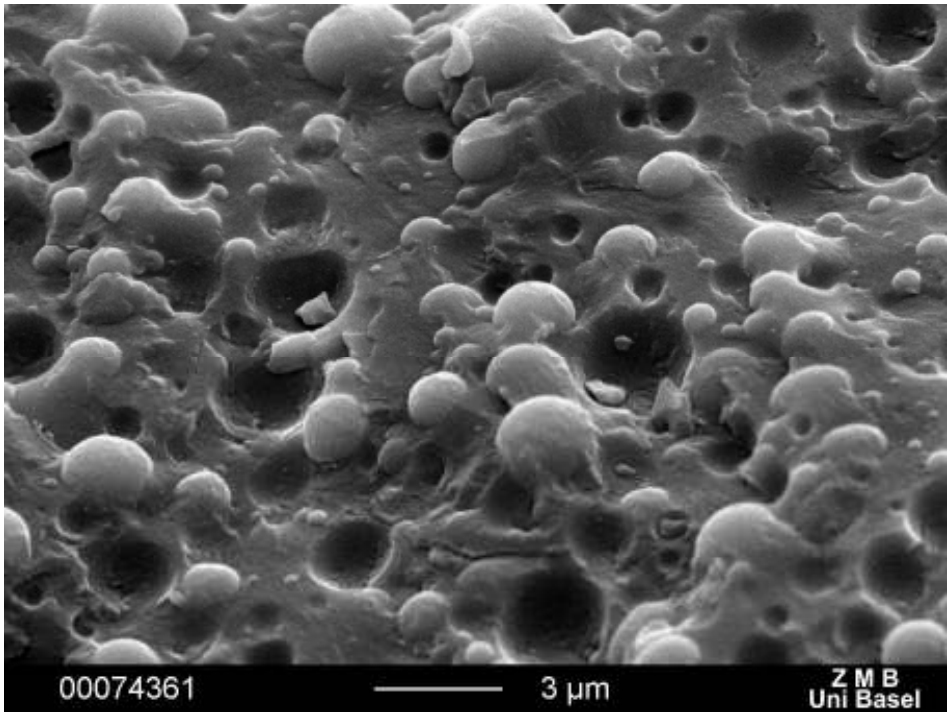


Figure 33. w/o-emulsion with miglyol 812N (E50): 50% of dispersed water phase

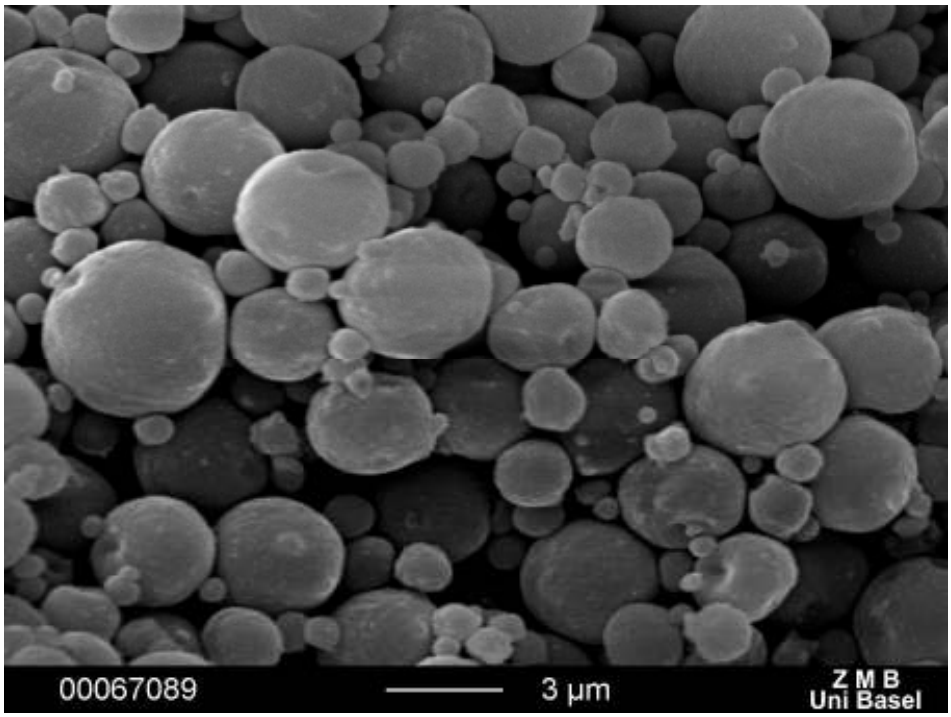


Figure 34. w/o-emulsion with miglyol 812N (E30): 30% of dispersed water phase

6.5.3 W/O-emulsion with Paraffinum liquidum

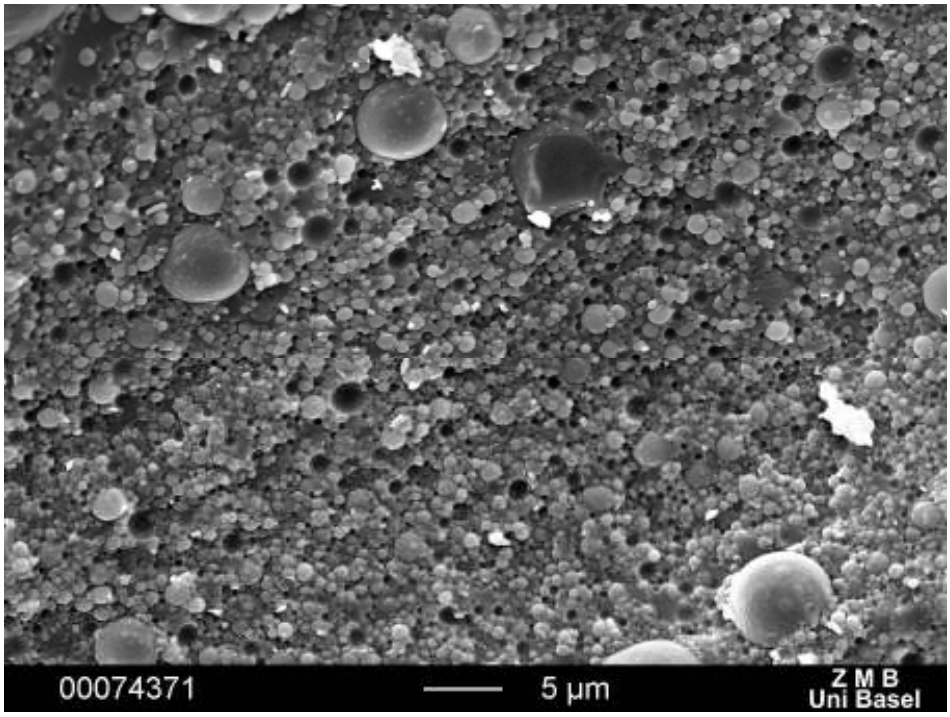


Figure 35. w/o-emulsion with paraffinum liquidum (E70): 70% of dispersed water phase

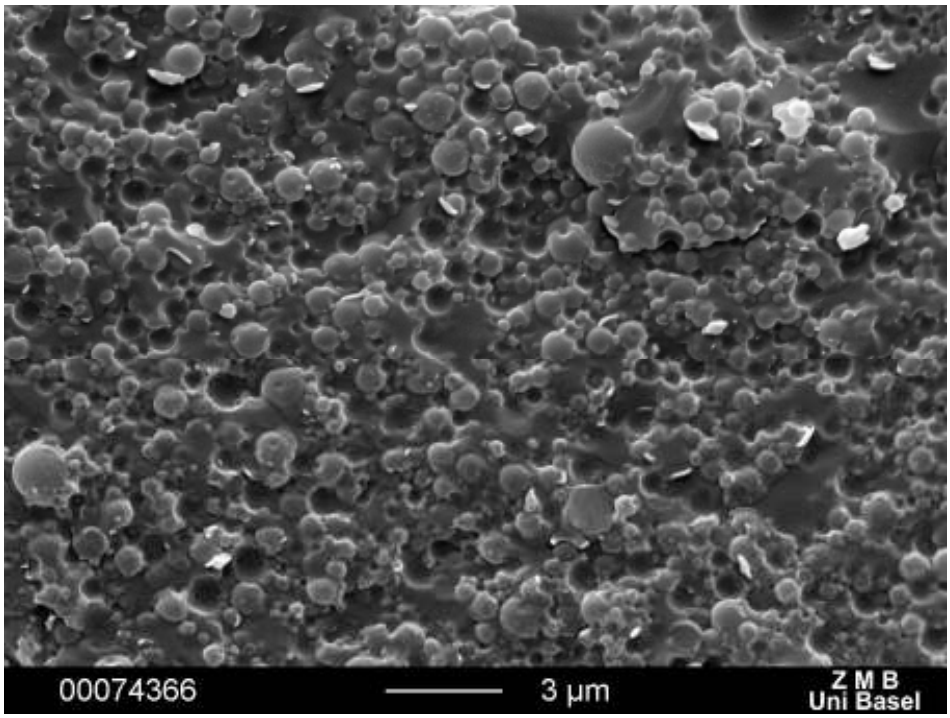


Figure 36. w/o-emulsion with paraffinum liquidum (E50): 50% of dispersed water phase

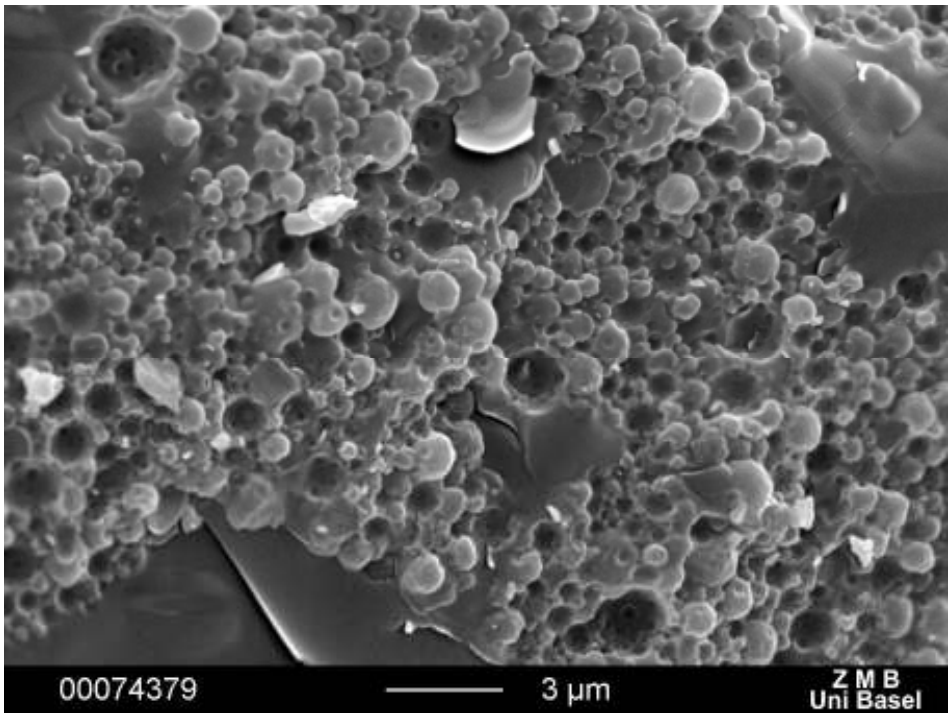


Figure 37. w/o-emulsion with paraffinum liquidum (E30): 30% of dispersed water phase

6.6 Composition of Employed Solutions

6.6.1 Benzyltrimethylammonium Chloride

Phosphate buffer of the mobile phase for HPLC quantification (pH 3.5; buffer capacity β = 0.5)	
sodium dihydrogenphosphate dihydrate	14.296 g
1-octansulfonic acid sodium salt monohydrate	0.2925 g
bidistilled water	Ad 1000.0 g

6.6.2 Caffeine

Phosphate buffer of the mobile phase for HPLC quantification (pH 7.4; buffer capacity β = 0.01)	
sodium dihydrogenphosphate dihydrate	3.1195 g
bidistilled water	Ad 1000.0 g

Solution for the determination of caffeine distribution coefficient between oil and water phase	
caffeine	10.0 g
sodium chloride	5.0 g
bidistilled water	Ad 1000.0 g

6.6.3 Ibuprofen

Phosphate buffer of the mobile phase for HPLC quantification (pH 2.1; buffer capacity β = 0.02)	
ortho-phosphoric acid 85%	8.4 ml
bidistilled water	Ad 1000.0 ml

Sodium acetate buffer used for ibuprofen in emulsions and distribution coefficient determinations (pH 4.5; buffer capacity β = 0.01)	
sodium acetate	1.60376 g
bidistilled water	Ad 1000.0 g

6.6.4 Receiver Solution of the Transport Experiments

Phosphate buffer for the transport experiments (pH 7.4; buffer capacity β = 0.05)	
sodium dihydrogenphosphate dihydrate	14.28 g
sodium azide	1.0 g
bidistilled water	Ad 1000.0 g

6.6.5 Preparation of Stratum Corneum by Trypsin Digestion

Phosphate buffer used for trysin digestion (pH 7.4; buffer capacity β = 0.05)	
sodium dihydrogenphosphate dihydrate	1.56008 g
sodium azide	16.364 g
bidistilled water	Ad 1000.0 g

References

7 References

[1] C. Surber, E.W. Smith, The mystical effects of dermatological vehicles. Dermatology 210(2) (2005) 157-168.
[2] D. Hummel, Struktur topischer Formulierungen mit Triglycerid, O/W-Tensid und Phospholipid als dispergierte Phase und der Einfluss strukturbedingter physikochemischer Parameter und Evaporationsprozesse auf die perkutane Absorption. Institute of Pharmaceutical Technology, University of Basel, Basel, 2001.
[3] H. Nalenz, Dependence of skin drug permeation on microstructure and time dependent alterations following application of more-phasic dermatolgocial formulations studied by the continuouse phase drug concentration concept. Institute of Pharmaceutical Technology, University of Basel, Basel, 2006.
[4] J. Hadgraft, M. Whitefield, P.H. Rosher, Skin penetration of topical formulations of ibuprofen 5%: An in vitro comparative study. Skin Pharmacology and Applied Skin Physiology 16(3) (2003) 137-142.
[5] C. Laugel, A. Baillet, M.P.Y. Piemi, J.P. Marty, D. Ferrier, Oil-water-oil multiple emulsions for prolonged delivery of hydrocortisone after topical application: comparison with simple emulsions. International Journal of Pharmaceutics 160(1) (1998) 109-117.
[6] C.B. Lalor, G.L. Flynn, N. Weiner, FORMULATION FACTORS AFFECTING RELEASE OF DRUG FROM TOPICAL FORMULATIONS .1. EFFECT OF EMULSION-TYPE UPON IN-VITRO DELIVERY OF ETHYL P-AMINOBENZOATE. Journal of Pharmaceutical Sciences 83(11) (1994) 1525-1528.
[7] F.P. Schwarb, G. Imanidis, E.W. Smith, J.M. Haigh, C. Surber, Effect of concentration and degree of saturation of topical fluocinonide formulations on in vitro membrane transport and in vivo availability on human skin. Pharmaceutical Research 16(6) (1999) 909-915.
[8] M.F. Coldman, B.J. Poulsen, T. Higuchi, ENHANCEMENT OF PERCUTANEOUS ABSORPTION BY USE OF VOLATILE - NONVOLATILE SYSTEMS AS VEHICLES. Journal of Pharmaceutical Sciences 58(9) (1969) 1098-&.
[9] K. Yamaguchi, T. Mitsui, Y. Aso, K. Sugibayashi, Analysis of in vitro skin permeation of 22-oxacalcitriol from ointments based on a two- or three-layer diffusion model considering diffusivity in a vehicle. International Journal of Pharmaceutics 336(2) (2007) 310-318.
[10] O. Diez-Sales, T.M. Garrigues, I. Herraez, R. Belda, A. Martin-Villodre, M. Herraez, In vitro percutaneous penetration of acyclovir from solvent systems and carbopol 971-P hydrogels: Influence of propylene glycol. Journal of Pharmaceutical Sciences 94(5) (2005) 1039-1047.
[11] C.C. Muller-Goymann, U. Alberg, Modified water containing hydrophilic ointment with suspended hydrocortisone-21-acetate - the influence of the microstructure of the cream on the in vitro drug release and in vitro percutaneous penetration. European Journal of Pharmaceutics and Biopharmaceutics 47(2) (1999) 139-143.
[12] A. Faller, M. Schünke, Der Körper des Menschen : Einführung in Bau und Funktion, Thieme, Stuttgart, 1999.
[13] B.W. Barry, Dermatological formulations : percutaneous absorption, Dekker, New York, 1983.
[14] H. Schaefer, T.E. Redelmeier, Skin barrier : principles of percutaneous absorption, S. Karger, Basel [etc.], 1996.
[15] K.A. Walters, Percutaneous absorption and transdermal therapy. Pharmaceutical Technology (1986) 30-46.
[16] L.C. Junqueira, J. Carneiro, Histologie : Zytologie, Histologie und mikroskopische Anatomie des Menschen : unter Berücksichtigung der Histophysiologie, Springer-Verlag, Berlin, 1991.
[17] G.K. Menon, New insights into skin structure: scratching the surface. Adv Drug Deliv Rev 54 Suppl 1 (2002) S3-17.
[18] H. Schaefer, Structure and Dynamics of the Skin Barrier. in: T. E. Redelmaier (Ed.), Schaefer, H; Redelmaier, T.E., Basel, 1996, pp. 1-42.
[19] A. Benninghoff, D. Drenckhahn, W. Zenker, Anatomie : makroskopische Anatomie, Embryologie und Histologie des Menschen, Urban und Schwarzenberg, München [etc.], 1994.
[20] C.M. Chuong, B.J. Nickoloff, P.M. Elias, L.A. Goldsmith, E. Macher, P.A. Maderson, J.P. Sundberg, H. Tagami, P.M. Plonka, K. Thestrup-Pedersen, B.A. Bernard, J.M. Schroder, P. Dotto, C.H. Chang, M.L. Williams, K.R. Feingold, L.E. King, A.M. Kligman, J.L. Rees, E. Christophers, What is the 'true' function of skin? Viewpoint 1. Experimental Dermatology 11(2) (2002) 159-163.

[21] B.C. Lippold, Biopharmazie : e. Einf. zu d. wichtigsten Arzneiformen, Wissenschaftliche Verlagsgesellschaft, Stuttgart, 1984.
[22] E. Houben, K. De Paepe, V. Rogiers, A keratinocyte's course of life. Skin Pharmacology and Physiology 20(3) (2007) 122-132.
[23] G.K. Menon, New insights into skin structure: scratching the surface. Advanced Drug Delivery Reviews 54 (2002) S3-S17.
[24] K.S. Stenn, in: L. Weiss (Ed.), Histology, Cell and Tissue Biology, Elsevier Science, New York, 1983, pp. 569-606.
[25] E. Candi, R. Schmidt, G. Melino, The cornified envelope: a model of cell death in the skin. Nature Reviews Molecular Cell Biology 6(4) (2005) 328-340.
[26] P. Corcuff, F. Fiat, A.M. Minondo, Ultrastructure of the human stratum corneum. Skin Pharmacol Appl Skin Physiol 14 Suppl 1 (2001) 4-9.
[27] B.W. Barry, Novel mechanisms and devices to enable successful transdermal drug delivery. European Journal of Pharmaceutical Sciences 14(2) (2001) 101-114.
[28] P.M. Elias, D.S. Friend, PERMEABILITY BARRIER IN MAMMALIAN EPIDERMIS. Journal of Cell Biology 65(1) (1975) 180-191.
[29] L. Norlen, Skin barrier structure and function: The single gel phase model. Journal of Investigative Dermatology 117(4) (2001) 830-836.
[30] D. Kessner, A. Ruettinger, M.A. Kiselev, S. Wartewig, R.H.H. Neubert, Properties of ceramides and their impact on the stratum corneum structure: A review - Part 2: Stratum corneum lipid model systems. Skin Pharmacology and Physiology 21(2) (2008) 58-74.
[31] B. Forslind, A DOMAIN MOSAIC MODEL OF THE SKIN BARRIER. Acta Dermato-Venereologica 74(1) (1994) 1-6.
[32] J.A. Bouwstra, F.E.R. Dubbelaar, G.S. Gooris, A.M. Weerheim, M. Ponec, The role of ceramide composition in the lipid organisation of the skin barrier. Biochimica Et Biophysica Acta-Biomembranes 1419(2) (1999) 127-136.
[33] S. Wartewig, R.H. Neubert, Properties of ceramides and their impact on the stratum corneum structure: a review. Part 1: ceramides. Skin Pharmacol Physiol 20(5) (2007) 220-229.
[34] Y.Z. Li, Y.S. Quan, L. Zang, M.N. Jin, F. Kamiyama, H. Katsumi, A. Yamamoto, S. Tsutsumi, Transdermal delivery of insulin using trypsin as a biochemical enhancer. Biol Pharm Bull 31(8) (2008) 1574-1579.
[35] C.R. Harding, A. Watkinson, A.V. Rawlings, I.R. Scott, Dry skin, moisturization and corneodesmolysis. Int J Cosmet Sci 22(1) (2000) 21-52.
[36] R. Daniels, U. Knie, Galenics of dermal products--vehicles, properties and drug release. J Dtsch Dermatol Ges 5(5) (2007) 367-383.
[37] L. Buhse, R. Kolinski, B. Westenberger, A. Wokovich, J. Spencer, C.W. Chen, S. Turujman, M. Gautam-Basak, G.J. Kang, A. Kibbe, B. Heintzelman, E. Wolfgang, Topical drug classification. Int J Pharm 295(1-2) (2005) 101-112.
[38] G.L. Flynn, in: G. S. Banker and C. T. Rhodes (Eds.), Modern Pharmaceutics, Vol. 72, Marcel Dekker, Inc., New York, Basel, 1996, pp. 239-298.
[39] C.S. Leopold, B.C. Lippold, ENHANCING EFFECTS OF LIPOPHILIC VEHICLES ON SKIN PENETRATION OF METHYL NICOTINATE IN-VIVO. Journal of Pharmaceutical Sciences 84(2) (1995) 195-198.
[40] K. Henning, Emulgieren - Demulgieren. Vol. 7, Seifen - Öle - Fette - Wachse, 2003, pp. 69 - 78.
[41] D. Myers, in: D. Myers (Ed.), Surfaces, Interfaces and Colloids: Principles and Applications, Wiley, Weinheim, 1999, pp. 253-294.
[42] E.L. Rowe, EFFECT OF EMULSIFIER CONCENTRATION AND TYPE ON PARTICLE SIZE DISTRIBUTION OF EMULSIONS. Journal of Pharmaceutical Sciences 54(2) (1965) 260-&.
[43] M. Gloor, How do dermatological vehicles influence the horny layer? Skin Pharmacol Physiol 17(6) (2004) 267-273.
[44] M.P. Aronson, M.F. Petko, HIGHLY CONCENTRATED WATER-IN-OIL EMULSIONS - INFLUENCE OF ELECTROLYTE ON THEIR PROPERTIES AND STABILITY. Journal of Colloid and Interface Science 159(1) (1993) 134-149.
[45] P. Clement, C. Laugel, J.P. Marty, In vitro release of caffeine from concentrated W/O emulsions: effect of formulation parameters. International Journal of Pharmaceutics 207(1-2) (2000) 7-20.
[46] J. Meyer, P. Allef, H. Fötsch, A novel PEG-free emulsifier designed for formulating w/o lotions with a light skin feel. Vol. 11, Seifen - Fette - Öle - Wachse, 2005, pp. 20 - 28.

[47] F.O. Opawale, D.J. Burgess, Influence of interfacial properties of lipophilic surfactants on water-in-oil emulsion stability. Journal of Colloid and Interface Science 197(1) (1998) 142-150.
[48] P. Kent, B.R. Saunders, The role of added electrolyte in the stabilization of inverse emulsions. Journal of Colloid and Interface Science 242(2) (2001) 437-442.
[49] I.I.U.o.P.a.A. Chemistry), D.o.P. Chemistry, Manual of Symbols and Terminology for Physocochemical Quantities and Units. Vol. Appendix II, Butterworths, London, 1972, p. 611.
[50] E. Melzer, J. Kreuter, R. Daniels, Ethylcellulose: a new type of emulsion stabilizer. European Journal of Pharmaceutics and Biopharmaceutics 56(1) (2003) 23-27.
[51] d.-P.I. PDI, Isolan PDI - Emulgator zur Formulierung von kosmetischen W/O-Cremes und -Lotionen. Goldschmidt GmbH, Mai 2003.
[52] J. Storsberg, A. Laschewsky, Polymer surfactants - novel active agents with exceptional properties. Vol. 12, Seifen - Fette - Öle - Wachse, 2004, pp. 14 - 18.
[53] S.R. Farinato, R. L.R., in: P. Becher (Ed.), Encyclopedia of emulsion technology, Vol. 1, Marcel Dekker, New York ; Basel, 1983, pp. 439-479.
[54] A. Martin, in: A. N. Martin and P. Bustamante (Eds.), Physical pharmacy : physical chemical principles in the pharmaceutical sciences, Lea & Febiger, Philadelphia, 1993, pp. 477-511.
[55] H. T., in: P. Sherman (Ed.), Emulsion science, Academic Press, London; New York, 1969, pp. 353-478.
[56] R.J. Haskell, Characterization of submicron systems via optical methods. Journal of Pharmaceutical Sciences 87(2) (1998) 125-129.
[57] I. Stoye, K. Schroder, C.C. Muller-Goymann, Transformation of a liposomal dispersion containing ibuprofen lysinate and phospholipids into mixed micelles - physico-chemical characterization and influence on drug permeation through excised human stratum corneum. European Journal of Pharmaceutics and Biopharmaceutics 46(2) (1998) 191-200.
[58] R. Brummer, T. Berg, S. Friedrich, K.P. Wittern, Definition of the status of development by analysis. Particle sizing, rheology, microscopy and dielectric spectroscopy. Seifen Fette Öle Wachse Journal, 2002, pp. 2-9.
[59] E. Esposito, E. Menegatti, R. Cortesi, Ethosomes and liposomes as topical vehicles for azelaic acid: A preformulation study. Journal of Cosmetic Science 55(3) (2004) 253-264.
[60] A. Manosroi, L. Kongkaneramit, J. Manosroi, Characterization of amphotericin B liposome formulations. Drug Dev Ind Pharm 30(5) (2004) 535-543.
[61] J. Schweitzer, Scanning Electron Microscope (SEM). University of Purdue, 2006.
[62] A. Seifert, K. Strenge, M. Schultz, H. Schmandke, DETERMINATION OF STABILITY OF FOOD EMULSIONS .1. THE INFLUENCE OF THE TYPE AND CONCENTRATION OF PROTEIN ON CREAMING STABILITY DETERMINED BY ANALYTICAL ULTRACENTRIFUGATION. Nahrung-Food 35(9) (1991) 989-998.
[63] R.D. Vold, K.L. Mittal, A.U. Hahn, Ultracentrifugal stability of emulsions. Surf. And Coll. Sci. 10 (1978).
[64] P. Guichardon, P. Moulin, F. Tosini, L. Cara, F. Charbit, Comparative study of semi-solid liposome purification by different separation methods. Separation and Purification Technology 41(2) (2005) 123-131.
[65] M. Rotenberg, M. Rubin, A. Bor, D. Meyuhas, Y. Talmon, D. Lichtenberg, Physico-chemical characterization of Intralipid emulsions. Biochim Biophys Acta 1086(3) (1991) 265-272.
[66] P.F.C. Lim, X.Y. Liu, L. Kang, P.C.L. Ho, S.Y. Chan, Physicochemical effects of terpenes on organogel for transdermal drug delivery. Int J Pharm 358(1-2) (2008) 102-107.
[67] I.F. Almeida, M.F. Bahia, Evaluation of the physical stability of two oleogels. Int J Pharm 327(1-2) (2006) 73-77.
[68] A. Vintiloiu, J.C. Leroux, Organogels and their use in drug delivery - A review. Journal of Controlled Release 125(3) (2008) 179-192.
[69] B. Anand, S.S. Pisal, A.R. Paradkar, K.R. Mahadik, Applications of organogels in pharmaceuticals. Journal of Scientific & Industrial Research 60(4) (2001) 311-318.
[70] M.A. Ruiz, B. Clares, M.E. Morales, V. Gallardo, Preparation, rheological study, and characterization of an organogel as a system for transdermal release of active principles. Pharmaceutical Development and Technology 12 (2007) 637-644.
[71] S. Murdan, A review of pluronic lecithin organogels as a topical and transdermal drug delivery system. H.Pharm. 12 (2005).
[72] M.H. Abraham, W.E. Acree, Characterisation of the water-isopropyl myristate system. International Journal of Pharmaceutics 294(1-2) (2005) 121-128.

[73] M.M. Saket, K.C. James, I.W. Kellaway, THE PARTITIONING OF SOME 21-ALKYL STEROID ESTERS BETWEEN HUMAN STRATUM-CORNEUM AND WATER. International Journal of Pharmaceutics 27(2-3) (1985) 287-298.
[74] B.W. Barry, Dermatological Formulations: Percutaneous Absorption, Marcel Dekker, New York, 1983, pp. 127-233.
[75] D. Bucks, H. Maibach, in: Bronaugh, R.L. and H. I. Maibach (Eds.), Percutaneous absorptION: drugs - cosmetics - mechanisms - methodology, Taylor & Francis, Boca Raton, 2005, pp. 65-83.
[76] J. Hadgraft, Skin, the final frontier. International Journal of Pharmaceutics 224(1-2) (2001) 1-18.
[77] J. Ring, H.H. Fröhlich, Wirkstoffe in der dermatologischen Therapie, Springer, Berlin, 1985.
[78] J.J. Martinez-Pla, Y. Martin-Biosca, S. Sagrado, R.M. Villanueva-Camanas, M.J. Medina-Hernandez, Evaluation of the pH effect of formulations on the skin permeability of drugs by biopartitioning micellar chromatography. Journal of Chromatography A 1047(2) (2004) 255-262.
[79] M.R. Prausnitz, S. Mitragotri, R. Langer, Current status and future potential of transdermal drug delivery. Nature Reviews Drug Discovery 3(2) (2004) 115-124.
[80] S.E. Cross, B.M. Magnusson, G. Winckle, Y. Anissimov, M.S. Roberts, Determination of the effect of lipophilicity on the in vitro permeability and tissue reservoir characteristics of topically applied solutes in human skin layers. Journal of Investigative Dermatology 120(5) (2003) 759-764.
[81] B.P. Wenkers, B.C. Lippold, Skin penetration of nonsteroidal antiinflammatory drugs out of a lipophilic vehicle: influence of the viable epidermis. J Pharm Sci 88(12) (1999) 1326-1331.
[82] K. Inoue, K. Ogawa, Y. Suzuki, J. Okada, A. Kusai, M. Ikeda, K. Nishimura, The skin permeation mechanism of ketotifen: Evaluation of permeation pathways and barrier components in the stratum corneum. Drug Development and Industrial Pharmacy 26(1) (1999) 45-53.
[83] J. Hadgraft, Skin deep. European Journal of Pharmaceutics and Biopharmaceutics 58(2) (2004) 291-299.
[84] H. Trommer, R.H.H. Neubert, Overcoming the stratum corneum: The modulation of skin penetration - A review. Skin Pharmacology and Physiology 19(2) (2006) 106-121.
[85] R.H.H. Neubert, R. Wepf, Stratum Corneum: Struktur und Morphologie einer Barriere. Vol. 17/2007, Pharmazeutische Zeitung, 2007.
[86] R. Daniels, Strategies for Skin Penetration Enhancement. Skin Care Forum - Online, 2004.
[87] R.H. Guy, J. Hadgraft, Physicochemical aspects of percutaneous penetration and its enhancement. Pharm Res 5(12) (1988) 753-758.
[88] P. Karande, S. Mitragotri, Dependence of skin permeability on contact area. Pharm Res 20(2) (2003) 257-263.
[89] H. Zhai, H.I. Maibach, Occlusion vs. skin barrier function. Skin Res Technol 8(1) (2002) 1-6.
[90] H.B. Zhai, H.I. Maibach, Effects of skin occlusion on percutaneous absorption: An overview. Skin Pharmacology and Applied Skin Physiology 14(1) (2001) 1-10.
[91] H. Zhai, H. Maibach, Effects of Occlusion: Percutaneous Absorption. Vol. 118, Cosmetics & Toiletries magazine, 2003, pp. 22-29.
[92] J.W. Fluhr, S. Lazzerini, F. Distante, M. Gloor, E. Berardesca, Effects of prolonged occlusion on stratum corneum barrier function and water holding capacity. Skin Pharmacology and Applied Skin Physiology 12(4) (1999) 193-198.
[93] H. Wagner, K.H. Kostka, W. Adelhardt, U.F. Schaefer, Effects of various vehicles on the penetration of flufenamic acid into human skin. European Journal of Pharmaceutics and Biopharmaceutics 58(1) (2004) 121-129.
[94] P. Treffel, P. Muret, P. Muretdaniello, S. Coumesmarquet, P. Agache, EFFECT OF OCCLUSION ON INVITRO PERCUTANEOUS-ABSORPTION OF 2 COMPOUNDS WITH DIFFERENT PHYSICOCHEMICAL PROPERTIES. Skin Pharmacology 5(2) (1992) 108-113.
[95] S. Makki, P. Muret, A.M. Said, P. Bassignot, P. Humbert, P. Agache, J. Millet, Percutaneous absorption of three psoralens commonly used in therapy: Effect of skin occlusion (in vitro study). International Journal of Pharmaceutics 133(1-2) (1996) 245-252.
[96] C.M. Chiang, G.L. Flynn, N.D. Weiner, G.J. Szpunar, BIOAVAILABILITY ASSESSMENT OF TOPICAL DELIVERY SYSTEMS - EFFECT OF VEHICLE EVAPORATION UPON INVITRO DELIVERY OF MINOXIDIL FROM SOLUTION FORMULATIONS. International Journal of Pharmaceutics 55(2-3) (1989) 229-236.
[97] S.X. Chen, R.T. Lostritto, Maintaining a near zero-order drug delivery from minidose reservoirs: Simultaneous drug diffusion and binary vehicle evaporation. Journal of Pharmaceutical Sciences 86(6) (1997) 739-746.

[98] S.E. Friberg, T. Huang, P.A. Aikens, Phase changes during evaporation from a vegetable oil emulsion stabilized by a polyoxyethylene {20} sorbitanoleate, Tween(R) 80. Colloids and Surfaces a-Physicochemical and Engineering Aspects 121(1) (1997) 1-7.
[99] A. Al Bawab, S.E. Friberg, C. Fusco, Evaporation of a model skin lotion with beta-hydroxy acids. Int J Cosmet Sci 26(6) (2004) 273-279.
[100] A. Al-Bawab, S.E. Friberg, Phase behavior of the alpha-hydroxyoctanoic acid/Laureth 4/white oil/water system and preliminary evaluation of the phase changes during evaporation of its emulsion. Journal of Cosmetic Science 53(3) (2002) 151-164.
[101] L.A.M. Ferreira, J. Doucet, M. Seiller, J.L. Grossiord, J.P. Marty, J. Wepierre, IN-VITRO PERCUTANEOUS-ABSORPTION OF METRONIDAZOLE AND GLUCOSE - COMPARISON OF O/W, W/O/W AND W/O SYSTEMS. International Journal of Pharmaceutics 121(2) (1995) 169-179.
[102] D. Hummel, G. Imanidis, Marks,R; Leveque, J.-L; Voegeli, R., London, 2002, pp. 119-124.
[103] M.S. Roberts, S.E. Cross, Y.G. Anissimov, Factors affecting the formation of a skin reservoir for topically applied solutes. Skin Pharmacology & Physiology 17(1) (2004) 3-16.
[104] S.M. Al-Saidan, Transdermal self-permeation enhancement of ibuprofen. Journal of Controlled Release 100(2) (2004) 199-209.
[105] L. Kikwai, N. Kanikkannan, R.J. Babu, M. Singh, Effect of vehicles on the transdermal delivery of melatonin across porcine skin in vitro. Journal of Controlled Release 83(2) (2002) 307-311.
[106] J. Ziegenmeyer, Haut - Vehikel - Pharmakon, die entscheidenden Kriterien für die percutane Absorption. Vol. 15, Pharmazie in unserer Zeit, 1986, pp. 129-143.
[107] W. Weitschiess, Einfluss der Galenik auf die Therapie von Hautkrankheiten. Pharmazeutische Zeitung, 2003.
[108] C. Valenta, U. Siman, M. Kratzel, J. Hadgraft, The dermal delivery of lignocaine: influence of ion pairing. International Journal of Pharmaceutics 197(1-2) (2000) 77-85.
[109] J. Hadgraft, C. Valenta, PH, pK(a) and dermal delivery. International Journal of Pharmaceutics 200(2) (2000) 243-247.
[110] P.G. Green, J. Hadgraft, G. Ridout, ENHANCED INVITRO SKIN PERMEATION OF CATIONIC DRUGS. Pharmaceutical Research 6(7) (1989) 628-632.
[111] D.A. Dimas, P.P. Dallas, D.M. Rekkas, Ion pair formation as a possible mechanism for the enhancement effect of lauric acid on the transdermal permeation of ondansetron. Pharmaceutical Development and Technology 9(3) (2004) 311-320.
[112] M. Dias, J. Hadgraft, M.E. Lane, Influence of membrane-solvent-solute interactions on solute permeation in skin. International Journal of Pharmaceutics 340(1-2) (2007) 65-70.
[113] E. Jaeckle, U.F. Schaefer, H. Loth, Comparison of effects of different ointment bases on the penetration of ketoprofen through heat-separated human epidermis and artificial lipid barriers. Journal of Pharmaceutical Sciences 92(7) (2003) 1396-1406.
[114] B.W. Barry, Mode of action of penetration enhancers in human skin. Vol. 6, Journal of Controlled Release, 1987, pp. 85-97.
[115] K. Moser, K. Kriwet, A. Naik, Y.N. Kalia, R.H. Guy, Passive skin penetration enhancement and its quantification in vitro. Eur J Pharm Biopharm 52(2) (2001) 103-112.
[116] J. Kalbitz, R. Neubert, W. Wohlrab, Modulation der Wirkstoffpenetration in die Haut. Vol. 51, Pharmazie, 1996, pp. 619-637.
[117] B.W. Barry, LIPID-PROTEIN-PARTITIONING THEORY OF SKIN PENETRATION ENHANCEMENT. Journal of Controlled Release 15(3) (1991) 237-248.
[118] M. Bach, B.C. Lippold, Percutaneous penetration enhancement and its quantification. Eur J Pharm Biopharm 46(1) (1998) 1-13.
[119] R. Panchagnula, H. Desu, A. Jain, S. Khandavilli, Effect of lipid bilayer alteration on transdermal delivery of a high-molecular-weight and lipophilic drug: Studies with paclitaxel. Journal of Pharmaceutical Sciences 93(9) (2004) 2177-2183.
[120] R. Daniels, Strategies for Skin Penetration Enhancement. Issue 37, Skin Care Forum, 2004.
[121] I. Brinkmann, C.C. Muller-Goymann, Role of isopropyl myristate, isopropyl alcohol and a combination of both in hydrocortisone permeation across the human stratum corneum. Skin Pharmacology and Applied Skin Physiology 16(6) (2003) 393-404.
[122] T. Ogiso, M. Iwaki, T. Paku, EFFECT OF VARIOUS ENHANCERS ON TRANSDERMAL PENETRATION OF INDOMETHACIN AND UREA, AND RELATIONSHIP BETWEEN PENETRATION PARAMETERS AND ENHANCEMENT FACTORS. Journal of Pharmaceutical Sciences 84(4) (1995) 482-488.
[123] I. Kakubari, N. Nakamura, T. Takayasu, H. Yamauchi, S. Takayama, K. Takayama, Effects of solvents on skin permeation of formoterol fumarate. Biol Pharm Bull 29(1) (2006) 146-149.

[124] B.W. Barry, Is transdermal drug delivery research still important today? Drug Discovery Today 6(19) (2001) 967-971.
[125] V.M. Knepp, F.C. Szoka, R.H. Guy, CONTROLLED DRUG RELEASE FROM A NOVEL LIPOSOMAL DELIVERY SYSTEM .2. TRANSDERMAL DELIVERY CHARACTERISTICS. Journal of Controlled Release 12(1) (1990) 25-30.
[126] B.A.I. van den Bergh, I. Salomons-de Vries, J.A. Bouwstra, Interactions between liposomes and human stratum corneum studied by freeze-substitution electron microscopy. International Journal of Pharmaceutics 167(1-2) (1998) 57-67.
[127] V.M. Knepp, R.S. Hinz, F.C. Szoka, R.H. Guy, Controlled drug release from a novel liposomal delivery system. I. Investigations of transdermal potential. Vol. 5, Journal of Controlled Release, 1988, pp. 211-221.
[128] A. Teichmann, S. Heuschkel, U. Jacobi, G. Presse, R.H. Neubert, W. Sterry, J. Lademann, 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 67(3) (2007) 699-706.
[129] M.B. DelgadoCharro, G. IglesiasVilas, J. BlancoMendez, M.A. LopezQuintela, J.P. Marty, R.H. Guy, Delivery of a hydrophilic solute through the skin from novel microemulsion systems. European Journal of Pharmaceutics and Biopharmaceutics 43(1) (1997) 37-42.
[130] U. Schmalfuss, R. Neubert, W. Wohlrab, Modification of drug penetration into human skin using microemulsions. Journal of Controlled Release 46(3) (1997) 279-285.
[131] M. Kreilgaard, Influence of microemulsions on cutaneous drug delivery. Adv Drug Deliv Rev 54 Suppl 1 (2002) S77-98.
[132] G.A. Simon, H.I. Maibach, The pig as an experimental animal model of percutaneous permeation in man: Qualitative and quantitative observations - An overview. Skin Pharmacology and Applied Skin Physiology 13(5) (2000) 229-234.
[133] U. Jacobi, M. Kaiser, R. Toll, S. Mangelsdorf, H. Audring, N. Otberg, W. Sterry, J. Lademann, Porcine ear skin: an in vitro model for human skin. Skin Research and Technology 13(1) (2007) 19-24.
[134] N. Sekkat, Y.N. Kalia, R.H. Guy, Biophysical study of porcine ear skin in vitro and its comparison to human skin in vivo. J Pharm Sci 91(11) (2002) 2376-2381.
[135] O. Lopez, A. delaMaza, L. Coderch, J.L. Parra, Study of the composition and structure of pig stratum corneum based on the action of different solubilizing agents. Colloids and Surfaces a-Physicochemical and Engineering Aspects 123 (1997) 415-424.
[136] F.P. Schmook, J.G. Meingassner, A. Billich, Comparison of human skin or epidermis models with human and animal skin in in-vitro percutaneous absorption. International Journal of Pharmaceutics 215(1-2) (2001) 51-56.
[137] T. Ngawhirunpat, P. Opanasopit, S. Prakongpan, Comparison of skin transport and metabolism of ethyl nicotinate in various species. European Journal of Pharmaceutics and Biopharmaceutics 58(3) (2004) 645-651.
[138] D.R. Friend, INVITRO SKIN PERMEATION TECHNIQUES. Journal of Controlled Release 18(3) (1992) 235-248.
[139] T.J. Franz, On the relevance of in vitro data. Vol. 64, J. Invest. Dermatol., 1975, pp. 190 - 195.
[140] Y.W. Chien, Transdermal controlled systemic medications, Marcel Dekker, New York, 1987, pp. 25-81.
[141] OECD, Guidance document for the conduct of skin absorption studies numer 28. 2004.
[142] A. Nangia, S. Patil, B. Berner, A. Boman, H. Maibach, In vitro measurement of transepidermal water loss: A rapid alternative to tritiated water permeation for assessing skill barrier functions. International Journal of Pharmaceutics 170(1) (1998) 33-40.
[143] T. Masada, W.I. Higuchi, V. Srinivasan, U. Rohr, J. Fox, C. Behl, S. Pons, EXAMINATION OF IONTOPHORETIC TRANSPORT OF IONIC DRUGS ACROSS SKIN - BASELINE STUDIES WITH THE 4-ELECTRODE SYSTEM. International Journal of Pharmaceutics 49(1) (1989) 57-62.
[144] D.A. Schwindt, K.P. Wilhelm, H.I. Maibach, Water diffusion characteristics of human stratum corneum at different anatomical sites in vivo. Journal of Investigative Dermatology 111(3) (1998) 385-389.
[145] F. Netzlaff, K.H. Kostka, C.M. Lehr, U.F. Schaefer, TEWL measurements as a routine method for evaluating the integrity of epidermis sheets in static Franz type diffusion cells in vitro. Limitations shown by transport data testing. Eur J Pharm Biopharm 63(1) (2006) 44-50.
[146] J. Hadgraft, M.E. Lane, Skin permeation: The years of enlightenment. International Journal of Pharmaceutics 305(1-2) (2005) 2-12.

[147] W.J. Pugh, M.S. Roberts, J. Hadgraft, Epidermal permeability - Penetrant structure relationships .3. The effect of hydrogen bonding interactions and molecular size on diffusion across the stratum corneum. International Journal of Pharmaceutics 138(2) (1996) 149-165.
[148] M.S. Roberts, K.A. Walters, Drugs and the pharmaceutical sciences, Vol. 91, Marcel Dekker, New York ; Basel etc., 1998, pp. 245 - 268.
[149] M.H. Abraham, H.S. Chadha, R.C. Mitchell, THE FACTORS THAT INFLUENCE SKIN PENETRATION OF SOLUTES. Journal of Pharmacy and Pharmacology 47(1) (1995) 8-16.
[150] M.S. Roberts, W.J. Pugh, J. Hadgraft, Epidermal permeability: Penetrant structure relationships .2. The effect of H-bonding groups in penetrants on their diffusion through the stratum corneum. International Journal of Pharmaceutics 132(1-2) (1996) 23-32.
[151] M. Dias, A. Farinha, E. Faustino, J. Hadgraft, J. Pais, C. Toscano, Topical delivery of caffeine from some commercial formulations. International Journal of Pharmaceutics 182(1) (1999) 41-47.
[152] H. Wagner, K.H. Kostka, C.M. Lehr, U.F. Schaefer, Correlation between stratum corneum/water-partition coefficient and amounts of flufenamic acid penetrated into the stratum corneum. J Pharm Sci 91(8) (2002) 1915-1921.
[153] D. van der Merwe, J.E. Riviere, Effect of vehicles and sodium lauryl sulphate on xenobiotic permeability and stratum corneum partitioning in porcine skin. Toxicology 206(3) (2005) 325-335.
[154] G.E. Parry, A.L. Bunge, G.D. Silcox, L.K. Pershing, D.W. Pershing, PERCUTANEOUS-ABSORPTION OF BENZOIC-ACID ACROSS HUMAN SKIN .1. INVITRO EXPERIMENTS AND MATHEMATICAL-MODELING. Pharmaceutical Research 7(3) (1990) 230-236.
[155] E. Khalil, K. Kretsos, G.B. Kasting, Glucose partition coefficient and diffusivity in the lower skin layers. Pharmaceutical Research 23(6) (2006) 1227-1234.
[156] M. Kreilgaard, E.J. Pedersen, J.W. Jaroszewski, NMR characterisation and transdermal drug delivery potential of microemulsion systems. J Control Release 69(3) (2000) 421-433.
[157] G.M. Golden, J.E. McKie, R.O. Potts, Role of stratum corneum lipid fluidity in transdermal drug flux. J Pharm Sci 76(1) (1987) 25-28.
[158] S. Hansen, A. Henning, A. Naegel, M. Heisig, G. Wittum, D. Neumann, K.H. Kostka, J. Zbytovska, C.M. Lehr, U.F. Schaefer, In-silico model of skin penetration based on experimentally determined input parameters. Part I: Experimental determination of partition and diffusion coefficients. European Journal of Pharmaceutics and Biopharmaceutics 68(2) (2008) 352-367.
[159] W.I. Higuchi, DIFFUSIONAL MODELS USEFUL IN BIOPHARMACEUTICS - DRUG RELEASE RATE PROCESSES. Journal of Pharmaceutical Sciences 56(3) (1967) 315-&.
[160] W.I. Higuchi, T. Higuchi, THEORETICAL ANALYSIS OF DIFFUSIONAL MOVEMENT THROUGH HETEROGENEOUS BARRIERS. Journal of the American Pharmaceutical Association 49(9) (1960) 598-606.
[161] J.T.H. Ong, E. Manoukian, RELEASE OF LONAPALENE FROM 2-PHASE EMULSION-TYPE OINTMENT SYSTEMS. Pharmaceutical Research 5(1) (1988) 16-20.
[162] K. Kriwet, C.C. Mullergoymann, MUTUAL INTERACTIONS BETWEEN DICLOFENAC DIETHYLAMINE AND PHOSPHOLIPIDS - INVESTIGATIONS ON THE MICROSTRUCTURE OF THE ARISEN SYSTEMS. Pharmazie 49(2-3) (1994) 187-191.
[163] P. Clement, C. Laugel, J.P. Marty, Influence of three synthetic membranes on the release of caffeine from concentrated W/O emulsions. Journal of Controlled Release 66(2-3) (2000) 243-254.
[164] D.I. Friedman, J.S. Schwarz, M. Weisspapir, SUBMICRON EMULSION VEHICLE FOR ENHANCED TRANSDERMAL DELIVERY OF STEROIDAL AND NONSTEROIDAL ANTIINFLAMMATORY DRUGS. Journal of Pharmaceutical Sciences 84(3) (1995) 324-329.
[165] P. Izquierdo, J.W. Wiechers, E. Escribano, M.J. Garcia-Celma, T.F. Tadros, J. Esquena, J.C. Dederen, C. Solans, A study on the influence of emulsion droplet size on the skin penetration of tetracaine. Skin Pharmacology and Physiology 20(5) (2007) 263-270.
[166] J.S. Schwarz, M.R. Weisspapir, D.I. Friedman, ENHANCED TRANSDERMAL DELIVERY OF DIAZEPAM BY SUBMICRON EMULSION (SME) CREAMS. Pharmaceutical Research 12(5) (1995) 687-692.
[167] G. Di Colo, C. Giannessi, E. Nannipieri, M.F. Serafini, D. Vitale, Influence of drug-surfactant and skin-surfactant interactions on percutaneous absorption of two model compounds from ointment bases in vitro. Vol. 50, Elsevier, International Journal of Pharmaceutics, 1989, pp. 27-34.

Curriculum Vitae

8 Curriculum Vitae

Miriam Reiser

Pharmacist

Personal Details

Day of Birth	January 28th, 1979
Place of Birth	Nürnberg
Nationality	German

PhD Thesis

November 2004 – November 2008	PhD Student at Institute of Pharmaceutical Technology (University of Basel) under the supervision of Prof. Dr. Georgios Imanidis
	Assistant in practical university courses of semi-solid dose forms

Supervision of the Following Master Thesis

March - July 2007	„Physicochemical characterization of lipophilic vehicle systems and its influence on transdermal permeation behaviour of benzyltrimethylammonium chloride, caffeine and ibuprofen as model compounds“(Ursula Stäger)
January - June 2008	„Effect of oil base, phase fraction, dose and evaporation on the transdermal permeation of caffeine as model compound in lipophilic vehicle systems“(Melanie Albert)

Presentation

25 - 29 March 2008	Perspectives in Percutaneous Penetration 2008, International Conference in La Grande Motte, France
	Poster: “Continuous phase drug concentration concept: Application to simple w/o-emulsions with ibuprofen as model compound“

Education

October 1998 - 2002	Pharmacy studies at Friedrich-Alexander-University, Erlangen-Nürnberg
June 1998	High school diploma („Abitur“) at Gymnasium Stein, Stein

Work Experience

January - October 2004	Responsible pharmacist at Sonnen-Apotheke, Rosstal
December 2003	Approbation as pharmacist
May 2003 - Oktober 2003	Intership at Hoffmann - La Roche AG in Kaiseraugst / Basel, Switzerland
October 2002 - April 2003	Intership at Germania-Apotheke, Nürnberg

Trainings

June 2008	Quality and GMP – Continuing Education Course, Pharmazentrum Basel - ETH Zürich
March 2007	Regulatory Affairs – Continuing Education Course, Pharmazentrum Basel - ETH Zürich
September / October 2005	Key Issues in Drug Discovery and Development, Pharmazentrum Basel - ETH Zürich
March 2005	Basics of HPLC, Agilent Technologies, Basel

Additional Skills

Languages	German (native speaker) English (fluent) French (basic knowledge)
PC literacy Applications	Proficient user Good command of Microsoft Office Applications (Excel, Power Point, Word)

I am grateful to my academic teachers:

Prof. Dr. Peter Gmeiner, Prof. Dr. Reinhard Troschütz, Prof. Dr. Wolfgang Kreis, Prof. Dr. Gisa Tiegs, Prof. Dr. Burkhard Hinz, Prof. Dr. Geoffrey Lee, Prof. Dr. Georgios Imanidis, Dr. Brian Cutting