DISSERTATION

Titel der Dissertation

Cubic gels, microemulsions and nanoemulsions for optimised skin penetration/permeation of active molecules and improved chemical stability

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„Wie der Wanderer den zurückgelegten Weg erst, wenn er auf der Höhe angekommen ist, im Zusammenhang überblickt und erkennt, so erkennen wir erst am Ende einer Periode unseres Lebens auch den Wert derselben.“
(Schopenhauer)

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1 Einleitung und Zielsetzung

Die Aufnahme von Arzneistoffen über die Haut wird schon seit langem durch bewährte Arzneiformen gewährleistet, wobei in den letzten Jahren kolloidale Trägersysteme immer mehr an Bedeutung gewonnen haben. Zu diesen Systemen zählen moderne Dermatika, wie Liposomen, Mikroemulsionen, Kubische Gele und Nanoemulsionen.

Vorteile wie hohe chemische und physikalische Stabilität, ausreichende Arzneistoff-Ladungskapazität für lipophile sowie hydrophile Moleküle, eine kontrollierte Freisetzungsrate und die Möglichkeit zum „Drug Targeting“ machen diese neuen innovativen Systeme zu einem interessanten und viel versprechenden Forschungsschwerpunkt in der Pharmazeutischen Technologie. Von besonderer Bedeutung sind die rheologischen Eigenschaften kolloidaler Systeme, die z.B. von Konzentration, Temperatur, Teilchengröße, Teilchenform etc. abhängen. Weiters sind diese Trägersysteme meist durch einfache Herstellungsmethoden produzierbar und deswegen in der pharmazeutischen Industrie gern genutzt.

Im Bereich der dermalen und transdermalen Anwendung findet der Einsatz kolloidaler Systeme auf Grund ihrer permeationsfördernden Eigenschaften großes Interesse. Kubische Gele, Mikroemulsionen und Nanoemulsionen bestehen aus Wasser, Öl und Tenside als auch Ko-Tenside. Meist werden Öle und Tenside verwendet, die als chemische Permeationsförderer agieren. Sie vermögen die Integrität des Stratum corneum, der physiologischen Hautbarriere, permanent zu zerstören und so den Eintritt von Xenobiotika jeder Art in die systemische Zirkulation zu ermöglichen. Jedoch limitiert die Zusammensetzung solcher Formulierungen oft das Einsatzgebiet. Viele der Trägersysteme enthalten hautirritative und toxische Inhaltsstoffe.

Im Rahmen der vorliegenden Dissertation sollten Kubische Gele, Mikroemulsionen und Nanoemulsionen entwickelt werden, die in erster Linie aus Inhaltsstoffen hoher dermaler Verträglichkeit und geringer Toxizität bestehen und sich durch eine hohe Wirkstofffreisetzung auszeichnen.

Der erste Teil der Arbeit bestand darin den Einfluss unterschiedlich lipophiler, fluorierter Modelldrogen auf die Mikrostruktur eines kürzlich neu entwickelten kubischen Systems, das aus einer Mischung von Polyoxyethylen (5)cetyl/oleylether und Polyoxyethylen(10)cetyl/oleylether, Wasser und Paraffinöl besteht, zu untersuchen [1,2]. Rheologische Messungen bei unterschiedlichen Temperaturen, sowie Hautdiffusionsversuche und Stabilitätsuntersuchungen von darin eingearbeiteten Wirkstoffen wurden durchgeführt.

Der zweite Teil beschäftige sich mit der Entwicklung besser hautverträglicher kolloidaler Trägersysteme, da Polyoxyethylen-Derivate zu leichten Hautirritationen führen können. Mikroemulsionen aus unterschiedlich zusammengesetzten Soja-Lezithinen sollten an Hand von Phasendiagrammen entwickelt werden. Eine hohe dermale Verträglichkeit und geringe Toxizität der Formulierungen standen im Vordergrund.
Ziel war es, den Einfluss von Lipoid S100, S75 und S45 auf das kolloidale System und dessen physikalisch-chemische Eigenschaften festzustellen. Parameter wie Partikelgröße, Polydispersitäts Index (PDI) und Viskosität wurden gemessen und die Hautpermeation von Modelldrogen und deren chemische Stabilität in den Zubereitungen wurden erforscht. Da Phosphatidylcholin in der Literatur mit einer hohen Membranaffinität und folglich als Membranpermeationsförderer gehandelt wird, stellte sich die Frage, ob unterschiedliche Anteile an Phosphatidylcholin (PC), Phosphatidylethanolamin (PE) und Lysophosphatidylethanolamin (LPE) zu einer Änderung der kolloidalen Mikrostruktur der Mikroemulsionen führen würden [3]. Daher war es auch von Interesse, ob die unterschiedlichen Lezithinzusammensetzungen einen Einfluss auf die Hautdiffusion der ausgesuchten Modelldrogen haben. Anschließend sollten die Systeme hinsichtlich der unterschiedlichen Ölkomponenten Ölsäure und Isopropylmyristat in Bezug auf die Hautdiffusion getestet werden. In einem nächsten Schritt wurden die unterschiedlichen Formulierungen mittels unterschiedlicher NMR-Methoden charakterisiert, um eine eventuelle Korrelation zwischen den physikalisch-chemischen Eigenschaften, Arzneistoffselbstdiffusion und Hautpermeation aufzuzeigen.

Der dritte Teil der Arbeit beschäftigte sich mit der Entwicklung hautfreundlicher, physikalisch stabiler Nanoemulsionen unter Verwendung eines Hochdruckhomogenisators, basierend auf bestehenden Standardrezepturen. Als hautfreundliche Tenside wurden Soja-Lezithin, ausgewählte Zuckertenside oder Polysorbat 80 verwendet. Der Vorteil von Nanoemulsionen gegenüber Mikroemulsionen besteht in erster Linie im geringeren Tensidgehalt.
Nach erfolgter physikalisch-chemischer Charakterisierung und anschließenden Stabilitätstests, sollte in einem weiteren Schritt die Oberfläche der Partikel positiv geladen werden. Als kationisches Additiv wurde das physiologisch vorkommende, anti-inflammatorisch wirkende Phytosphingosin eingearbeitet. Ziel war es durch die positive Ladung eine erhöhte Arzneistoffpermeation von Modelldrogen zu bewirken, da in vorangegangenen Studien bewiesen wurde, dass positive öberflächenmodifizierte Nanoemulsionströpfchen Wirkstoffe besser durch die Haut transportieren können als negativ geladene Partikel [4, 5].

Der letzte Teil der Arbeit beschäftigte sich mit den anti-inflammatorischen wirkenden Phytosphingosin und N-Palmitoylethanolamin und deren Bindungskapazität an liposomalen DPPC-Modellmembranen. Mit Micro-Differential-Scanning-Calorimetry sollten Wechselwirkungen nachgewiesen werden, die sich durch Verschiebungen der charakteristischen thermischen Phasenübergänge des Modelllipids DPPC auszeichnen.

2 Introduction and Aim of the study

During the last years drug transport via the dermal and transdermal route has been supported by approved dosage forms, whereas colloidal systems became more and more important. Examples for such systems are liposomes, microemulsions, cubic systems and nanoemulsions.

Advantages, such as high chemical and physical stability, sufficient drug loading capacity, controlled release characteristics and the possibility of drug targeting favour colloidal carriers in the field of pharmaceutical technology. The rheological properties of these systems depending on concentration, temperature, particle size and shape are of particular importance. Moreover, the feasibility of scaling up production with simple methods of production and reasonable overall costs makes colloidal carriers interesting candidates in the pharmaceutical industry.

Vehicles in the field of dermal and transdermal application are of great interest due to their potential permeation enhancing effect. Cubic gels, microemulsions and nanoemulsions consist of water, oil and surfactant as well as co-surfactant. Usually, oil and surfactants with potential of chemical enhancement were used. They are able to interrupt the physiological stratum corneum barrier and therefore to allow the permeation of xenobiotics in the systemic circulation. However, the ingredients of such formulations are limiting the field of application. Various components of carrier systems are often skin irritating and toxic.

The purpose of the present work was to develop cubic systems, microemulsions and nanoemulsions having a high permeation rate of active agents using highly skin friendly and non-toxic ingredients.

In the first part the influence of selected fluorinated drugs with different solubility on the microstructure of an already evaluated cubic gel system should be investigated. The cubic gel consisted of a mixture of polyoxyethylen(5) cetyl/oleylether and polyoxyethylen(10)cetyl/oleylether, water and paraffin-oil [1,2]. Rheological measurements at different temperatures, in vitro standard

diffusion experiments as well as the chemical stability of the incorporated fluorinated drugs should be performed.

The second part of the study focused on the developing of higher skin friendly colloidal drug delivery systems, due to the skin irritation potential of polyoxyethylen-derivatives. Microemulsions consisting of differently composed soybean lecithins should be evaluated by means of pseudo ternary phase diagrams. These systems should be characterised by high skin compatibility and non-toxicity. It was the aim to investigate the influence of Lipoid S100, Lipoid S75 and Lipoid S45 on one hand on the physicochemical properties of the colloidal systems like particle size, polydispersity index (PDI), viscosity and on the other hand on the skin permeation and chemical stability of the incorporated model drugs. In literature data phosphatidylcholine is discussed to have a strong ability to adsorb on biomembranes and to enhance biomembrane permeability [3]. Therefore, it was the question, whether different amounts of phosphatidylcholine (PC) molecules, phosphatidylethanolamine (PE) and lysophosphatidylethanolamine are able to improve physicochemical behaviours in order to increase skin permeation. Additionally, the influence of different oil components oleic acid and isopropyl myristate should be investigated on skin permeation. As next step the microstructure of the systems should be analysed by different multinuclear NMR-techniques, in order to find a correlation between skin diffusion, physicochemical properties and drug self-diffusion coefficients.

The third part of the study focused on the investigation of highly skin compatible physically stable nanoemulsions using high pressure homogenisation, based on standard o/w nanoemulsions. Therefore skin friendly surfactants like soybean lecithins, selected sugar esters or polysorbate 80 were used, respectively. A major advantage of nanoemulsions compared to microemulsions is the low surfactant concentration. After physicochemical characterisation and stability studies it was the main objective to get a positive surface charge of the particles. Phytosphingosine, a physiological component served as cationic additive. In recent published reports an improved skin permeability of positively modified nanoemulsion particles was indicated compared to negatively charged

[4, 5]. The objective was to enhance drug permeation of active agents across skin by phytosphingosine as cationic compound.

In the last part the anti-inflammatory phytosphingosine and N-palmitoylethanolamine were tested on their ability of binding or penetrating into the layers of active phospholipids used as model membranes. In DSC-studies interactions are indicated by changes in the characteristic phase transition temperature.

3 Background

3.1 Skin

3.1.1 Structure and morphology of the skin

The skin is the outer barrier of living tissue of plant, animal and human. It is the largest organ of the integumentary system made up of multiple layers of epithelial tissues, and guards the underlying muscles, bones, ligaments and internal organs. Because it interfaces with the environment, skin plays a very important role in protecting the body against pathogens. Its other functions are insulation, temperature regulation, sensation and synthesis of vitamin D and the protection of vitamin B folates [6]. The skin consists of epidermis, dermis and subcutis as well as of the appendages like hair follicle, sebaceous and sweat glands.

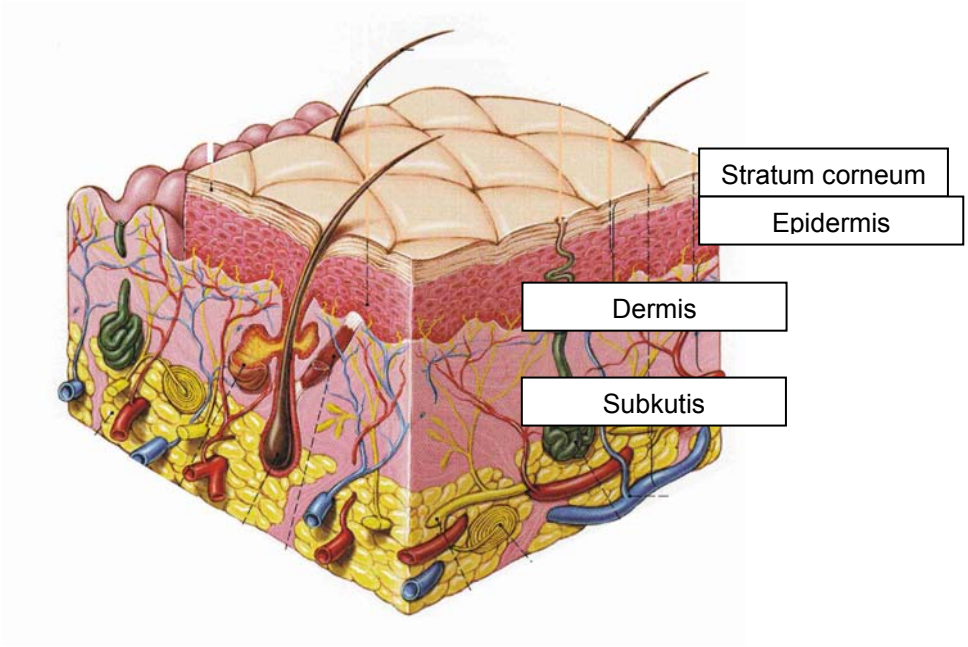

Figure 1: Skin layers: epidermis, dermis and subcutis, showing a hair follicle, sweat gland and sebaceous gland (http://www.sinnesphysiologie.de/hvsinne/haut/hautin.htm, access date: 05.06)

The epidermis consists of stratum basale, stratum spinosum, stratum garnulousm and stratum corneum. The keratinocytes are built in the stratum basale by cell differentiation and under different differentiation processes they are moving into the stratum corneum, the outermost layer. The stratum

corneum (SC) controls absorption and is the final product of epidermal differentiation. It is a highly complex organised and extremely adaptable, biologically death, but dynamic 10-15 µm thick skin layer [7]. It is composed mainly of corneocytes, cells that are containing keratin, a protein that helps keep the skin hydrated by preventing water evaporation. The thickness of the stratum corneum varies according to the amount of protection and/or grip required by a region of the body. In generally it consists of 15–20 cell layers built on corneocytes and intercellular lipids and is a multi-layered brick and mortar like structure [8] (Figure 2). It consists of lipid bilayer with alternating hydrophilic and hydrophobic areas. This is an efficient barrier against chemicals that are insoluble in fat and against those, which are insoluble in water. The intercellular lipids consist of ceramides (30 %), free fatty acids (30 %) as well as cholesterol and cholesterol esters. Unlike all other biological membranes, those in the stratum corneum do not contain phospholipids. The most remarkable of all the ceramides is the ester-linked ω-hydroxyacids, the acylceramide [7]. This acylceramide is one of the principal carriers of linolic acid in the epidermis and acts as a “molecular rivet” in the intercellular lipid lamellae of the SC [9].

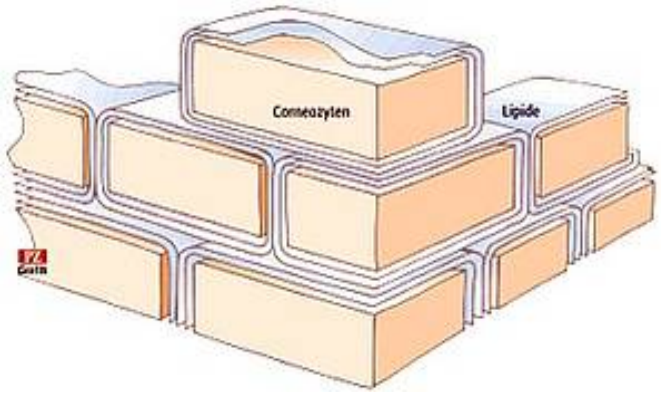

Figure 2: The multi-layered brick and mortar model of the stratum corneum [10]

The high degree of order of the intercellular lipids is important for the barrier function of the stratum corneum. The SC-lipids are building lipid bilayers, which are between 4-5 nm thick. For the stability and barrier function of the SC not only the brick mortar model is deciding, but also the clamp structure of the corneocytes, the corneodesmosomes and the tight junctions [6]. Furthermore

the epidermis consists of melanocytes, sensory Merkel-cells, immunology active Langerhans-cells and lymphocytes beside a multiplicity of keratinocytes (90%).

The dermis is tightly connected to the epidermis by a basement membrane, and harbours many nerve endings that provide the sense of touch and heat. It contains the hair follicles, sweat glands, sebaceous glands, apocrine glands and blood vessels. The blood vessels in the dermis provide nourishment and waste removal to its own cells. The dermis is about 0.1 to 0.5 cm thick and consist of collagenous fibres (70%), providing a scaffold of support and elastic connective tissue, providing elasticity [6].

The subcutis or hypodermis is directly underlying the dermis and is mainly composed of adipose tissue. Its physiological function includes insulation and storage of nutrients and mechanical protection.

3.1.2 Mechanism of dermal drug delivery

The high degree of order of the intercellular lipids is important for the barrier function of the stratum corneum. There are a number of routes possible by which a molecule can cross the stratum corneum (Figure 3):

- Intercellular route
- Transcellular route
- Transappendage route either through sweat glands or hair follicle
- Corneodesmosomale route

According new cognitions the transcellular route seems to bee implausible, because of molecules have to penetrate alternating lipophilic and hydrophilic layers [7]. Moreover, it seems that small polar molecules are penetrating through the corneodesmosomale route considering the high number of corneodesmosoes pro corneocytes. Corneodesmosomes are molecular joints between cells consisting of transmembranproteins playing a major role for the cohesion of corneocytes [7].
The intercellular pathway is possible for lipophilic/apolar molecules through lateral diffusion along the lipophilic hydrocarbon chains of the stratum corneum

lipids [8]. In recent years, the transappendage route has become interesting for research. It seems to play a more important role as so far assumed [11,12].

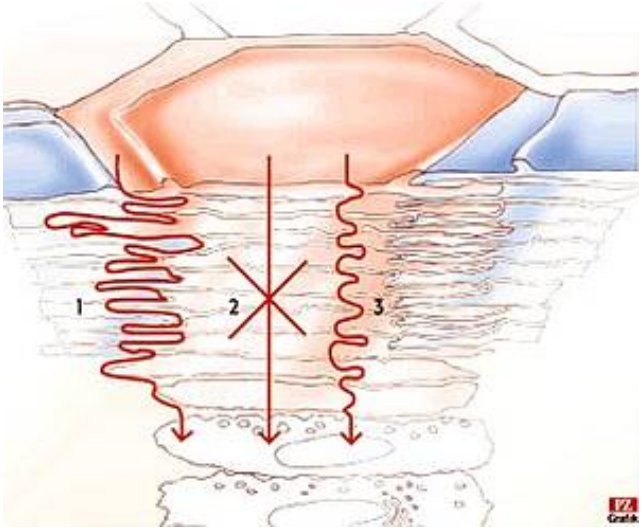

Figure 3: transport routes across the stratum corneum according to new cognitions of the skin barrier structure 1: intercellular pathway; 2: transcellular pathway; 3: corneodesmosomale pathway (http://www.pharmazeutische-zeitung.de/index.php; access date: 09.2008)

Moreover, the rate at which permeation occurs is largely dependent on the physicochemical characteristics of the penetrant, the concentration of permeant applied, the partition coefficient of the permeant between the SC and the vehicle as well as the diffusivity of the compound within the stratum corneum [6].

Drug diffusion across the stratum corneum obeys Fick`s first law (equation 1), where steady-state flux (J) is related to the diffusion coefficient (D) of the drug in the stratum corneum over a diffusional path length or membrane thickness (h), the partition coefficient (P) between the SC and the vehicle and the applied drug concentration (c_0) which is assumed to be constant:

$$\frac{dm}{dt} = J = \frac{DC_0P}{h} \qquad (1)$$

The influence of solubility and partition coefficient of a drug on diffusion across the SC has been extensively studied. Molecules showing intermediate partition

coefficient (log $P_{ocatonol/water}$ of 1-3) have adequate solubility within the lipid domains of the SC to permit diffusion through these domains, whereas still having sufficient hydrophilic nature to allow partitioning into the viable tissues of the epidermis [13].

3.1.3 Penetration enhancement

Chemical penetration enhancers increase skin permeability by reversibly damaging or altering the physicochemical nature of the stratum corneum to reduce its diffusion resistance. Therefore, scientists are searching for possibilities to enhance drug permeation across skin since many years. The effectiveness of a permeation enhancer is mostly dependent on its physicochemical properties. These properties are responsible for the penetration route of the molecules across the SC, the polar or apolar pathway. The enhancer activity of many classes of chemicals has been tested including water, surfactants, essential oils and terpens, Azone analogues, dimethyl sulfoxide (DMSO), alcohols and fatty acids [13, 14]. On of the most researched compound is oleic acid, which is forming pools within the lipid domains to create permeable pores that provide less resistance for polar molecules [8, 13, 15]. On the other hand Azone appears to influence the permeation as well on the polar as on the apolar pathway due to its chemical structure [7]. In addition some chemicals have been identified as penetration retarders. To sum up the mechanism by which enhancers effect skin permeability are:

- Disruption of the intercellular bilayer structure
- Interaction with the intercellular proteins of the stratum corneum
- Improvement of partitioning of a drug, co-enhancer, or co-solvent into the stratum corneum

One of the problems associated with many chemical penetration enhancers is their irritation and toxicity potential. Therefore, the use for clinical applications is limited. In recent years there have been move towards investigations of potential enhancers classified as GRAS (Generally Regarded As Safe), such as essential oils and terpens, and polymeric enhancers [16].

Also a number of electrical methods of penetration enhancement have been evaluated. These include iontophoresis, phonophoresis, electroporation and photomechanical waves [13]. In addition synergy between chemical enhancers and electrically assisted methods have been described [17].

Beside the penetration enhancement by stratum corneum modification the enhancement can also be improved by optimisation of drug and vehicle properties. Thereby drug selection, pro-drug & ion pairs, drug-vehicle interactions, chemical potential of drug, complexes, liposomes vesicles and particles are all different techniques to enhance drug permeation [13].
An overview of techniques to optimise drug permeation across skin is given is given in Figure 4.

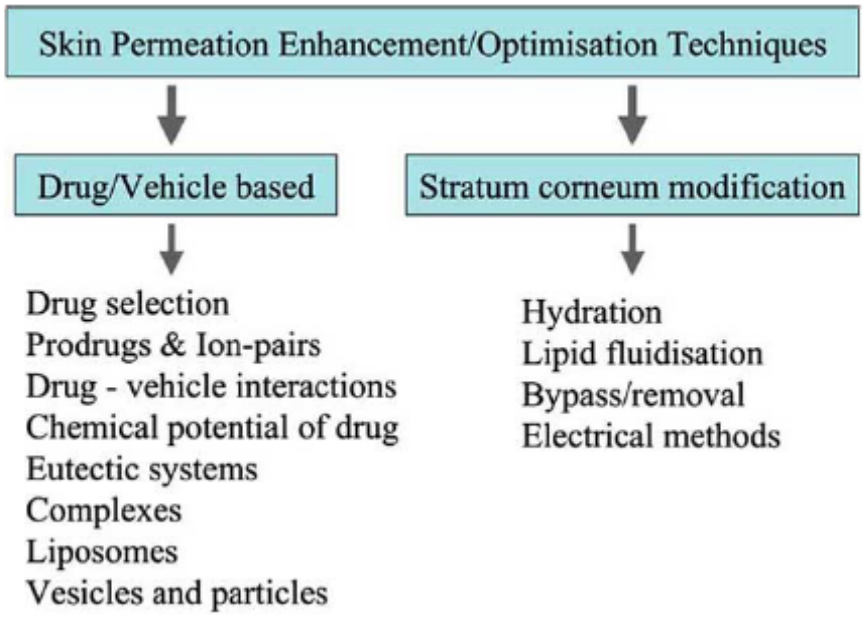

Figure 4: Techniques to optimise drug permeation across the skin [13]

3.2 Colloidal drug delivery systems

Colloidal carriers have attracted the main interest because they are promising systems for drug delivery across skin. The important requirements such as non-toxicity, sufficient drug loading capacity, possibility of drug targeting, controlled release characteristics and chemical and physical storage stability can all be fulfilled by these systems [18]. The common characteristics of all colloidal systems are the submicron particle size. The carriers might differ in materials, composition, drug loading and application spectrum. But in the first place, nanosized carriers are treated as hopeful means to increase the solubility and therefore the bioavailability of poorly water-soluble active ingredients [19].

3.2.1 Cubic gels

Cubic “ringing” gels have an interesting thermodynamically stable structure consisting of curved bi-continuous lipid bilayer in three dimensions, separating two congruent networks of water channels (Figure 5). The water pore diameter of the fully swelled phase is about 5 nm and the phase is very viscous [20].

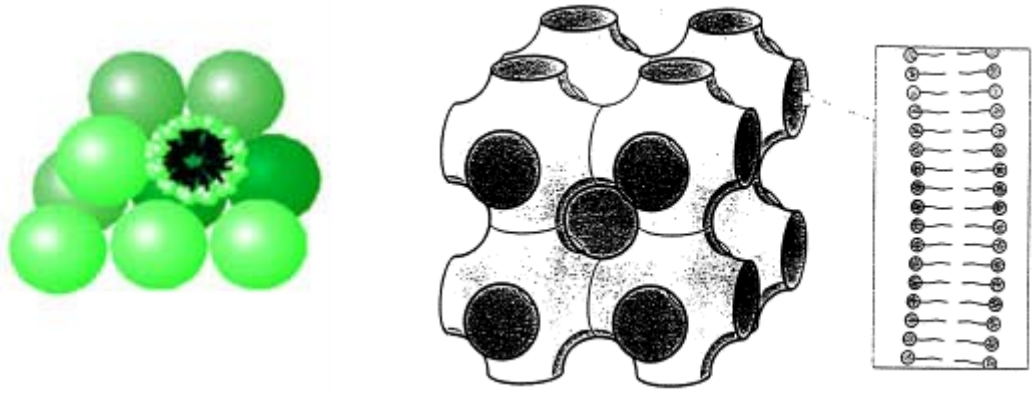

Figure 5: Cubic gel structures [20, 21]

The unique structure of cubic phase has been extensively studied using various spectroscopic techniques and their resemblance to bio-membranes has prompted many scientists to study behaviour of active molecules in cubic

phases [2]. Cubic phases are structurally closely related to microemulsions and can be prepared at water concentrations from 55 to 85% with 15-45% of lipid, usually GMO (glyceryl monooleate). The diffusion of drugs from a cubic phase typically shows controlled release from such matrices as indicated by Higuchi`s square root of time release kinetics. Incorporation of a drug in a cubic phase can cause phase transformation to a lamellar or reversed hexagonal phase depending on the polarity and concentration of the drug, which may affect the release profile. Biodegradability, phase behaviour, ability to deliver drugs of varying sizes and polarity and the ability to enhance the chemical and/or physical stability of incorporated drugs and proteins make cubic phases excellent candidates for use as drug delivery vehicles [20, 22]. However, cubic gels have also disadvantages. One of the major is the extremely high viscosity of the formulation that may be limited to specific applications such as periodontal, mucosal, vaginal and short acting oral drug delivery. Beside, also the skin compatibility and toxicity of the ingredients should be taken into consideration. Therefore, a careful analysis of risks and benefits is necessary.

3.2.2 Microemulsions

Microemulsions are colloidal dispersions composed of an oil phase, aqueous phase, surfactant and co-surfactant at appropriate ratios [15]. They are generally defined as isotropic, transparent and thermodynamically stable liquids with ultra low interfacial tension, large interfacial area and the capacity to solubilize both aqueous and oil-soluble compounds [15, 23-25]. The essential distinction between normal emulsion and microemulsions is their particle size and stability. Usually the droplet diameter is within the range of 10 -100 nm [25]. The use of lower alkanols as co-surfactants (butanol, pentanol, hexanol) is necessary to produce transparent solutions comprising dispersions of either water in oil (w/o), oil in water (o/w) or bi-continuous structures (Figure 6).

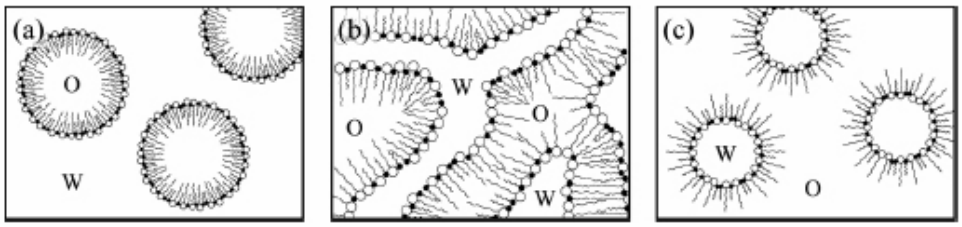

Figure 6: Structures of microemulsions; a) o/w microemulsion; b) bi-continuous structure; c) w/o microemulsion [26]

The co-surfactants lower the interfacial tension between oil and water sufficiently low for almost spontaneously formation of the system. The miscibility of oil, water and amphiphile (surfactant plus co-surfactant) depends on the overall composition, which is system specific. Ternary and quarterny phase diagrams (Figure 7) can describe the phase manifestations and are essential in the study of microemulsions [23, 24, 27, 28]. Such characteristics textures are commonly referred as Winsor phases and the classification is distinguished in a single phase region of common micelles or oil in water (o/w) microemulsions (Winsor I), a reverse micelle phase or water-in-oil (w/o) microemulsions (Winsor II), a middle microemulsion phase, so called bicontinuous phase (Winsor III) and a single phase with oil, water and surfactant homogeneously mixed (Winsor IV)[24].

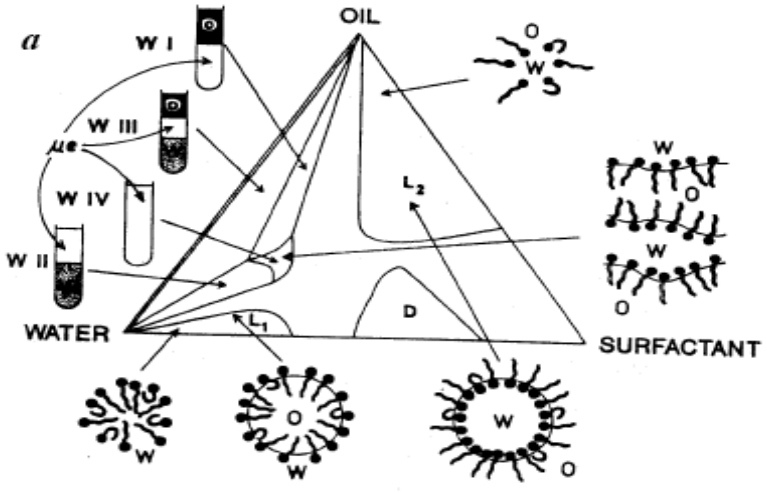

Figure 7: Schematic ternary phase diagram representing Winsor classification. L_1 single phase region of normal micelles or o/w microemulsions; L_2 reverse micelles or w/o microemulsions; D anisotropic lamellar liquid crystalline phase [24]

In the last years many reports are dealing with microemulsions and there pharmaceutical use [3, 29, 30]. Three main mechanisms have been proposed to explain the advantages of microemulsions for the dermal and transdermal use:

- The high solubility potential for lipophilic and hydrophilic drugs, which might increase the thermodynamic activity towards the skin
- Ingredients of microemulsions can act as permeation enhancers
- Enhancing effect on dermal and transdermal ability over conventional formulation

However, there are also several factors limiting the uses of microemulsions in the pharmaceutical and medicinal fields. Toxicity and bio-incompatibility of surfactants and co-surfactants and requirement of high concentrations of surfactant make the findings of skin friendly ingredients for microemulsions necessary. Therefore, the use of natural occurring surfactants was proposed such as soybean-lecithins and unsaturated fatty acid esters as oil component [3, 31, 32].

3.2.3 Nanoemulsions

Another interesting group of drug delivery vehicles are nanoemulsions. They are part of a broad class of multiphase colloidal dispersions. Although some lyotropic liquid crystalline phases, also known as micellar phases, mesophases and microemulsions may appear to be similar to nanoemulsions in composition and nanoscale structure, such matrices are actually quite different. Nanoemulsions do not form spontaneously, because an external shear has to be applied to rupture larger droplets into smaller ones [33]. They can be prepared by spontaneous emulsification such as phase inversion temperature (PIT) emulsification or phase inversion composition, or by using a high shear device [34]. Generally, nanoemulsions can be defined as oil in water emulsions with mean particle size diameters ranging from 20 to 200 nm [35]. The particles which are formed exhibit a liquid, lipophilic core separated from the surrounding aqueous phase by a monomolecular layer of phospholipids (Figure 8). Due to their lipophilic interior, nanoemulsions are more suitable for the transport of lipophilc compounds than liposomes.

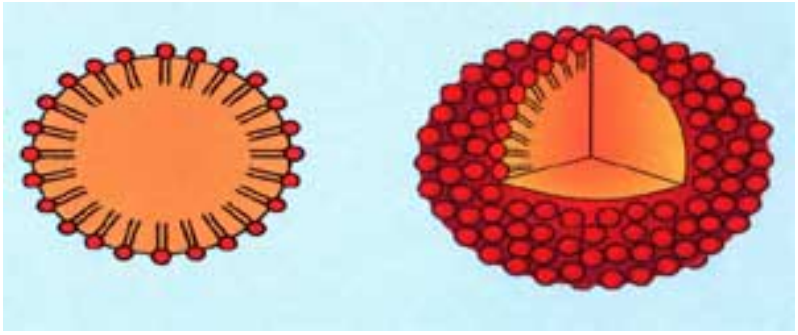

Figure 8: Structure of nanoemulsion droplet (www.pharmoscorp.com/img/bd_fig2.jpg, access date: 11.2008)

The attraction of nanoemulsions for application in health care as well as in cosmetics is due to the following advantages [37]:

- The very small droplet size causes a large reduction in the gravity force and the Brownian motion may be sufficient for overcoming gravity. This means that no creaming or sedimentation occurs on storage.
- Nanoemulsions are suitable for efficient delivery of active ingredients across the skin, especially for non polar active compounds. The large surface area of the emulsions system allows rapid penetration of actives.
- Nanoemulsions can be prepared using reasonable surfactant concentrations. Usually a surfactant concentration of 5-10% may be sufficient, whereas microemulsions require a surfactant concentration of 20 % and higher.
- Nanoemulsions may be applied as a substitute for liposomes and vesicles, which are much less stable

Although, nanoemulsions are proposed for numerous applications in pharmacy as drug delivery systems, one of the main problems is the Ostwald ripening, which is perhaps the most serious problem with nanoemulsions. This results from the difference in solubility between small and large particles [37-39]. Ostwald ripening can be overcame for example by addition of a second disperse phase component or by modification of the interfacial film at the o/w interface [37].

3.3 Investigated compounds

The investigated compounds were chosen with regard to skin compatibility and toxicity. They are naturally occurring ingredients such as soybean-lecithins, phytosphingosine and N-palmitoylethanolamine and promising candidates for drug delivery systems.

3.3.1 Soybean-Lecithins

Lecithin is a naturally-occurring substance composed of phosphoric acid, choline, fatty acid, glycerol, glycolipids, triglycerides and phospholipids such as phosphatidylcholine, phosphatidylethanolamine, lysophosphatidylcholine and phosphatidylinositol. They are occurring in animal and plant tissues and belong to the group of glycerophospholipids.
In aqueous solution its phospholipids can form bilayer structures, micelles or lamellar structures, depending on hydration and temperature. Therefore lecithins are used as surfactants that are classified as amphiphile, due to their hydrophilic and lipophilic parts, which determine their surface active properties. The physiological impact is the enhancement of the skin barrier function and the stabilising effect of the natural transepidermal water loss [40]. Lecithins usually are non-irritating and non-sensitizing for animal and human skin [41]. Moreover lecithin compounds present an affinity to cellular membranes thus leading to an increased absorption of several drugs [31].

Soybean-lecithin is isolated from soy beans and the main phospholipids are phosphatidylcholine (50-90%), phosphatidylethanolamine (5-10%) phosphatidylinositol and phosphatidic acid (Figure 9).

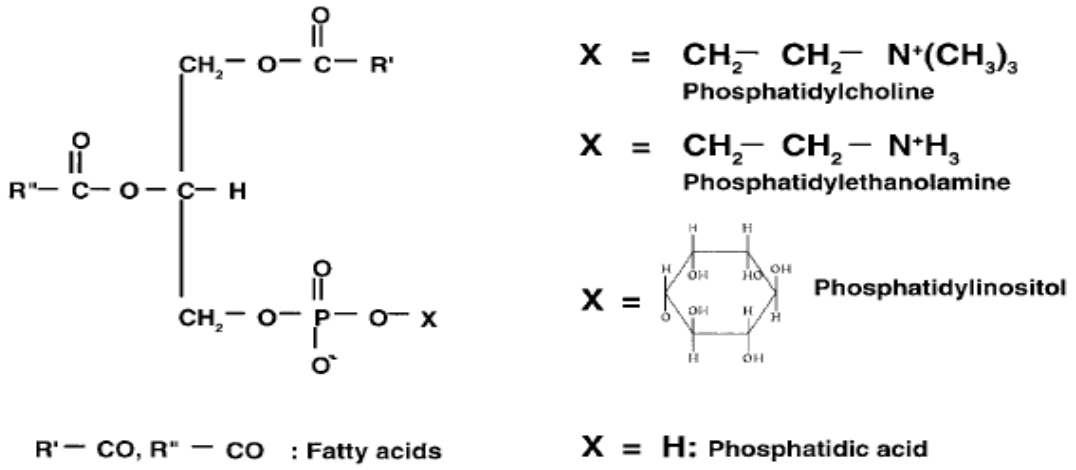

Figure 9: Molecular structures of main phospholipids [42]

For the investigated drug delivery systems of the present work different commercially available soybean-lecithins are used, namely Lipoid S100, Lipoid S75 and Lipoid S45. The lecithins exhibited different amounts of phosphatidylcholine (PC), lysophosphatidylcholine (LPC) and phosphatidylethanolamine (PE).

Table 1: Compositions of the main components within the different soybean-lecithins as supplied by the manufactory

Lecithin	PC % (w/w)[b]	LPC %(w/w)[b]	PE %(w/w)[b]
Lipoid S100	98.00	0.70	< 0.1
Lipoid S75	72.70	1.80	8.50
Lipoid S45	48.80	1.60	15.40

3.3.2 Sugar surfactants

Sugar surfactants are getting great interest because they display some properties that are different from traditional non-ionic surfactants of the alkyl ethoxylate type. In addition, the sugar-based surfactants are made from renewable raw materials (at least the polar part), they are easily biodegradable, and they are mild on skin. These positive properties have stimulated increased research, and it can predicted that these types of surfactants will be increasingly used in commercial products [43].

Sucrose fatty acid esters (SE) are non-ionic surfactants consisting of sucrose as hydrophilic group and a fatty acid as liphophilic group (Figure 10). Usually the products are mixtures of 70-80% monoesters and 20-30% bi-, tri- and polyesters.

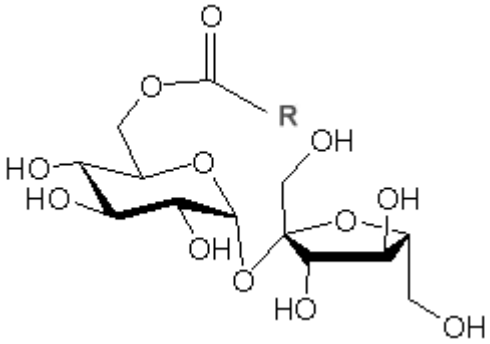

Figure 10: Chemical structure of sucrose monoester (R= fatty acid)

They are available with a wide range of HLB (hydrophilic-lipophilic balance), controlled by the degree of esterification and the type of fatty acid used and exhibit good emulsifier capacity and therefore surfactant functionality [44, 45].

Sucrose esters have the potential of inserting between the lipophilic tails with their long hydrocarbon chain allowing the sucrose ring to interact with the polar lipid head groups. The interaction with these groups can modify hydrogen bonds and ionic forces, distributing the hydration spheres of the lipids, thus resulting in alteration of the head group domain. Among the group of sucrose esters sucrose laureate is described to have the best enhancer activity [45].

3.3.3 Oleic acid

Oleic acid, a monosaturated omega-9 fatty acid, belongs to the lipids that are naturally occurring in the stratum corneum (Figure 11) and is abundant in some vegetable oils (e.g., olive, palm, peanut, and sunflower seed) [40].

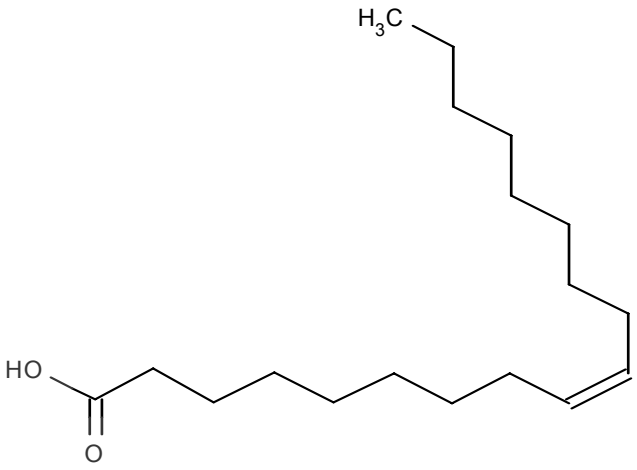

Figure 11: Molecular structure of oleic acid ($C_{18}H_{34}O_2$); molecular Mass: 282.4 g/mol

Since a couple of years extensive investigations are made concerning the mechanism of action [8, 46-48]. It was found that oleic acid disrupts the structure of the bilayers by forming pools due to their *cis* double bond. It is likely that the *cis* double bond requires some considerable energy and therefore pool formation and phase separation is favoured [8].

3.3.4 Isopropyl myristate

Isopropyl myristate (IPM) is the ester of isopropanol and myristic acid and is often used in topical preparations as well as in the cosmetic field, where good absorption through the skin is desired (Figure 12).

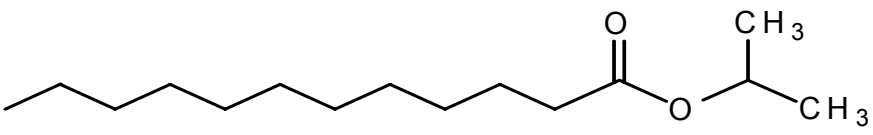

Figure 12: Chemical structure of Isopropyl myrsistate ($C_{17}H_{34}O_2$); Molecular Mass: 270.45 g/mol

Among the past years many studies were performed using IPM as penetration enhancer due to its well topical tolerability [15, 49]. Some researcher reported that IPM incorporation into SC results in densely packed bilayer lipids and a loss of order of the corneocyte-bonded lipids [50].
Moreover IPM is an ideal emollient for skin softening. It can form a thin layer to maintain moisture, which additionally favourite IPM as a good ingredient in drug delivery systems.

3.3.5 Phytosphingosine

Phytosphingosine (PS), a free sphingoid base, is naturally present in the human body and is found in the stratum corneum (Figure13). It inhibits the growth of micro-organisms on the skin, reduces effectively the signs of acne and acts as natural anti-inflammatory agent. Because of these properties, PS is considered to be part of the skin's natural defence system [51].

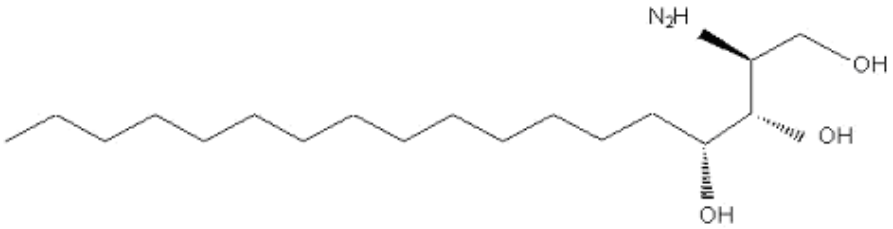

Figure 13: Chemical structure of phytosphingosine ($C_{18}H_{39}NO_3$); molecular Mass: 317.15 g/mol

At physiological skin pH, PS is protonated at the amino-group and influences the surface charges of the nanoparticles, as previously reported [5]. This indicates that PS is located at the oil/water interface and by protonating the amino-group the droplets became positive, which provides increased skin permeation [4, 5].

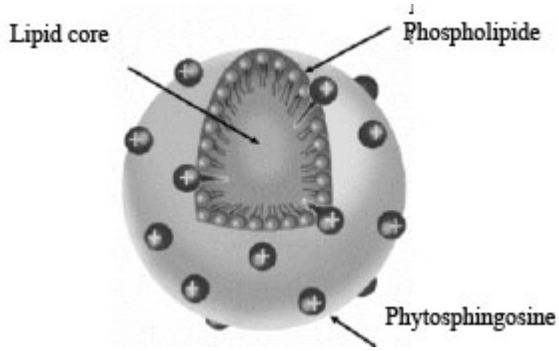

Figure 14: Design of a phytosphingosine positive charged nanoparticel [36]

In summary, phytosphingosine seems to have a wide range of product applications and interesting physicochemical properties, which makes it an interesting candidate for topical use.

3.3.6 N-Palmitoylethanolamine

N-palmitoylethanolamine (PEA) is a naturally occurring C16:0 fatty acid derivative where the carboxylate function is amidated by the primary amine of ethanolamine (Figure15). It is found in the in the most mammalian tissue and is isolated from soybean lecithins and egg yolk [52].

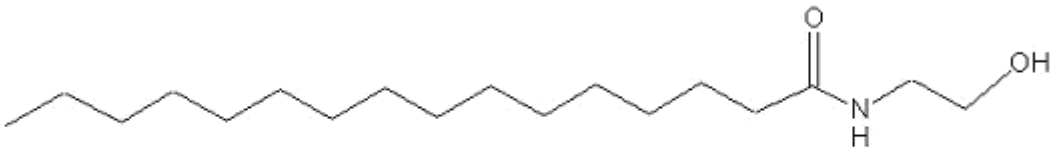

Figure 15: Structure of N-palmitoylethanolamine ($C_{18}H_{37}NO_2$); molecular Mass: 299.49 g/mol

Besides his interesting biological properties like inhibition of sperm fertilizing capacity and gap-junction conductance, PEA is also of considerable interest because of its properties as anti-inflammatory and anti-viral agent [53]. PEA is a endogenous ligand for cannabiniod receptors that are already found to be expressed in human skin [54]. Cannabinoid receptors bind endogenous Cannabinoids such as PEA and anti-analgetic effects can be achieved.

Consequently, these findings make PEA an interesting candidate for topical delivery systems.

4 Publications

4.1 Effect of selected fluorinated drugs in a “ringing” gel on rheological behaviour and skin permeation

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Abstract

The purpose of the present study was to investigate the influence of different drugs exhibiting different solubility on the viscoelastic properties and on the skin diffusion profile of a ringing gel. In a preliminary rheology study with the placebo gel predominating elastic properties were confirmed and a temperature influence was indicated. Fluconazole, fludrocortisone-acetate, flumethasone-pivalate, flutamide and flufenamic-acid each 1% (w/w) were incorporated into the preparation and oscillatory measurements were performed at temperatures of 25°, 28°, 32° and 37 °C. In all drug containing formulations a high elastic G' value predominated the viscous G'' value. The highest G' value could be obtained with the incorporated flumethasone-pivalate. Additionally in almost all cases the G' values decreased with increasing temperature compared to the placebo gel.

Additionally in vitro standard diffusion experiments using Franz-type cells and porcine skin were performed. Following rank order of the cumulative drug release after 48 hours was obtained: fluconazole > flufenamic-acid > flumethasone-pivalate > flutamide > fludrocortisone-acetate. Furthermore an excellent chemical stability of all incorporated drugs was confirmed over 10 weeks.

Keywords: Fluconazole; Fludrocortisone-acetate; Flumethasone-pivalate; Flutamide; Flufenamic-acid; Skin permeation; Rheology; Chemical stability;

1. Introduction

For topical treatment of dermatological diseases as well as for skin care a wide array of vehicles ranging from solids to semisolids and liquid preparations are available. A major advantage using various drugs over the dermal or transdermal way is to eliminate negative side effects caused by using chemical drugs on the peroral route. Within the semisolid preparations, the use of transparent gels has expanded both in pharmaceuticals and in cosmetics. One group of very interesting formulations are “cubic gels”, which might be structurally very closely related to microemulsions. Like microemulsions cubic gels are defined as a dispersion consisting of oil, surfactants, co-surfactants and aqueous phase, which are thermodynamically stable. Microemulsions and cubic gels have several advantages such as enhanced drug solubility, good thermodynamic stability, enhancing effect of transdermal ability over conventional formulations [1]. General forms are gels with a ringing behaviour. On one hand they are interesting because of their colloid–chemical structure, which is accessable only with physical-chemical experiments like Small Angle X-Ray Scattering or NMR- self diffusion experiments. On the other hand they are important as dermal drug delivery systems with optimal release characteristics and matrix behaviour. Recently a cubic gel has been characterised by NMR-self diffusion experiments and rheology [2]. In the present paper fluorinated drug candidates were selected, where it will be very easy in further studies to analyse the diffusion coefficients of the drugs with high sensitivity by F^{19} NMR studies.

The aim of the study was to investigate the influence of different lipophilic drugs like fluconazole, fludrocortisone-acetate, flumethasone-pivalate, flutamide and flufenamic-acid on rheology parameters of this cubic gel. Furthermore in vitro skin permeation studies and chemical stability of these selected drugs will be analysed.

2. Material and methods

2.1. Materials

Fluconazole (CAS: 86386-73-4), flufenamic-acid (CAS: 530-78-8), flutamide (CAS: 13311-84-7) and flumethasone-pivalate (CAS: 2002-29-1) were purchased from Kemprotec (UK). Fludrocortisone-acetate (CAS: 514-36-3) was purchased from Sigma-Aldrich (St.Louis, USA). Polyoxyethylene-5-cetyloleylether and polyoxyethylene-10-cetyloleylether were kindly donated by Cognis (Düsseldorf, Ge). All other chemicals used were of analytical reagent grade and used as received without any further purification.

2.2 Formulations

The formulation was prepared by mixing 1.5 g of the surfactant mixture of polyoxyethylene-5-cetyloleylether and polyoxyethylene-10-cetyloleylether (1+1), 2.75 g of water and 0.8 g of paraffin liquid as the oily component at temperatures up to 70-80°C. A clear semisolid stable very elastic “ringing” gel was formed [2].

The fluorinated drugs fluconazole, fludrocortisone-acetate, flumethasone-pivalate, flutamide and flufenamic-acid (each 1% w/w) were incorporated into the systems.

For the formulations with fluconazole, flutamide and flufenamic-acid transparent highly viscous formulations were built up. For fludrocortisone-acetate and flumethasone-pivalate a turbid but also highly viscous ringing gel formulation was built up.

2.3 Rheological measurements

Oscillatory shear experiments were performed on a Haake rheometer Rotovisco RT20 (Haake, Karlsruhe, Germany, thermo controller Haake F6/8). The rheometer tool was a thermostatically controlled tool with a 20-mm diameter plate/plate (PP20/Ti). The experiments were performed at four temperatures

25°, 28°, 32° and 37°C. The sample amount was between 0.5-1g. By this modus the induced response is measured (strain) when a sinusoidal stress is applied to the sample. After the identification of the linear viscoelastic region, samples were investigated over a frequency of 0.1 to 10 Hz. The parameters obtained were the complex modulus G* consisting of the elastic modulus G' and the viscous modulus G'', tan δ, and ω the angular velocity of oscillatory stress. According their correlations the parameters are calculated by:

$$G' = G^* \cos(\delta)$$

$$G'' = G^* \sin(\delta)$$

$$\tan \delta = G''/G'$$

$$\omega = 2\pi v$$

The measurements were performed in triplicate. The values in the figures are the average values of three experiments.

2.4 Solubility in the acceptor medium

Of each drug the solubility in 0.012 M phosphate buffer (pH, 7.4) was analysed. Therefore an excess of each drug was added to the buffer and stirred by a magnetic bar for 24 hours at room temperature. Afterwards a filtration through a Minisart RC4 filter was performed and 20 µl of the filtration were injected into the HPLC system.
The experiments were performed in triplicate.

2.5 Skin preparation

Porcine abdominal skin was shaved and then prepared with a dermatome (GB 228R, Aesculap) set at 1.2 mm. The skin was stored in a freezer at -20°C until use. Two hours prior to the experiment the samples were thawed.

2.6 Diffusion cell preparation

Permeation studies for the investigated drugs were performed using Franz-type diffusion cells having a permeation area of about 1 cm². The receptor

compartment was filled with 2 ml of 0.012 M phosphate buffer (pH, 7.4). Excised skin was mounted in the cell, stratum corneum uppermost, with the dermal side facing the receptor compartment. The diffusion cells were thermostated at skin surface temperature of 32°C and stirred by magnetic bars. At defined time intervals 200 µl (2, 4, 6, 8, 24, 28, 32 and 48 h) were removed for analysis and replaced with fresh acceptor medium. Approximately 0.6 g of each formulation was applied. Three parallel experiments were performed for each formulation.

2.7 Chemical stability

From each drug containing gel formulation stability studies were performed. All formulations were stored in tubes under room temperature for 10 weeks. The drug amounts were analysed at the day of preparation (starting point) and afterwards weekly.
About 10 mg of the formulation were dissolved in 1 ml of methanol and vigorously shaken for 60 min. Then the solution was centrifuged for 6 min (3000G) and afterwards 20 µl were injected into the HPLC system.

2.8 HPLC analysis

All samples were quantified for their drug content by HPLC (Perkin Elmer, US) consisting of an automatic auto sampler ISS-200, a pump and an UV-diode array detector. For all drugs the stationary phase was a Nucelosil 100 - 5C18 column (240 mm x 4 mm; ARC-Seibersdorf Austria).
At least 8 different standard solutions per drug in the mobile phase were prepared and calibrations curves were calculated on the basis of peak area measurements using standard samples. They were generated with a correlation coefficient between 1.0 and 0.9996 for all drugs.

Fluconazole
For the quantification of fluconazole we used a modified method described by Wang et al. (2003) [3]. The mobile phase consisted of 0.012 M phosphate buffer

(pH, 7.4) and methanol (55:45 v/v) and 1 mmol octanesulfonic-acid. The detection wavelength was 260 nm and the flow rate was 1.0 ml/min. The retention time was approx. 5.60 min. The concentration range of the standard solution for fluconazole was between 15.23 and 121.9 µg/ml.

Fludrocortisone-acetate

For the quantification of fludrocortisone-acetate the mobile phase consisted of acetonitrile and water (40:60 v/v) as described by Cisternino et al. (2003) [4]. The detection wavelength was set at 240 nm and the flow rate was 0.8 ml/min. The retention time was approx. 12.10 min. For fludrocortisone-acetate the concentration range of the standard solution was between 3.00 and 133.65 µg/ml.

Flumethasone-pivalate

For the quantification of flumethasone-pivalate a previously reported method was used [5]. The mobile phase consisted of acetonitrile and water (40:60 v/v) and the detection wavelength was set at 240 nm. The flow rate was 1.0 ml/min and the retention time was approx. 6.20 min. The concentration range of the standard solution was between 0.92 and 103.63 µg/ml.

Flutamide

The mobile phase consisted of acetonitrile, methanol and water (30:25:45 v/v/v) and the detection wavelength was 300 nm as described by Leibinger [6]. The flow rate was set at 0.5 ml/min and the retention time was approx. 20.18 min. For flutamide the standard concentration range was between 1.53 and 96.24 µg/ml.

Flufenamic-acid

For the quantification of flufenamic-acid the mobile phase consisted of methanol, water and glacial acid (80:20:1 v/v/v). The detection wavelength was set at 245 nm, the flow rate was 1.0 ml/min and the retention time was approx. 6.15 min. The concentration range of the standard solution was between 1.32 and 76.5 µg/ml.

2.9 Statistical data analysis

Results are expressed as the means of at least three experiments ± SD. Statistical data analysis was performed using the *t*-test with $P<0.05$ as a minimal level as significance.

3. Results

3.1 Formulations

The gels with fluconazole, flutamide and flufenamic-acid were transparent and semisolid, whereas the gels with flumethasone-pivalate and fludrocortisone-acetate exhibited a significant turbidity. All formulations showed a “ringing” effect.

3.2 Solubility

The solubility of the selected drugs was analysed in phosphate-buffer (pH, 7.4), which is the most commonly acceptor fluid. In order to obtain sink conditions, the solubility in the acceptor medium was analysed and presented in Table 1. As a general rule, the concentration of the permeant should not be allowed to exceed 10% of saturation solubility [7].

3.3 Rheological investigations

Temperature can largely influence the viscoelastic properties of cubic phase systems [8]. In order to determine the temperature influence on the viscoelastic properties first changes in the ratio of viscous and elastic properties of the placebo gel were investigated with increasing temperature (Fig. 1). From the aspect of rheology, tan δ is defined as G’’/G’. As seen, the elastic modulus of the samples is dominating due to tan δ far below 1. Attention should be paid to the fact that tan δ is almost independent of the shearing frequency, which is completely different from results at the moderate and high temperatures. The

result demonstrates a dominant elastic behaviour exhibiting strong crystal like properties of the cubic gel at the investigated temperatures. This is confirmed by a recent publication on a similar gel [8]. Except at the temperature of 37°C the shape of the curves from 15°C to 32°C is very similar. One reason for the difference at 37°C might be the approximation to the melting point of the cubic gel.

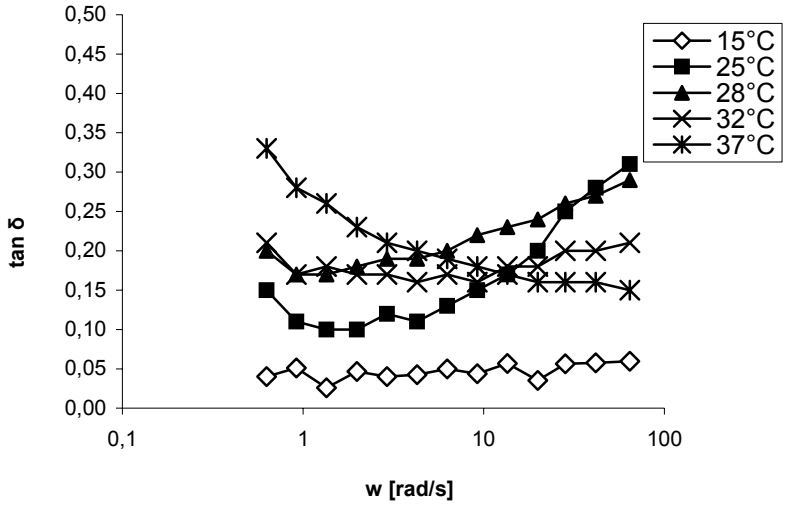

Figure 1 tan δ (G''/G', the ratio of viscous and elastic components) as function of temperature and shear frequency for the cubic placebo-gel. Indicated values are means (± SD) of three experiments.

Next step should be the rheological investigations of the drug containing gels in terms of G' and G'' at 25°, 28°, 32° and 37°C compared to the placebo gel. In Fig 2(A-F) the influence of the incorporated drugs on G' and G'' at different temperatures are presented. Although only 1 % (w/w) of model drugs were incorporated, significantly differences in the elastic modulus G' and viscous modulus G'' were observed compared to the placebo gel. The temperature exhibited an influence mainly on the elastic behaviour. One would expect a decrease of G' as well as of G'' with increasing temperature, however the placebo gel (Fig. 2F) showed an increasing G' until 32°C and then a "one third" decrease of G' at 37°C compared to G' at 32°C.

The pattern of the block-diagrams (Fig. 2A-F) shows no influence of fluconazole on the G' values compared to the placebo gel. This indicates only a little

interaction between the incorporated drug and the microstructure of the gel. On the other hand fludrocortisone–acetate, flumethasone-pivalate, flutamide and flufenamic-acid caused increased G' values at all tested temperatures compared to the placebo gel (Fig. 2F).

The highest G' values at 25°C were $5.9x10^5$ Pa and $5.7x10^5$ Pa at 10.2 Hz measured with flumethasone-pivalate and flufenamic-acid, respectively. This fact implies a significant interaction of the incorporated drug with the microstructure of the cubic gel system. However, with further increase of the temperature the G' value of fludrocortisone-acetate, flumethasone-pivalate and flufenamic-acid decreased to a value in the range of G' of the placebo gel. At skin temperature of about 32°C the gel with incorporated flutamide exhibited a different influence. In this case the G' value of the flutamide gel showed the lowest G' in comparison to the placebo gel. Moreover the elastic G' and viscous G'' value decreased further at 37°C and have about the same viscous and elastic properties. This behaviour might be due to the vicinity to the melting point of the gel-system. This is in agreement with literature data [8].

Regarding the viscous G'' values an influence of the drugs could be observed too. The G'' values of fluconazole-gels are in the same range as the G'' values of the placebo gel, whereas the G'' values of fludrocortisone-acetate-gels, flufenamic-acid-gels and fludrocortisone-acetate-gels were significantly lower.

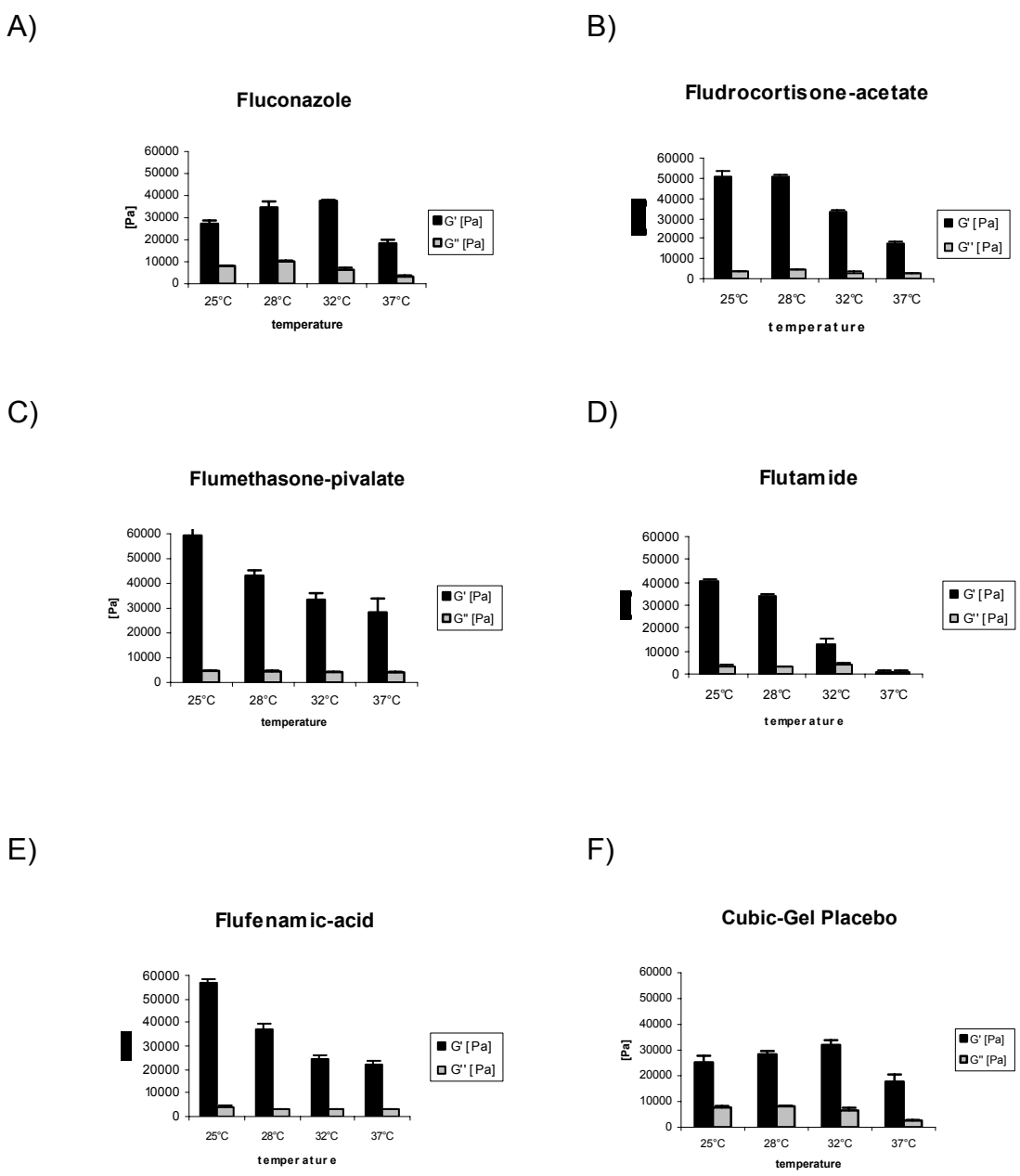

Figure 2(A-F) Comparison of the elastic modulus G' and viscous modulus G'' of the drug containing cubic gel (each 1% drug) to the placebo gel at 25°C, 28°C, 32°C and 37°C measured at 10,2 Hz. Fluconazole (A), fludrocortisone-acetate (B), flumethasone-pivalate (C), flutamide (D), flufenamic-acid (E), placebo gel (F). Indicated values are means (± SD) of three experiments.

3.4 Skin diffusion

The release data obtained in the present work are presented in Fig. 3. As shown in Fig. 3 the cumulative permeation rate of fludrocortisone-acetate,

flumethasone-pivalate and flutamide are nearly in the same range between 9 µg and 19 µg /cm^2 after 48 h of diffusion and were not significantly different. Whereas the cumulative amount released of flufenamic-acid is about 21-fold higher after the same diffusion time. Interestingly the lag time was about 10 hours in these four cases. For fluconazole the highest cumulative permeation rate was measured, which could be expected from the solubility data in the acceptor medium (Table 1). The amount was about 6-fold higher compared to the tested flufenamic-acid and about 125-fold higher compared to the other tested drugs.

Table 1 Saturation solubility of fluconazole, fludrocortisone-acetate, flumethasone-pivalate, flutamide and flufenamic-acid in mg/ml 0.012 M phosphate buffer (pH, 7.4); n=3

Drug	**Solubility ± SD mg/ml**
Fluconazole	5,961 ±0,398
Fludrocortisone-acetate	0,026 ±0,008
Flumethasone-pivalate	0,053 ±0,001
Flutamide	0,020 ±0,001
Flufenamic-acid	1,536 ±0,026

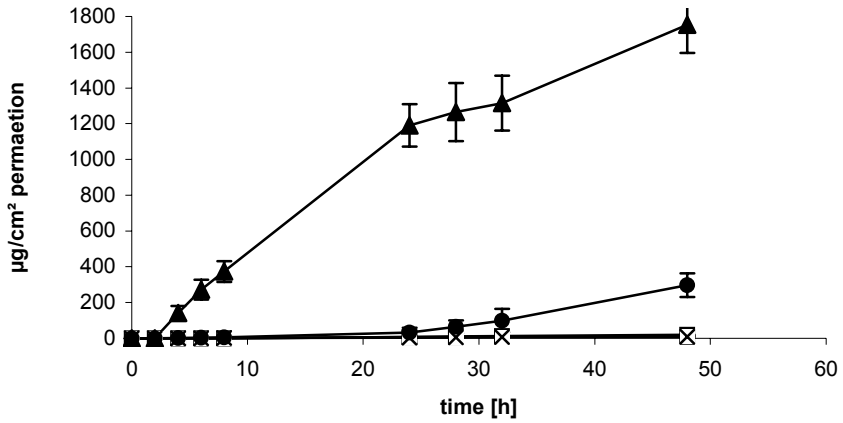

Figure 3 Skin diffusion profiles of fluconazole, flufenamic-acid, fludrocortisone-acetate, flumethasone-pivalate and flutamide through porcine skin in µg/cm² over 48 h of diffusion. Fluconazole (♦), flufenamic-acid (●), fludrocortisone-acetate (▲), flumethasone-pivalate (■) and

flutamide (x). Indicated values are means (±SD) of three experiments.

3.5 Chemical stability

The chemical stability of fluconazole, fludrocortisone-acetate, flumethasone-pivalate, flutamide and flufenamic-acid is presented in Fig. 4. As indicated there was no significant decrease of the drug amount in the presented gel during the whole observation period. Moreover on the HPLC-chromatograms no degradation products could be detected. Beside this, the visual observation of all formulations showed no signs of microbial contamination.

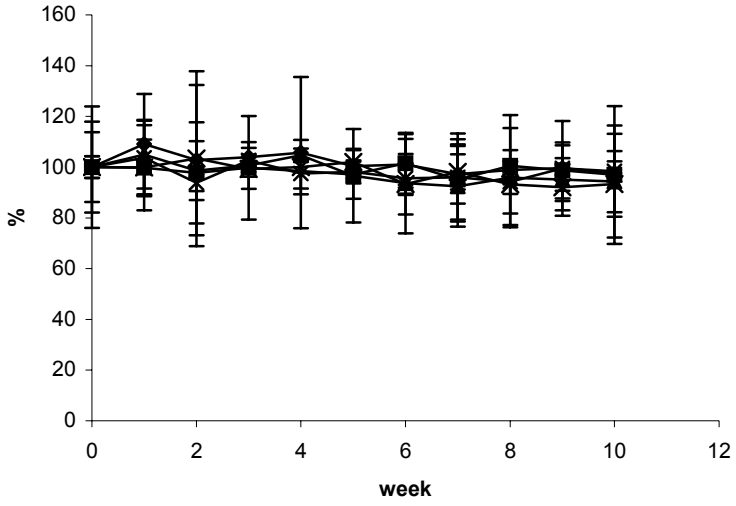

Figure 4 Drug amount of fluconazole (♦), flufenamic-acid (●), flutamide (x), flumethasone-pivalate (■) and fludrocortisone-acetate (▲) in % (w/w) incorporated in the cubic-gel over an observation period of 10 weeks. Indicated values are means (±SD) of three experiments.

4. Discussion

A transparent “ringing gel” consisting of surfactants, paraffin oil and water has been used as vehicle in order to investigate in vitro skin permeation-studies, chemical stability and rheological behaviour of selected different fluorinated drugs fluconazole, fludrocortisone-acetate, flumethasone-pivalate, flutamide and flufenamic-acid.

Incorporation of drug in cubic phase can cause phase transformation to lamellar

or reversed hexagonal phase depending on the polarity and concentration of the drug, which may affect the release profile [9]. Depending on the physicochemical properties of the drug , it may reside in the lipid bilayer or the aqueous channels of the gel and the location of the drug in the gel may influence its release [9].

As already described in other reports gel microemulsions are good vehicles for fluconazole drug delivery [10]. These results could also be confirmed by our studies. Therefore for fluconazole an orally active antifungal agent which is used in the treatment of superficial and systemic candidiasis and in the treatment of cyrptococcal infections the presented cubic gel showed excellent vehicle properties. The released cumulative amount of fluconazole from the presented cubic gel was even higher compared to a similar gel microemulsion described in an other publication [10], where mice skin was used, which is known as much more permeable compared to porcine skin [11].

Regarding the lipophilic non steroidal anti-inflammatory flufenamic-acid with potent anti-inflammatory and analgesic effects mediated by the inhibition of prostaglandin synthesis the presented cubic gel showed also excellent vehicle properties. For the corticosteroid fludrocortisone-acetate, which is commercially available in compressed tablets [4] the cumulative amount released was in the same range as the mostly dermal used flumethasone-pivalate. Fludrocortisone-acetate is currently used for the treatment of several diseases from endocrine and non-endocrine origin [12]. For flumethasone-pivalate a difluorinated corticosteroid with anti-inflammatory and vasoconstrictive properties available in products like Locacorten®, Vioform® etc. the cubic gel might be a possibility for a new drug vehicle.

Another agent with a hair growth potential is the nonsteroidal anti-androgen flutamide. This drug was introduced as a new potent compound for treatment or prostatic carcinoma. The systemic administration of flutamide causes several unwanted side effect, such as reducing libido and impairing spermatogenesis in men and feminizing male fetuses in pregnant women. Topical administration, therefore, is an important goal for such a drug, especially if indicated for skin disorders [13]. The release studies of flutamide from the cubic gel showed reasonable but lower skin permeation compared to a previous published report

[13] where phosphate buffer and ethyl alcohol were used as acceptor medium. This additive could be one of the reasons for the higher cumulative released amount of flutamide to our results.

To explain a probable mechanism by which cubic gels, structurally closely related to microemulsions, enhance the release and percutaneoeus absorption of drugs efficiently, the histological and histochemical structure of the stratum corneum must be taken into consideration.

A dermally applied microemulsion is expected to penetrate the stratum corneum and to exist intact in the whole horny layer, altering both the lipid and the polar pathways [10]. The lipophilic domain of the cubic gel can interact with the stratum corneum in many ways. The drug dissolved in the lipid domain of the microemulsion can directly partition into the lipids of the stratum corneum, or the lipid vesicles themselves can intercalate between the lipid chains of the stratum corneum, thereby destabilizing its bilayer structure. These interactions will lead to increased permeability of the lipid pathway to the drugs.

On the other hand, the hydrophilic domain of the cubic gel can hydrate the stratum corneum to a greater extent, and plays an important role in the percutaneous uptake of drugs. When the aqueous fluid of the cubic gel enters the polar pathway, it will increase the interlamellar volume of the stratum corneum lipid bilayers, resulting in the disruption of its interfacial structure. Since some lipid chains are covalently attached to corneocytes, hydration of these proteins will also lead to the disorder of lipid bilayers. Similarly, swelling of the intercellular proteins may also disturb the lipid bilayers; a lipophilic penetrant can then permeate more easily through the lipid pathway of the stratum corneum.

In fact, not such mechanism could be considered in explaining the superiority of the microemulsion or cubic gel over the other vehicles, but the combined effect of both the lipophilic and hydrophilic domains was responsible for its enhancing activity [10].

But not only the excellent vehicle properties of the cubic gel might be an advantage, but also the high chemical stability of the incorporated drugs could be an important factor for using the cubic gel as drug delivery system. As mentioned in earlier reports the chemical stability of various drugs incorporated

in this new gel formulation was very high [14]. This could also be confirmed by our studies. High chemical stability of the model drugs could be obtained over the whole observation period. However, the here investigated drugs showed also good chemical stability properties in other matrices like tablets, solutions and powders [4, 10, 15].

As mentioned above as well the chemical structure of the incorporated drugs as the temperature has an important effect on rheological behaviour of the microstructure of the cubic gel. At moderate temperature, the rheological properties and inner structure of cubic liquid crystal change hugely. Furthermore, the influence of the incorporated drugs on the viscoelastic properties was dependent on their chemical structure and can probably be related to the micro-structure of the cubic gel. This effect might be confirmed also by the low aqueous solubility of the model drugs. As mentioned above the cubic gel with the incorporated flumethasone-pivalate and fludrocortisone-acetate exhibited a significant turbidity. This could be an indication that drug particles may be suspended in the aqueous component of the gel, which might influence the elastic behaviour of the gel system. This fact could be a reason for the high elastic G´ of those two incorporated drugs. Therefore additional biophysical experiments like micro-DSC and NMR-self-diffusion experiments are in progress to specify more the changes in liquid crystalline microstructure and molecular movement.

5. References

1. H. Chen, X. Chang, D. Du, J. Li, H. Xu, X. Yang, Microemulsion-based hydrogel formulation of ibuprofen for topical delivery, Int. J. Pharm. 315 (2006) 52-58.

2. H. Kählig, C.Valenta, U. Dampfhart, B. G. Auner, Rheology and NMR self-diffusion experiments as well as skin permeation of diclofenac-sodium and cyproterone acetate of new gel preparations, J. Pharm. Sci. 94 (2005) 288-296.

3. Wang, Y.P., Shuming; Shi, Yanping, Determination of fluconazole injection and its related substance by HPLC, Zhongguo Yiyuan Yaoxue Zazhi, (2003) 23(5) 268-270.

4. S. Cisternino, J. Schlatter, J.L. Saulnier, Stability of Fludrocortisone acetate solutions prepared from tablets and powder, Eur. J. Pharm. Biopharm. 55 (2003) 209-213.

5. L. Gagliardi, D. De Orsi, M. R. Del Giudice, F. Gatta, R. Porra, P. Chimenti, D. Tonelli, Development of a tandem thin-layer chromatography -high- performance liquid chromatography method for the identification and determination of corticosteroids in cosmetic products, Analytica Chimica Acta, 457 (2002) 187-198.

6. J. Leibinger, M. Kapas, New and validated high-performance liquid chromatographic method for determination of hydroxyflutamide in human plasma, J. Pharm. Biom. Anal. 14 (1996) 1377-1381.

7. K.R. Brain, K.A.W., A.C. Watkinson, Investigation of skin permeation in vitro, in: M.S. Roberts, K.A. Walters, Dermal Absorption and Toxicity Assessment. Marcel Dekker, New York, 1998, pp.161-187.

8. H. Wang, G. Zhang, Z. Du, Q. Li, W. Wang, D. Liu, X. Zhang, Effect of

temperature on dynamic rheological behaviour of discontinuous cubic liquid crystal, J. Colloid Interface Sci. 300 (2006) 348-353.

9. J. Shah, Y.Sadhale, D. Chilukuri, Cubic Phase gels as drug delivery systems, Adv. Drug Del. Rev. 47 (2001) 229-250.

10. H.M.El Laithy, K.M.F.El-Shaboury, The development of cutina lipogels and gel microemulsions for topical administration of fluconazole, AAPS Pharm. Sci. Tech. 3 (2002) 1-9.

11. R.C. Wester, H.I. Maibach, In vivo animal models for percutaneous absorption, in: R.L. Bronaugh, H.I. Maibach (Eds.), Percutaneous Absorption, Marcel Dekker, New York, 1985, pp. 251-266.

12. S. Gibaud, N. Al Awwadi, C. Ducki, A. Astier, Poly (ε-caprolactone) and Eudragit® microparticles containing fludrocortisone-acetate, Int. J. Pharm. 269 (2004) 491-508.

13. A. Sintov, S. Sserfimovich, A. Gilhar, New topical antiandrogenic formulations can stimulate hair growth in human bald scalp grafted onto nice, Int. J. Pharm. 194 (1999) 125-134.

14. C. Valenta, B. Auner, I. Loibl, Skin permeation and stability studies of 5-aminolevulinic acid in a new gel and patch preparation, J. Contr. Rel. 107 (2005) 495-501.

15. Miranda A, I. Carabella, M. Millan, Stability study of flutamide in solid state and in aqueous solution, Drug Dev. Ind. Pharm. 28 (2002) 413-22.

4.2 Skin compatible lecithin drug delivery systems for fluconazole: effect of phosphatidylethanolamine and oleic acid on skin permeation

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Abstract

The purpose of the present study was to evaluate skin compatible drug delivery systems for fluconazole. Pseudo-ternary phase diagrams were constructed composed of different soybean lecithins/oil/isopropanol and water. The role of the various lecithin compositions is expressed in different resulting isotropic areas. Based on these phase diagrams two systems were chosen as drug delivery vehicle for fluconazole. On one hand the influence of phosphatidylethanolamine and on the other hand the influence of the oil component was investigated on the skin permeation of fluconazole. The more phosphatidylethanolamine the higher was the fluconazole skin permeation independent of the hydrophilicity of the system. The influence of oleic acid and isopropylmyristate as an oil component resulted in a preference of penetration enhancing effect of the oleic acid containing microemulsion.

Keywords: Microemulsions; Fluconazole; Soybean-lecithin; Skin permeation; Phosphatidylethanolamine

1. Introduction

Microemulsions are clear isotropic, thermodynamically stable liquids with ultralow interfacial tension, large interfacial area and the capacity to solubilize both aqueous and oil-soluble compounds (Paul et al., 2001). The different resulting structures that were formed by mixing various ratios of surfactants have been studied and systematized in detail during the last years (Aboofazeli and Lawrence, 1993; 1994; 1995; Wang et al., 2006). These self-assembled structures are tuneable by changing the ratio and the concentrations of the ingredients.

In order to affect the flux during the skin several permeation enhancers were used as ingredients in such formulations. Oleic acid and several other fatty acids are able to interrupt the lipid barrier in the stratum corneum by forming separate domains, which interfere with the continuity of the multilamellar stratum corneum and may induce highly permeable pathways (Peltola et al., 2003; Hadgraft, 2001; Ongpipattanakul et al., 1991). Also isopropylmyristate another non toxic ingredient is well known as permeation enhancer in transdermal formulations, although the mechanism of its action is poorly understood (Goldberg-Cettina et al., 1995).

The aim was to construct pseudo-ternary phase diagrams consisting of lecithin/isopropanol/oil and water in order to see how different commercially available soybean lecithins influences the formation of microemulsions and transport of the entrapped lipophilic drug through skin. Also the influence of oleic acid or isopropylmyristate as an oil component was compared.

2. Material and methods

2.1. Materials

Fluconazole (CAS: 86386-73-4) was purchased from Kemprotec (UK). Soybean-lecithins with different concentrations of phosphatidylcholine (PC), lysophosphatidylcholine (LC) and phosphatidylethanolamine (PE), namely Lipoid S45 (CAS: 745303-1), Lipoid S75 (CAS: 776099-1) and Lipoid S100

(CAS: 790535-5) were kindly donated by Lipoid GmbH (Ludwigshafen, Ge). Isopropanol was supplied by Merck (Hohenbrunn, Ge). Isopropylmyristate (IPM) was purchased from Sigma-Aldrich (St.Louis, USA) and Oleic acid (OA, EG.Nr: 204-007-1) was supplied from Herba Chemosan (Vienna, Au).

2.2 Construction of pseudo-ternary phase diagrams

In order to construct pseudo-ternary phase diagrams appropriate amounts of three different lecithins (S100, S75 and S45), OA and isopropanol were weight out to vials and stirred at room temperature until a clear solution was obtained. Distilled water was added drop-by-drop and stirred to attain equilibrium. After being equilibrated, the mixture was assessed visually and under cross polarizers for the absence of a liquid crystalline (LC) phase in order to determine the boundaries of microemulsions identified by isotropic phases and birefringent liquid crystalline domains.

2.3 Microscopic characterisation of microemulsions

Polarizing light microscopy studies were carried out at room temperature using an Optiphot- microscope (Nikon GmbH, Ge) in order to get more information on the microscopic structure of the systems.

2.4 Formulations

The formulations were prepared as already described and the compositions are presented in Table 1. System I consisted of 25% oil, 25% surfactant, 25% co-surfactant and 25% water. System II consisted of 40% oil, 25% surfactant, 25% co-surfactant and 10% water. Both systems were prepared also with OA and IPM.

Table 1 Composition of the microemulsions in %, (w/w)

Formulation Code	IPM	OA	S100	S75	S45	ISO	Water	Flu
System I								
I-1	25	--	25	--	--	25	25	1
I-2	25	--	--	25	--	25	25	1
I-3	25	--	--	--	25	25	25	1
I-4	--	25	25	--	--	25	25	1
I-5	--	25	--	25	--	25	25	1
I-6	--	25	--	--	25	25	25	1
System II								
II-1	40	--	25	--	--	25	10	1
II-2	40	--	--	25	--	25	10	1
II-3	40	--	--	--	25	25	10	1
II-4	--	40	25	--	--	25	10	1
II-5	--	40	--	25	--	25	10	1
II-6	--	40	--	--	25	25	10	1

Abbreviations: IPM: isopropylmyristate; OA: oleic acid; S100: Lipoid S100 (consisting of 98.40 %PC, 0.3% LC and < 0.1% PE); S75: Lipoid S75 (consisting of 69.90% PC, 2.20% LC and 8.40% of PE); S45: Lipoid S45 (consisting of 54.90 PC, 1.0% LC and 16.90 % PE); ISO: isopropanol; Flu: fluconazole

2.5 In vitro skin permeation study

Porcine abdominal skin was shaved and then prepared with a dermatome (GB 228R, Aesculap) set at 1.0 mm. The permeation of fluconazole was investigated as described in a prior study (Hoeller and Valenta, 2007).

2.6 HPLC analysis

For the quantification of fluconazole by HPLC (Perkin Elmer, US) an already experienced procedure was used (Hoeller and Valenta, 2007). Calibrations curves were calculated on the basis of peak area measurements of using standard solutions. They were generated with a correlation coefficient of 0.9993. The concentration range of the standard solution for fluconazole was between 13.66 and 109.3 µg/ml.

2.7 Statistical data analysis

Results are expressed as the means of at least three experiments ± SD. Statistical data analysis for Figure 3A-B were performed using the Kruskal-Wallis test ($p<0.05$).

3. Results

3.1 Microscopic characterisation of microemulsions

The microscopic pictures are presented in Figure 1A-F. Microemulsions were identified obviously on their clear isotropic texture, which appears under polarized light as black planes (Fig. 1A). Such phases are usually produced at very high surfactant concentrations and low water content. As the surfactant concentration was further diluted with water fluent drop aggregates existed, designated as Fig. 1B. Typical ‘mosaic’ textures (Fig. 1C) appeared mostly at a very low surfactant concentration of about 10-20 %. At a higher surfactant concentration of about 25-30 % various liquid crystalline structures were obtained (Fig. 1D-F). Such structures showed birefringence under polarized light and appeared mostly at a mixing ratio with low oil content (10-20 %). At a surfactant/co-surfactant concentration of about 30% typically lamellar liquid crystalline structures were obtained, while at higher surfactant concentrations hexagonal phases were produced.

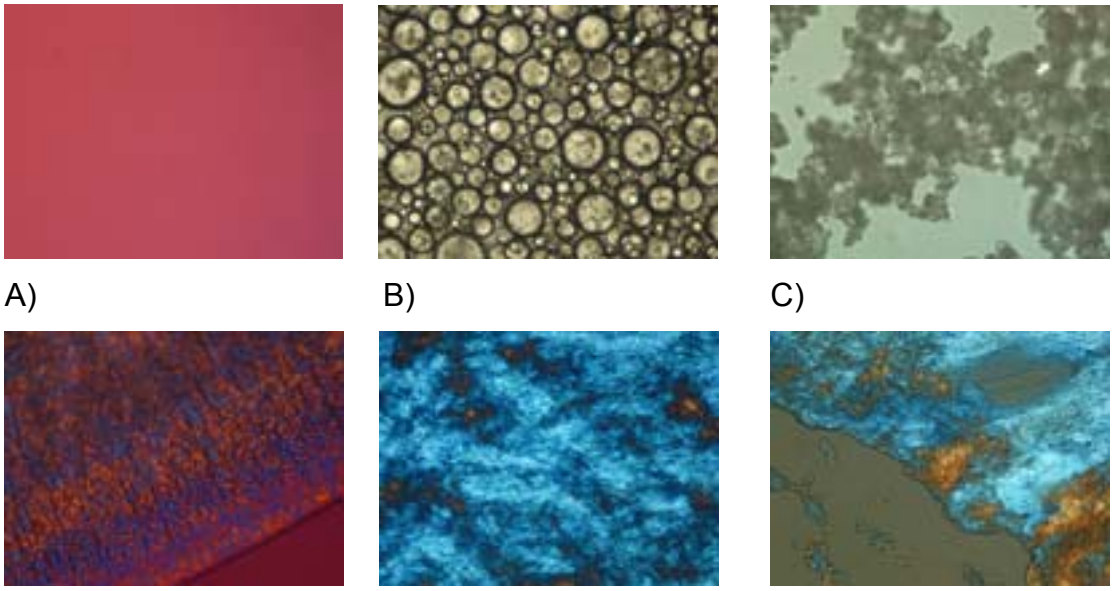


Figure 1(A-F) Micrographs of the phase textures obtained by the construction of the pseudo-ternary phase diagrams (Surfactant/co-surfactant/oil/water). A) isotropic texture: 50/40/10, B) fluent drop aggregates: 10/10/80, C) mosaic texture: 20/30/50, D-F) liquid crystalline textures: 30/10/60; 30/10/60; 30/10/60

3.2 Pseudo-ternary phase diagrams

The overlaid pseudo ternary phase-diagrams are presented in Figure 2. The largest isotropic area was obtained by Lipoid S75, which contains 69.90% of phosphatidylcholine, followed by the narrowed area induced by Lipoid S100 with the highest content of phosphatidylcholine. A Comparison of the isotropic area from S45 (lowest amount of PC) with S75 and S100 showed an extension in the water and oil region. The possible percent compositions for isotropic regions are from about 45 to 85 % of surfactant/co-surfactant and from about 5 to 55 % of oil and from about 5 to 30 % of water.

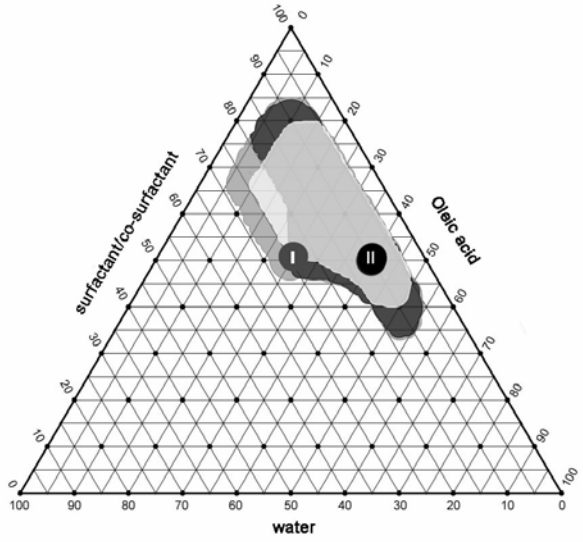


Figure 2 Overlaid pseudo-ternary phase diagrams of the oil-surfactant/co-surfactant-water systems using three different lipid compositions produced at room temperature (the marked area represents the microemulsion region); Pale grey: Lipoid S75; black: Lipoid S100; white: Lipoid S45

Point I: System I consisting of 25 % oil, 25 % lipid, 25 % isopropanol and 25 % water.

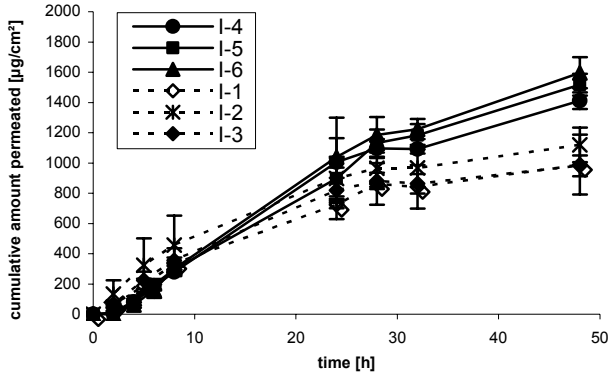
Point II: System II consisting of 40 % oil, 25 % lipid, 25 % isopropanol and 10 % water.

3.3 In vitro skin permeation study

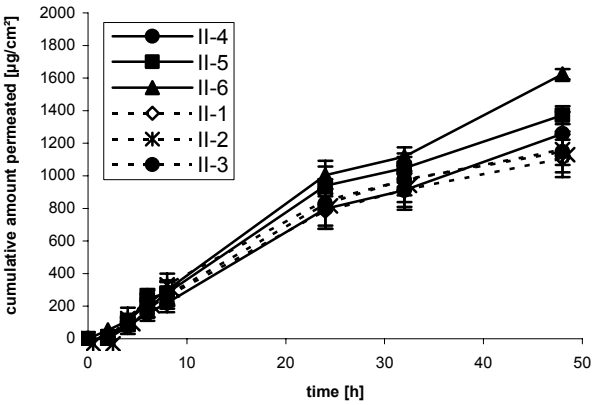
The results are presented in Figure 3A-B and the cumulative amount of the permeation of fluconazole is included in Table 2.

System I: The microemulsion with OA showed higher cumulative amount of fluconazole after 48 h of diffusion through skin than those of IPM (Fig. 3A). The rank order of cumulative amount permeated in case of OA is: I-6 > I-5 > I-4. The highest permeation could be obtained from I-6. This is the formulation with the lowest content of PC (S45), but a high content of PE (19.90 %). On second position is the lecithin (S75) with still 8.4 % PE. The formulation with the highest amount of PC, but almost no PE showed lower skin permeation (S100). Regarding the formulation with IPM the highest permeability was obtained from I-2. For I-1 and I-3 about the same amount of fluconazole was released. A comparison of the permeation of I-1, I-2 and I-3 with I-6 with OA indicated a significant higher cumulative amount of fluconazole of the latter (Kruskal-Wallis-Test $p < 0.05$).

System II: In System II a preference of higher cumulative amount of fluconazole through skin can be seen of OA containing preparation (Figure 3B). Comparing II-3 containing IPM with II-6 containing OA indicated a significant higher cumulative amount permeated of fluconazole from II-6 (Kruskal-Wallis test $p < 0.05$). The rank order of the results regarding the lecithin compositions are in case with OA: II-6 > II-5 > II-4 and in case with IPM: II-2 > II-3 > II-1. Again microemulsion II-6 containing the highest amount of PE exhibited the highest cumulative amount of fluconazole through skin in contrast to II-4 and II-5. All cumulative amounts permeated of fluconazole after 48 h of diffusion are presented in Table 2.



A)



B)

Figure 3 A-B Fluconazole diffusion through excised porcine skin after 48h of diffusion times from microemulsion systems II and I. A) Microemulsion system I; B) Microemulsions system II; n=3

Table 2 Comparison of the cumulative drug amount (µg/cm²) of fluconazole permeated after 48 h of diffusion time through porcine skin, n=3

System I	Cumulated drug amount (µg/cm²)		System II	Cumulated drug amount (µg/cm²)	
I-1	988,99	±197,44	**II-1**	1106,81	±114,90
I-2	1119,97	±114,90	**II-2**	1160,76	±93,89
I-3	981,66	±68,51	**II-3**	1147,01	±124,56
I-4	1411,97	±55,34	**II-4**	1258,80	±133,87
I-5	1517,24	±71,35	**II-5**	1372,57	±54,48
I-6	1596,65	±102,89	**II-6**	1622,84	±31,32

4. Discussion

Lecithin has been shown to enhance skin penetration by interfering with skin lipids and therefore induce a change of the skin lipid fluidity (Paolino et al., 2002). It was the question whether the composition of different lecithins has an impact on building the isotropic phase as well as on skin permeation. The results of the phase diagrams show different sizes of isotropic area. Lipoid S75 achieved the biggest area. The component lysophosphatidylcholine and phosphatidylethanolamine may be responsible for this. Lipoid S45 contains also these ingredients and they may influence the effective critical packing parameter in a different manner as described also in previous studies (Aboofazeli and Lawrence, 1994). Recently in one study the effect of PC and PE was investigated with respect to the transepidermal water loss (TEWL). In one publication PC and PE in a concentration of 10mg/cm² significantly reduced the TEWL and PE was approximately twofold more effective than PC (Raney and Hope, 2006). The data are consistent with formation of extensive hydrophobic interactions between the skin and the outwardly facing acyl chains of the inverted hexagonal phase adapted by PE. In our case the amount of

applied PE was about 25 mg/cm² calculated from the applied formulation of 0.6 g containing 25 % Lipoid S45 and containing about 17 % PC. The here applied doses would be sufficient to induce such hydrophobic interactions between skin and phosphatidylethanolamine. This could be one possible reason for the increase of fluconazole permeation.

OA versus IPM were checked for their percutaneous enhancing effect. The highest permeation of fluconazole was achieved with OA as oil component. This observation seems compatible with the main mechanism suggested for the action of OA as permeation enhancer, by increasing stratum corneum permeability. Therefore a facilitated membrane diffusion could be achieved (Touitou et al., 2002; Moser et al., 2001). It has been shown that OA is more effective to highly hydrophilic compounds, which supports the use in combination with the hydrophilic fluconazole. In our case this hypothesis was confirmed because in the more hydrophilic microemulsion I the penetration enhancer effect of OA was more pronounced than in the more lipophilic microemulsion II.

5. Conclusion

It is possible to create skin compatible lecithin microemulsions having high fluconazole diffusion through porcine skin, whereas the phosphatidylethanolamine content seems to play a major role in increasing the skin permeability.

6. References

Aboofazeli, R., Lawrence, M.J., 1993. Investigations into the formation and characterization of phospholipid microemulsions. I. Pseudo-ternary phase diagrams of systems containing water-lecithin-alcohol-isopropylmyristate. Int. J. Pharm. 93,161-175.

Aboofazeli, R., Lawrence, M.J., 1994. Investigations into the formation and characterization of phospholipid microemulsions. II. Pseudo-ternary phase diagrams of systems containing water-lecithin-alcohol-isopropylmyristate and alcohol: influence of purity of lecithin. Int. J. Pharm. 106, 51-61.

Aboofazeli, R., Lawrence. M.J., 1995. Investigations into the formation and characterization of phospholipid microemulsions. IV. Pseudo-ternary phase diagrams of systems containing water-lecithin-alcohol-and oil; the influence of oil. Int. J. Pharm. 125, 107-116.

Goldberg-Cettina, M., Liu, P., Nightingale, J., Kurihara-Bergstrom, T., 1995. Enhanced transdermal delivery of estradiol in vitro using binary vehicles of isopropylmyristate and short-chain alkanols. Int. J. Pharm. 114, 237-45.

Hadgraft, J., 2001. Skin, the final frontier. Int. J. Pharm. 224, 1-18.

Hoeller S., Valenta C., 2007. Effect of selected fluorinated drugs in a ‘ringing’ gel on rheological behaviour and skin permeation. Eur. J. Pharm. Biopharm. 66, 120-126

Moser, K., Kriwet, K., Naik, A. Kulia, Y.W., Guy, R.H.. 2001. Passive skin permeation enhancement and its quantification in vitro. Eur. J. Pharm. Biopharm. 52 (2),103-112.

Ongpipattanakul, B., Burnette, R.R., Potts, R. O., Francoeur, M.L. 1991. Evidence that oleic acid exists in a separate phase within stratum corneum lipids, Pharm. Res. 8, 350 - 354

Paolino, D., Ventura, C.A., Nistico, S., Puglisi, G., Fresta, M., 2002. Lecithin microemulsions for the topical administration of ketoprofen: percutaneous adsorption through human skin and in vivo human skin tolerability. Int. J. Pharm. 244, 21-31.

Paul, B. K., Moulik, S. P., 2001. Uses and applications of microemulsions. Curr. Sci. 80, 990-1001.

Peltola, S., Saarinen-Savolainen, P., Kiesvaara, J., Suhonen, T.M., Urtti, A., 2003. Microemulsions for topical delivery of estradiol. Int. J. Pharm 254, 99-107.

Raney Sameersingh, G., Hope, M. J., 2006. The effect of bilayer and hexagonal H (II) phase lipid films on transepidermal water loss. Experimental dermatology 15, 493-500.

Touitou, E., Godin, B., Karl, Y., Bujanover, S., Becker, Y., 2002. Oleic acid, a skin penetration enhancer, affects Langerhans cells and corneocytes. J. Controlled Release 80, 1-7.

Wang, H., Zhang, G., Du, Z., Li, Q., Wang, W., Liu, D., Zhang, X., 2006. Effect of temperature on dynamic rheological behaviour of discontinuous cubic liquid crystal. J. Colloid Interface Sci. 300, 348-353.

4.3 Lecithin based nanoemulsions: A comparative study of the influence of non-ionic surfactants and the cationic phytosphingosine on physicochemical behaviour and skin permeation

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Abstract

Charged drug delivery systems are interesting candidates for the delivery of drugs through skin. In the present study, it was possible to create negatively and positively charged oil/water nanoemulsions by using sucrose laureate and polysorbate 80 as non-ionic surfactants. The positively charged nanoemulsions were generated by adding cationic phytosphingosine (PS). The relationship between the physicochemical properties of the nanoemulsions was shown by particle size and zeta potential measurements. These properties were dependent on the type of non-ionic surfactant and the concentration of PS. Furthermore the cationic PS had a positive impact on the skin permeation rates (flux) of the incorporated model drugs fludrocortisone acetate and flumethasone pivalate. An enhancement factor between 1.1 and 1.5 was obtained in relation to the control. The interaction of pre-impregnated porcine skin with positively and negatively charged nanoemulsions was confirmed by DSC analysis. The generated DSC-curves showed a slight difference in the phase transition temperature assigned to the characteristic lipid transition. However, it was not possible to assign the effect to one of the ingredients in the multicomponent system.

Keywords: nanoemulsions, sucrose ester, polysorbate 80, particle size, zeta potential, skin permeation, DSC

1. Introduction

Nanoemulsions possess numerous advantages including the possibilities of controlled drug release and drug targeting, and the incorporation of a great variety of therapeutic actives (Calderilla-Fajardo et al., 2006). Usually the droplets achieve a size range between 50-200 nm. Unlike microemulsions they are stabilized by non-ionic surfactants and/or polymers exhibiting a steric stabilisation effect. Due to their small particle sizes, nanoemulsions are able to penetrate easily through the skin layers and enhance skin penetration of incorporated drugs (Tadros et al., 2004). Another important advantage is the low surfactant concentration compared to microemulsions (Tadros et al., 2004). Several preparation techniques such as spontaneous emulsification, phase inversion temperature (PIT) emulsification, phase inversion composition and high pressure homogenisation are known. The colloid-chemical structure depends thereby on these processes (Sonneville-Aubrun et al., 2004).

Due to their effectiveness for drug solubilization nanoemulsions offer an alternative for the administration of poorly water soluble drugs. This leads to improved efficacy and compliance because of reduced side effects (Kelmann et al., 2007). Nevertheless the use of safe and skin friendly ingredients is desirable for such colloidal formulations. As already reported, Lecithin, a natural mixture can fulfil these requirements, generally it is non-irritating and non-sensitizing for animal and human skin (Fiume, 2001; Paolino et al., 2002). Moreover lecithin compounds present an affinity to cellular membranes thus leading to an increased absorption of several drugs (Paolino et al., 2002). Sucrose esters are also non-irritant and biodegradable surfactants and therefore suitable for pharmaceutics and cosmetics. As previously published sucrose esters are promising candidates for enhanced drug permeability through skin showing lipid extraction and fluidization of the stratum corneum. Among the group of sucrose esters sucrose laureate has already been used to enhance skin permeability (Cazares-Delgadillo et al., 2005; Calderilla-Fajardo et al., 2006).

Cationic compounds can also have a positive effect on skin permeation, since the skin carries a negative surface charge due to the negatively charged residues of proteins (Yilmaz and Borchert, 2005). One candidate for this

purpose could be the physiological phytosphingosine (PS), a natural compound in the human body as well as in the stratum corneum. PS exhibited anti-inflammatory and antimicrobial effects both being interesting for topical use (Yilmaz and Borchert, 2005; M.Farwick et al., 2007).

The aim of the present study was to design physically stable nanoemulsions consisting of lecithin, vitamin E, PS, sucrose laureate and polysorbate 80, respectively. After the physicochemical characterisation by particle size and zeta potential measurements the influence of surfactant type and PS on skin permeation rates of the model drug fludrocortisone acetate was investigated. In order to confirm the influence of the cationic PS on skin diffusion additional permeation studies were performed using flumethasone pivalate in the polysorbate 80 nanoemulsion. Furthermore information about the possible interactions with skin lipids should be provided by DSC measurements of porcine skin samples after exposure to the various nanoemulsion formulations.

2. Materials and Methods

2.1 Materials

Fludrocortisone acetate (CAS: 514-36-3) was purchased from Sigma-Aldrich (St.Louis, USA). Flumethasone pivalate (CAS: 2002-29-1) was supplied from Kemprotec (Middlesbrough, U.K.). Phytosphingosine (PS) was kindly provided by Degussa (Cosmoferm BV, NL). Lipoid S-75 was obtained from Lipoid GmbH (Ludwigshafen, Ge), containing 69.9% phosphatidylcholine, 8.4% phosphatidylethanolamine, 2.2% lysophosphatidylcholine according to manufacturer's specifications. Sucrose laureate (SL, Ryoto Sugar Ester® L-1695) was gently donated by Mitsubishi-Kasei Food Corporation (Tokio, J). Polysorbate 80 and the antioxidant α-tocopherol were supplied by Pauli GmbH & Co.KG (Vienna, A). PCL-liquid (Etylhexanoate) was purchased from Symrise GmbH & Co.KG (Holzminden, Ge). All other chemicals used were of analytical reagent grade and used as received without any further purification.

2.2 Formulations

The nanoemulsions were prepared by mixing the separately prepared aqueous and oily phases. The aqueous phase containing sucrose laureate or polysorbate 80 and distilled water was heated up to 50 °C under slight mixing. When PS was incorporated it was dissolved in the oil phase, containing PCL-Liquid, Lipoid S75, α-tocopherol and model drug (1%). The two phases were merged and pre-homogenised for 3 min with an ultra-Turrax (Omni 500) at 2500 rpm and continuously stirred for 24 h at room temperature. Afterwards the raw formulation was pre-homogenised once more for 3 min at 50°C and further homogenised with a high-pressure homogeniser (EmulsiFlex-C3, Avestin) for 12 homogenisation cycles at 600 bars. In Table 1 all formulations performed with code names and their percentage compositions are presented. The meaning of the code names is detailed below Table 1.

Table 1 Composition of the nanoemulsion formulations and abbreviations

Excipients	Nanoemulsion composition (%w/w)					
	NL	NL-0.4PS	NL-0.6PS	NT	NT-0.4PS	NT-0.6PS
Lipid Phase						
PCl-Liquid	20	20	20	20	20	20
Lipoid S75	4	4	4	4	4	4
alpha-Tocopherol	1	1	1	1	1	1
Phytosphingosine (PS)	--	0.4	0.6	--	0.4	0.6
Fludrocortisone acetate	1	1	1	1	1	1
Aqueos phase						
Sucrose laureate L-1695	1	1	1	--	--	--
Polysorbate 80	--	--	--	1	1	1
Distilled water to	100	100	100	100	100	100

Abbreviations:

NL, sucrose laureate nanoemulsion without PS

NL-0.4PS, sucrose laureate nanoemulsion with 0.4% PS

NL-0.6PS, sucrose laureate nanoemulsion with 0.6% PS

NT, polysorbate 80 nanoemulsion without PS

NT-0.4PS, polysorbate 80 nanoemulsion with 0.4% PS

NT-0.6PS, polysorbate 80 nanoemulsion with 0.6% PS

2.3. Nanoemulsion characterisation

2.3.1. Particle size

The mean particle size and size distribution were determined by photon correlation spectroscopy with a Zetasizer Nano ZS (Malvern, UK) at 25°C. Each nanoemulsion was diluted to the appropriate concentration with distilled water to weak opalescence. The size distribution was represented by the polydispersity index (PDI) values. Thereby PDI values lower than 0.25 indicate a close size distribution providing good stability of nanoemulsions due to the reduced Ostwald ripening (Yilmaz and Borchert, 2005). The measurements were performed using a He-Ne laser at 633 nm. The diameters were determined immediately after nanoemulsion preparation and checked over 10 weeks during storage at room temperature.

2.3.2. Zeta Potential

The surface charge was analysed using a Zetasizer Nano ZS (Malvern, UK) at 25°C by measuring the zeta potential (ZP) of the preparations. For this purpose the samples were diluted with distilled water. The ZP characterises the surface charge of particles and thus gives information about repulsive forces between particles and droplets. Absolute higher values than 30 mV usually indicate good stability of the system (Müller, 1996). Also ZP values were measured after nanoemulsion preparation and checked over 10 weeks during storage at room temperature.

2.3.3. Skin permeation experiments

In vitro permeation studies with porcine abdominal skin were performed using Franz-type diffusion cells. Porcine abdominal skin was chosen as a model based on the similarity in permeability and morphology to human skin (Cazares-Delgadillo et al., 2005). Abdominal porcine skin was shaved and then prepared with a dermatome (GB 228R, Aesculap) set at 1 mm and afterwards stored in a freezer at -20°C until use. Two hours prior to the experiment the samples were thawed.

The skin was clamped between the donor and the receptor chamber of Franz-type diffusion cells having a permeation area of 1.13 cm². The receptor compartment was filled with 2 ml of 0.012 M phosphate buffer (pH, 7.4). The recently investigated solubility of the model drugs in 0.012 phosphate buffer (Hoeller and Valenta, 2007) guaranteed sink conditions. The diffusion cells were thermostated at skin surface temperature of 32°C and stirred by magnetic bars. The formulation (0.6 g) was gently placed in the donor chamber. Samples of 200 µl were removed at defined time intervals for analysis and replaced immediately by an equal volume of fresh buffer. At least three parallel experiments were performed for each formulation. Each 20 µl of the samples were analysed for their drug content by HPLC (Perkin Elmer, US) consisting of an automatic auto sampler ISS-200, a pump and an UV-diode array detector. A previously reported method was used (Cisternino et al., 2003). Briefly, the used column was a Nucelosil 100 – 5 C18 column (240 mm x 4 mm; ARC-Seibersdorf Austria) and the mobile phase consisted of acetonitrile and water (40:60 v/v), respectively. The detection wavelength was set at 240 nm and the flow rate was 0.8 ml/min for fludrocortisone acetate and 1.0 ml/min for flumethasone pivalate. Calibration curves were calculated on the basis of peak area measurements of the used standard solutions. The calibration curves had a correlation coefficient of 0.9998 and 0.9991, respectively. The concentration range of the standard solutions was between 3.87 - 124 µg/ml for fludrocortisone-acetate and between 3.48 -110.9 µg/ml for flumethasone pivalate.

Additionally, the steady state flux $J\ [\mu cm^{-2}h^{-1}]$ across the skin was calculated from the slope of linear portion of the cumulative amount permeated through the porcine skin per unit area versus time plot.

2.3.4. Differential scanning calorimetry (DSC)

DSC (Perkin Elmer DSC-7) was used to characterise the thermal transition of porcine skin samples of one individual that were either pre-impregnated with water or positively and negatively charged nanoemulsion formulations. The method used is already described (Valenta et. al., 2001). Briefly, prior to DSC

measurements about 25 mg of porcine skin were impregnated with 2 ml of water or differently charged nanoemulsions in a petri dish for 12 h. Porcine skin impregnated with water served as control. Afterwards the skin samples were blotted dry and then hermetically sealed within an aluminium holder and heated from 30 to 120°C at a heating rate of 5°C/min. The generated DSC-curves were compared to the control in terms of transition temperature and linear onset.
Gravimetric analysis of all samples prior to DSC experiments showed average water content of about 72.91 % (w/w) ± 5.83.

2.3.5. Statistical data analysis

Results are expressed as the means of minimum three experiments ± S.D. Statistical data analysis was performed using the *t*-test with $P<0.05$ as a minimal level as significance.

3. Results

3.1. Formulations

A visual inspection of the different formulations immediately after production showed liquid, whitish and well distributed nanoemulsions in all cases.

3.2. Nanoemulsion characterisation

The physicochemical properties of the formulations after 12 homogenisation cycles are shown in Table 2. As seen, the mean particle size depended on the type of surfactant and was strongly influenced by the presence of PS. In the sucrose laureate nanoemulsion the addition of PS increased the particle sizes in following manner: the higher the PS content, the higher the particle sizes of the nanoemulsions. In contrast to this in the polysorbate 80 nanoemulsion an increase from 0.4 % to 0.6 % PS decreased the particle size from 216 to 176 nm, which is nearly the size of the PS free polysorbate 80 nanoemulsion. As expected the surface charge of the nanoemulsions shifted from negative to

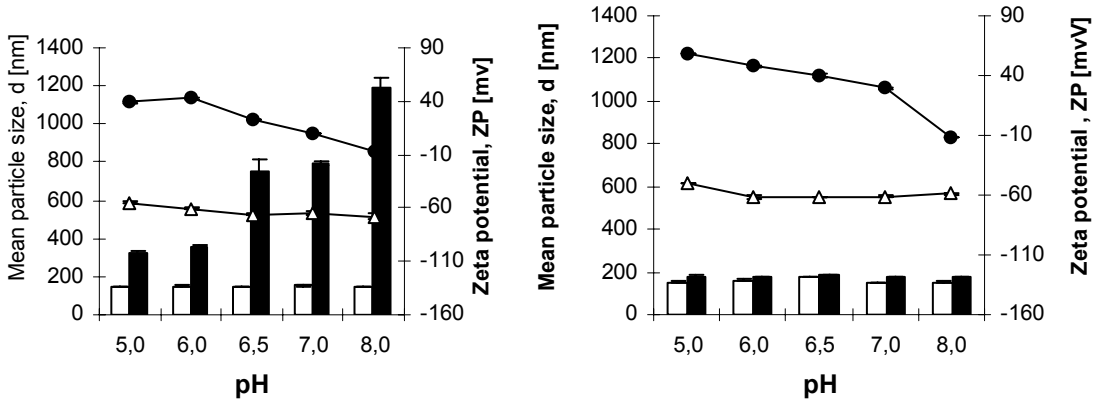
positive by adding PS. The absolute values being above 45 mV indicate a good electrochemical stability. The PDI values lower than 0.25 pointed to close size distributions.

Table 2

Physicochemical properties of the nanoemulsions after 12 homogenisation cycles with a high pressure homogeniser. PDI, polydispersity index; PS, phytosphingosine. Indicated values are the average (±S.D.) of three experiments.

	sucrose laureate nanoemulsion			polysorbate 80 nanoemulsion		
Property	NL	NL-0.4PS	NL-0.6PS	NT	NT-0.4PS	NT-0.6PS
Particle size (nm)	161±0.7	215±2.8	254±2.2	170±3.8	216±26.6	176±2.1
Zeta potential (mV)	-62±0.4	+46±0.4	+48±0.7	-55 ±0.7	+45±0.7	+48±1.1
PDI	0.12-0.22	0.22-0.25	0.06-0.10	0.15-0.18	0.13-0.18	0.10-0.14
pH	7.21±0.05	8.96±0.19	6.69±0.12	5.3±0.08	6.7±0.06	7.1±0.12

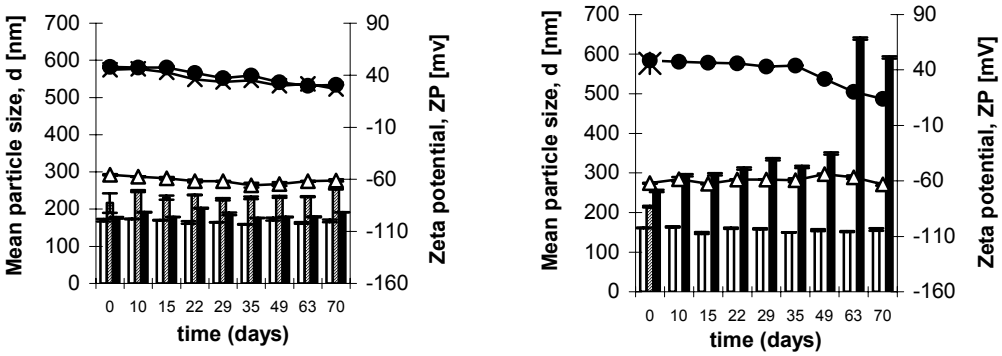
A set of experiments was performed over a pH-range from 5 to 8. Whereas the PS free nanoemulsions with sucrose laureate and polysorbate 80 showed a constant high ZP and a uniform particle size over the whole pH-range, the PS containing nanoemulsions exhibited a completely different behaviour (Fig 1 a, b). When 0.6 % PS was incorporated in sucrose laureate nanoemulsion (Fig. 1a, black bars) the particle size increased dramatically up to pH 6.5. The biggest particle sizes of about 1200 nm were seen at pH 8.0. When the pH value was decreased from 8 to 5, the ZP values increased from about –10 mV to above +40mV in the nanoemulsions with PS. In case of the polysorbate 80 nanoemulsion (Fig. 1b, black bars) the particle size was slightly increased but uniformly also by addition of 0.6% PS over the whole observed pH-range, which is an indication for a higher stability. The ZP changes followed the same pattern as in the sucrose laureate nanoemulsion at higher pH to negative.



a) b)

Figure 1 a, b Effect of pH on the mean particle size and zeta potential (ZP) from sucrose laureate and polysorbate 80 nanoemulsions. Indicated values are means (± S.D.) of three experiments. a) sucrose laureate nanoemulsions: white bars: mean particle size of NL; black bars: mean particle size of NL-0.6PS; -▲- zeta potential of NL; -●- zeta potential of NL-0.6PS b) polysorbate 80 nanoemulsions: white bars: mean particle size of NT; black bars: mean particle size of NT-0.6PS; -▲- zeta potential of NT; -●- zeta potential of NT-0.6PS

The stability assessments of the tested nanoemulsions are shown in Fig. 2 a, b. As seen, a constant ZP and uniform particle size over an observation period of 10 weeks are indicated in the PS free nanoemulsions (Fig. 2 a, b, white bars) as well as in the PS containing nanoemulsion with polysorbate 80 (Fig. 2a, grey and black bars). Although, the particle size is slightly increased in the PS containing preparation the nanoemulsion can be quoted as physically stable. In general, the polysorbate 80 nanoemulsion show particle sizes in the range from 165-250 nm independent of the surface charge.



a) b)

Figure 2 a, b Mean particle size and zeta potential (ZP) after storage of sucrose laureate and polysorbate 80 nanoemulsions over an observation period of 10 weeks. Indicated values are means (± S.D.) of three experiments. If no bars are shown, phase separation occurred. a) polysorbate 80 nanoemulsions: white bars: mean particle size of NT; grey lined bars: mean particle size of NT-0.4PS;black bars: mean particle size of NT-0.6PS ;-▲- zeta potential of NT -X- zeta potential of NT-0.4PS; -●- zeta potential of NT-0.6PS b) sucrose laureate nanoemulsions: white bars: mean particle size of NL; grey lined bars: mean particle size of NL-0.4PS; black bars: mean particle size of NL-0.6PS; -▲- zeta potential of NL; -X- zeta potential of NL-0.4PS; -●- zeta potential of NL-0.6PS

In contrast phase separation was observed in the sucrose laureate nanoemulsion containing 0.4 % PS after 10 days. On the contrary the formulation with 0.6 % PS exhibited improved physical stability. However, after seven weeks of storage a pronounced particle agglomeration occurred simultaneously with a dramatic decrease of the ZP (Fig. 2b, black bars).

3.3. Skin permeation experiments

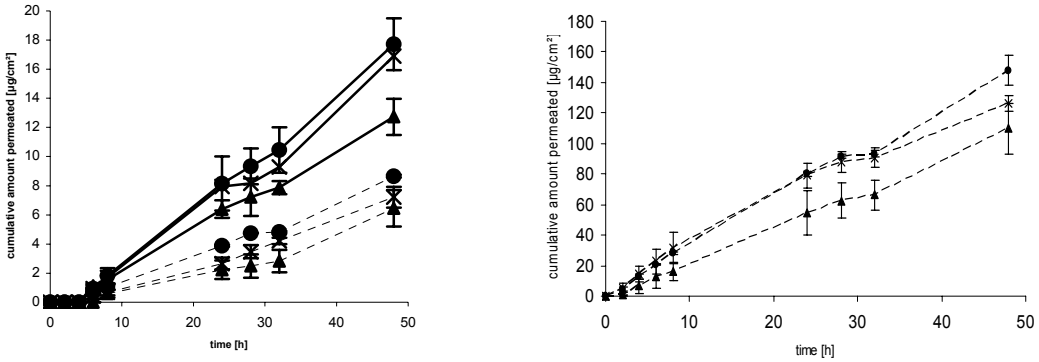
Firstly, in standard diffusion experiments the skin permeation rates (flux) of the model drug fludrocortisone acetate were investigated. As seen in Fig. 3A two groups can be distinguished: one group having lower fluxes of fludrocortisone acetate is based on the nanoemulsion with sucrose laureate and a second group exhibiting higher diffusion rates is based on polysorbate 80 nanoemulsion. Moreover, PS was able to increase the skin permeation rates of

fludrocortisone acetate compared to the control independent of the formulation. The addition of 0.6 % PS led to an increase in the permeation rates of 1.5 times from sucrose laureate nanoemulsion and 1.4 times of the polysorbate 80 nanoemulsion compared to the control (Table 3).

Table 3
Skin permeation rates (flux) of fludrocortisone acetate and flumethasone pivalate in different nanoemulsions and influence of phytosphingosine (PS) through porcine skin, n=3. (* indicate a statistical significance, $p < 0.05$)

Model drug	Nanoemulsion	Flux (J, µg/cm²/h)	Enhancement factor of PS compared to control
Fludrocortisone acetate	NL	0.126±0.027	control
	NL-0.4PS	0.150±0.010	1.1
	NL-0.6PS	0.189 ±0.012 *	1.5*
	NT	0.263 ±0.043	control
	NT-0.4PS	0.353 ±0.018 *	1.3*
	NT-0.6PS	0.377 ±0.038 *	1.4*
Flumethasone pivalate	NT	2.290±0.313	control
	NT -0.4PS	2.698±0.117	1.18
	NT -0.6 PS	3.073±0.104*	1.34*

To confirm the influence of PS on the skin rates a set of formulation with the polysorbate 80 nanoemulsion containing flumethasone pivalate as other model drug was investigated. Again the positive impact of the cationic PS on the flux was demonstrated, although to a lesser extent than in the experiments using fludrocortisone acetate (Table 3 and Fig. 3B). The enhancement factor for flumethasone pivalate by the addition of 0.6 % PS was 1.34.



A) B)

Figure 3 A-B) Permeation profiles of fludrocortisone acetate (A) and flumethasone pivalate (B) incorporated in positively and negatively charged nanoemulsions through porcine skin determined by HPLC. Indicated values are means (± S.D.) of three experiments. A) full line: -●- NT-0.6PS; -x- NT-0.4PS; -▲- NT; dashed line - - ● - - NL-0.6PS; - - x - - NL-0.4PS, - -▲- - NL; B) dashed line — — ● — — NT-0.6PS; — — x — — NT-0.4PS; — — ▲— — NT

3.4. Differential scanning calorimetry

To further explore the effect of different nanoemulsions on lipid components of porcine skin DSC studies were conducted. All samples were taken from the same piece of porcine skin. In literature data it was proposed that porcine transitions occurred near 60°, 70° and 100°C in hydrated samples, which were due to intercellular lipid, protein-intercellular lipid and keratin, respectively (Potts, 1989; Valenta et al., 2001; Kim et al., 2008). In our case porcine skin pre-impregnated with water for 12 h showed an endothermic transition peak T_m occurring near 80°C. The pre-impregnation of porcine skin with differently charged nanoemulsions resulted in a slight difference of T_m (Fig. 4 and Table 4). The maximal thermal transition is shifted from about 80°C to 74°C after treatment with negatively charged nanoemulsions independent of the non-ionic surfactant. Impregnation of porcine skin with positively charged nanoemulsions resulted in a T_m shift from 80°C to 76°C with the polysorbate 80 containing

nanoemulsion, whereas no influence of the positively charged sucrose laureate nanoemulsion was observed.

Table 4 Thermal changes following pre-impregnation of porcine skin with positively and negatively charged nanoemulsions. Control: porcine skin pre-impregnated with water for 12 h, n≥4

Treatment	DSC T_m (°C)	Linear Onset (°C)
Control	80.01 ±0.45	65.29 ±0.20
NL	74.38 ±4.53	65.54 ±5.17
NL-0.6PS	80.66 ±0.31	64.14 ±0.19
NT	74.90 ±2.95	64.47 ±2.72
NT-0.6PS	76.85 ±3.81	66.52 ±2.35

[a] Temperature of the transitions maximum T_m

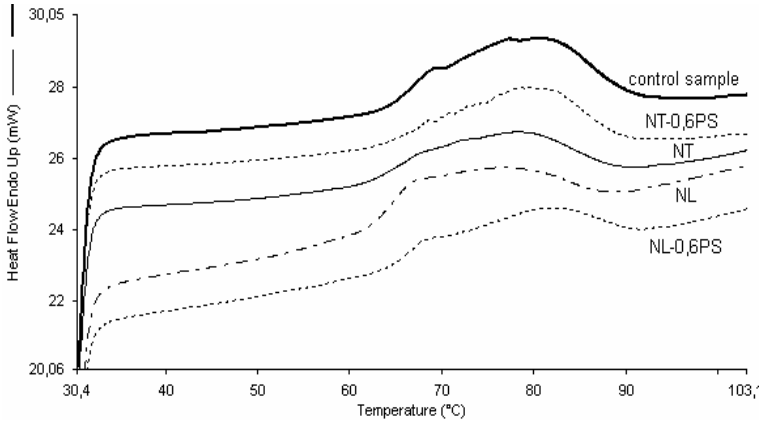


Figure 4 Differential scanning calorimetry (DSC) analysis of porcine skin treated with negatively and positively charged nanoemulsions. Gravimetric analysis of the samples prior to DSC studies showed average water content of about 73% (w/w). Graphs are representative of n≥4 replicate samples.

4. Discussion

This study investigated differently charged nanoemulsions containing either sucrose laureate or polysorbate 80 in terms of particle size and zeta potential. It is known that non-ionic surfactants stabilise emulsion due to a strong steric

repulsion mechanism (Piemi et al.,1999). As the results indicate polysorbate 80 improved the physicochemical stability of the formulation in comparison to the sucrose laureate nanoemulsions. Due to ethylene oxide groups and the long hydrocarbon chain a stronger steric stabilisation effect can be achieved. Therefore mean particle size and zeta potential values of the polysorbate 80 containing nanoemulsions were physically stable over 10 weeks showing no flocculation, creaming, coalescence and Ostwald ripening.

Additionally we tested the hypothesis that PS, a known sphingoid base, can increase skin permeability by its cationic charge on the negatively charged skin (Yilmaz and Borchert, 2005). The obtained data confirmed the pH dependent physicochemical properties of the tested PS nanoemulsion. The electrical surface charge of the particles is produced by ionisation of PS forming an interfacial film. It depends mainly on the extent of the ionisation of PS (Fig.1 a, b).

Percutaneous penetration implies several steps. The release of a drug through skin will depend on the physicochemical properties of the drug itself combined with the influence of the vehicle to alter the drug penetration profile (Piemi et al., 1999; Cazares-Delgadillo et al., 2005). The positively charged nanoemulsions containing PS were found to be more effective in terms of skin diffusion of fludrocortisone acetate and flumethasone pivalate through porcine skin than the negatively charged ones (Table 3 and Fig. 3A-B).

As mentioned the interaction of nanoemulsions with skin depends upon a number of factors including also the electrical charge of the droplets. The results obtained suggest that positively charged particles of the nanoemulsion systems are able to carry efficiently fludrocortisone acetate and flumethasone pivalate into the skin and subsequently promote the penetration of the drugs through skin. The degree of skin binding is probably more important with the positively charged particles than with the negatively one as it is known that the skin is negatively charged at neutral pH (Conrads and Zahn, 1987). This hypothesis is supported additionally by the results of Piemi et al, 1999. It can therefore be deduced that the binding of the charged particles to the skin can be attributed to a specific electrostatic interaction due to the positive charge of the particles in case of the phytosphingosine containing nanoemulsions (Piemi et

al., 1999). Nevertheless, more penetration studies need to be performed to confirm the influence of the charge of the vehicle and the binding skin process, especially in the case of positively or negatively charged nanoemulsions in order to establish a possible structure-binding relationship.

Moreover the penetration enhancement of drugs is closely related to the nature of the surfactants used in the formulation. Many penetration enhancers are capable of inserting themselves between the hydrophobic tails of the bilayer, thus disturbing their packing, increasing their fluidity, and subsequently, leading to an easier diffusion of lipid penetrants. Sucrose laureate interferes with their long hydrocarbon chain between the lipophilic tails allowing the sucrose ring to interact with the polar lipid head groups (Calderilla-Fajardo et al., 2006). There are two possible mechanisms of penetration enhancement suggested for polysorbate 80. On one hand it penetrates into the intercellular regions of stratum corneum, increases fluidity and eventually solubilises and extracts lipid components, and on the other hand an interaction and binding with keratin filaments may result in a disruption within the corneocytes. In this case keratin filaments and their associated water molecules could be disrupted, whereas a solubilising ability of the aqueous layer could result and an allowed drug transport through the corneocytes might be possible (Nokhodchi et al., 2003). Consequently one reason for the improved skin permeation of fludrocortisone acetate caused by using polysorbate 80 will be the protein domains involved.

In DSC-studies a slight difference was observed between the skin samples pre-impregnated with differently charged nanoemulsions and the water-impregnated control. The decreased transition temperatures showed an interaction with lamellar skin lipids structure at temperature near 60-80°C indicating a higher fluidity of skin. Due to the higher skin permeation of PS containing nanoemulsions we expected a further shift of the transition temperature to lower values. However the opposite was observed (Tab. 4). Therefore, the higher skin permeation might be caused by additional other mechanisms than by skin lipid interactions. It is also known that the water content plays a major role in the transition temperature of lipids and proteins (Potts, 1989). For that reason it was seen to it that all samples had equal water content prior to DSC-measurements.

5. Conclusion

Overall, we succeeded in creating nanoemulsions with the skin friendly mild surfactant sucrose laureate and in enhancing the skin fluxes of the model drug fludrocortisone acetate and flumethasone pivalate with PS. In addition the nanoemulsion with conventional polyoxyethylene derivatives exhibited higher diffusion rates and a prolonged physicochemical stability. Furthermore PS is an interesting multifunctional additive for drug delivery systems due to its enhancement effect on skin permeation and above all its hydrating and anti-inflammatory power.

6. References

Calderilla-Fajardo, S.B., Cazares-Delgadillo, J., Villalobos-Garcia, R., Quintanar-Guerrero, D., Ganem-Quintanar, A., 2006. Influence of sucrose esters on the in vivo percutaneous penetration of octyl methoxycinnamate formulated in nanocapsules, nanoemulsion and emulsion. Drug Dev. Ind. Pharm., 32, 107-113.

Cazares-Delgadillo, J., Naik, A., Kalia, Y.N., Quintanar-Guerrero, D., Ganem-Quintanar, A., 2005. Skin permeation enhancement by sucrose esters: A pH-dependent phenomenon. Int. J. Pharm., 297, 204-212.

Cisternino S., Schlatter, J., Saulnier, J.L., 2003. Stability of Fludrocortisone-acetate solutions prepared from tablets and powder. Eur. J. Pharm. Biopharm., 55, 209-213.

Conrads A. and Zahn H., 1987. A study of the interaction of sodium dodecyl sulphate with the proteins of human heel stratum corneum, Int. J. Cosm. Sci. 9(1), 29-46

Farwick, M., Watson, R. E. B., Rawlings, A.V., Wollenweber, U., Lersch, P., Bowden, J.J., Bastrilles J.Y., Griffiths C.E.M., 2007. Salicyloly-phytosphingosine: a novel agent for the repair of photoaged skin. Int. J. Cosmetics Sci., 29, 319-329.

Fiume, Z., 2001. Final report on the safety assessment of lecithin and hydrogenated lecithin. Int. J. Toxicology, 20, 21-45.

Hoeller S. and Valenta C., 2007. Effect of selected fluorinated drugs in a "ringing" gel on Rheological behaviour and skin permeation, Eur. J. Pharm. Biopharm., 66 ,120-126.

Kelmann, R. G., Kuminek, G., Teixeira H.F., Koester L.S., 2007.

Carbamazepine parenteral nanoemulsions prepared by spontaneous emulsification process. Int. J. Pharm., 342, 231-239.
Kim, Y.-C., Park, J.-H., Ludovice P.J., Prausnitz, M.R., 2008. Synergistic enhancement of skin permeability by N-lauroylsarcosine and ethanol. Int. J. Pharm., 352, 129-138.

Müller, R. H., 1996. Zetapotential und Partikelladung in der Laborpraxis. Paperback APV, Band 37,Wissenschaftliche Verlagsgesellschaft mbH Stuttgart

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

Paolino D., Ventura, C.A., Nistico, S., Puglisi, G., Fresta M., 2002. Lecithin microemulsions for the topical administration of ketoprofen: percutaneous adsorption through human skin and in vivo human skin tolerability. Int. J. Pharm., 244, 21-31.

Piemi, M. P. Y., Korner, D., Benita, S., Marty, J.P., 1999. Positively and negatively charged submicron emulsions for enhanced topical delivery of antifungal drugs. J. Contr. Rel., 58, 177-187.

Potts, R. O. 1989. Physical characterization of the stratum corneum: The relationship of mechanical and barrier properties to lipid protein structure. In. Hadgraft, J.; Guy, R.H. (Eds). Transdermal Drug Delivery.: pp. 23-57.

Sonneville-Aubrun, O., Simonnet, J.T., L´ Alloret, F., 2004. Nanoemulsions: a new vehicle for skincare products. Adv. Colloid Interface Sci., 108-109, 145-149.

Tadros, T., Izquierdo, P., Esquena, J., Solans, C., 2004. Formation and stability of nano-emulsions. Adv. Colloid Interface Sci., 108-109, 303-318.

Valenta, C., Nowak M., Hadgraft, J., 2001. Influence of phloretin and 6-ketocholestanol on the permeation of progesterone through porcine skin. Int. J. Pharm., 217, 79-86.

Valenta, C., Siman, U., Kratzel, M., Hadgraft, J., 2000. The dermal delivery of lignocaine: influence of ion pairing. Int. J. Pharm., 197, 77-85.

Yilmaz E. and Borchert H.-H., 2005. Design of a phytosphingosine-containing, positively-charge nanoemulsion as a colloidal carrier system for dermal application of ceramides. Eur. J. Pharm. Biopharm., 60, 91-98.

4.4 Multinuclear NMR characterisation and dermal delivery of fluorinated drugs in soybean-microemulsion systems

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Abstract

The present study evaluated the effect of different commercially available soybean lecithins in microemulsion systems in terms of microstructure transformation, physicochemical properties and transport of selected entrapped fluorinated drugs through skin.

Physicochemical characterisations by particle size and polydispersity index (PDI) measurements were performed and a direct correlation with NMR self-diffusion coefficients of the individual components was found. An increase of lysophosphatidylcholine (LPC), phosphatidylethanolamine (PE) and lysophosphatidylethanolamine (LPE) in the phospholipid mixtures increased the mean particle sizes and PDI. Bicontinous microemulsion structures were proven by ^{1}H and ^{31}P NMR in the placebo microemulsions. Reasonable permeation of the lipophilic drugs of all microemulsions systems was confirmed in standard diffusion studies using porcine skin. This could be due to the incorporation of the drugs in the surfactant structure of the lecithin based bicontinous micro textures, as proven by ^{19}F NMR self-diffusion studies.

Keywords: ^{1}H NMR, ^{19}F NMR, ^{31}P NMR, skin permeation, microemulsion, phospholipid

1. Introduction

Liquid crystalline, micellar and emulsion forming systems are widely used in pharmaceutical preparations. The easy formation and excellent solubilisation capacity favour microemulsions over other pharmaceutically used systems. The dispersed phase, lipophilic or hydrophilic (o/w, w/o or bicontinuos type) can act as a potential reservoir of lipophilic or hydrophilic drugs which are partitioned between the dispersed and the continuous phase [1].

A disadvantage of microemulsions is their relatively high concentration of surfactant and the requirement need for the presence of a co-surfactant. The potential of a topical formulation to be used as a transdermal drug delivery device should be evaluated not only in terms of carrier capacity and percutaneous drug absorption, but also in terms of tolerability and toxicity of the proposed formulation [2]. Therefore, the microemulsion components in the present study were chosen according to their biocompatibility and toxicity in order to produce highly eudermic formulations.

Microemulsions stabilised by lecithins will be highly regarded as potential delivery vehicles, since lecithin is a natural surfactant with a strong ability to adsorb on biomembranes and to enhance biomembrane permeability [3]. Moreover, lecithins are found generally to be non-irritating and non-sensitising for human skin [4]. Lecithin exhibits a very strong hydrophobicity due to long hydrocarbon chains as well as a strong hydrophilicity due to the zwitterionic polar head groups, which have dipole moments. There is a close balance between hydrophilic and lipophilic properties [5]. To obtain optically clear microemulsions using lecithin as surfactant a short chained alcohol is needed as a co-surfactant [6]. Therefore, isopropanol was used. The permeation enhancer isopropylmyristate (IPM), which usually has no irritating effects and good dermal compatibility, was used, as oily phase.

The drug release kinetics depend on their structure (droplet size, volume fraction of the dispersed phase etc.) as well as on many other physicochemical factors such as the solubility of the drug in both media, the properties of interfacial layers of surfactants, interfacial tension etc. [7]. Therefore, the choice of an appropriate vehicle to maximise skin permeation is necessary.

Phospholipid-based formulations are known to improve the permeation efficacy of active molecules. The entrapment of lipids strongly affects the microemulsion structures and the locus of the drug on interface [8]. Also, the compositions of commercially available lecithins may affect the microstructure and thus the permeation of the incorporated drugs [9]. In this study we are therefore evaluating the physical properties of microemulsion systems containing different commercially available soybean lecithins regarding their phosphatidylcholine content, their composition and their capability to transport different entrapped lipophilic drugs through the skin. In order to evaluate the microstructure the resulting systems should also be characterised by generating self-diffusion coefficients of the individual components of the vehicle by ^{1}H and ^{31}P NMR. Moreover ^{19}F NMR should be used for measuring the self-diffusion of the low concentrated fluorinated drugs and, if possible, should be related to the skin permeation.

2. Material and methods

2.1. Materials

Fluconazole (CAS: 86386-73-4), flufenamic acid (CAS: 530-78-8), flutamide (CAS: 13311-84-7) and flumethasone pivalate (CAS: 2002-29-1) were purchased from Kemprotec (UK). Lipoid S100, Lipoid S75 and Lipoid S45 were kindly donated by Lipoid GmbH (Ludwigshafen, Germany). Isopropanol (IP) was supplied by Merck (Hohenbrunn, Germany). Isopropylmyristate (IPM) was purchased from Sigma-Aldrich (St.Louis, USA).

2.2 Formulations

The oily phase consisting of lecithin and IPM was mixed with the co-surfactant isopropanol and water was added gradually under magnetic stirring at room temperature until the system was transparent. The components of the commercially available lecithins are listed in Table 1. The microemulsions consisted of 25% IPM, 25% isopropanol, 25% water and 25% Lipoid S100 (A),

Lipoid S75 (B) and Lipoid S45 (C) respectively (each % w/w, Table 2). The fluorinated drugs fluconazole, flumethasone pivalate, flutamide and flufenamic acid (each 1% w/w) were solved in the oily phase and thereby incorporated into the systems.

Table 1 Compositions of the main components within the different soybean-lecithins

Lecithin[a]	PC		LPC		PE		LPE	
	% (w/w)[b]	% (mol)[c]	% (w/w)[b]	% (mol)[c]	% (w/w)[b]	% (mol)[c]	% (w/w)[b]	% (mol)[c]
Lipoid S 100	98.00	100	0.70	0.8	< 0.1	-	-	-
Lipoid S 75	72.70	100	1.80	3.3	8.50	12.9	-	3.7
Lipoid S 45	48.80	100	1.60	3.9	15.40	34.9	-	7.4

[a] Abbreviations: PC = phosphatidylcholine, LPC = lysophosphatidylcholine, PE = phosphatidylethanolamine, LPE = lysophosphatidylethanolamine; [b] as supplied by the manufacturer; [c] Integration values of the ^{31}P NMR spectra, relative to PC

Table 2 Compositions of the microemulsions in % (w/w)

	Microemulsions		
	A	B	C
Isopropylmyristate	25.0	25.00	25.00
Lipoid S100	25.0	--	--
Lipoid S 75	--	25.00	--
Lipoid S 45	--	--	25.00
Isopropanol	25.0	25.00	25.00
Water	25.0	25.00	25.00

2.3 Characterisation and physical stability of the microemulsions

Microscopic observation to characterise the structure of the microemulsion systems were made using an optical polarising microscope. The mean particle

size and polydispersity index (PDI) were estimated by photon correlation spectroscopy with a Zetasizer Nano ZS (Malvern, UK) at 25°C. The measurements were performed using a He-Ne laser at 633 nm. The diameters were determined after microemulsions preparation and were checked over 10 weeks during storage at room temperature.

The viscosity of the placebo microemulsions was measured using a Haake MARS Rheometer with a plate/plate (PP60/Ti). Flow curves were performed at the following parameters: controlled rate (CR) modus, γ= 1-100; 25°C ±0.2°C.

2.4 NMR measurements

NMR experiments were performed on a BRUKER Avance DRX 600 NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) operating at 600.13 MHz for ^{1}H, 564.69 MHz for ^{19}F, and 242.94 MHz for ^{31}P, respectively. Either a 5 mm triple inverse probe (^{1}H, ^{13}C, broadband) with triple axis gradient coils was used or a 5 mm quadruple observe probe (^{1}H, ^{13}C, ^{19}F, ^{31}P) with z-axis gradient coil. For spectrometer stability reasons the H_2O phase in the microemulsions was replaced by D_2O. The diffusion coefficients at a constant temperature of 25°C were derived from the signal attenuation in a series of stimulated echo spectra with increasing gradient amplitudes by fixed diffusion delay according to Wu et al. [10]. To overcome thermal convection effects, either only orthogonal gradients were used or the sample tubes were rotated [11]. Typical experimental parameters were chosen as in the reference [12]. Heteronuclear diffusion coefficients were measured with a similar experimental setup using the following parameters: for ^{19}F the individual signals were set on resonance, the sweep width to 5.6 kHz, the time domain to 22000 data points, the relaxation delay to 5 s, and the number of scans to 8; for ^{31}P the sweep width was set to 4.8 kHz, the time domain to 29000 data points, the relaxation delay to 5 s, and the number of scans to 16. To collapse the proton coupling in the ^{19}F as well as in the ^{31}P NMR spectra, WALTZ-16 composite pulse decoupling was performed during acquisition with a γB_1 of 1 kHz. For ^{19}F NMR, CCl_3F (δ = 0 ppm), for ^{31}P 85% H_3PO_4 (δ = 0 ppm) was used as external reference, respectively. The processing and the analysis of the self-diffusion

measurements were done within the Topspin Software, Version 2.1 (Bruker BioSpin, GmbH, Rheinstetten, Germany). For the diffusion ordered spectroscopy (DOSY) representation, all Fourier transformed spectra of the stimulated echo series were fitted independently using a Levenberg-Marquardt algorithm. All experiments were repeated at least 5 times, the accuracy was ±4%.

2.5 In vitro permeation studies

Porcine abdominal skin was shaved and prepared with a dermatome (GB 228R, Aesculap) set at 1.2 mm. The skin was stored in a freezer at -20°C until use. Two hours prior to the experiment the samples were thawed.
Standard diffusion experiments using the same protocol and HPLC analysis for all drugs were performed as recently reported [13]. Briefly, the diffusion cells were thermostated at skin surface temperature of 32°C and stirred by magnetic bars for 8h. Samples of 200 µl were removed at defined time intervals (2, 4, 6, and 8h) for analysis and replaced by fresh receptor medium. Minimum three parallel experiments were performed.

2.6 Statistics

For statistical data analysis of Tables 3 and 7 the non parametric Kruskal-Wallis Test was performed with $p<0.05$ as a minimal level of significance. Results are expressed as the mean of at least four parallel experiments ± S.D.

3. Results and Discussion

3.1 Formulations

Much attention has been paid on phospholipid-based microemulsions and their pharmaceutically acceptability, recently [14]. Their non-toxicity makes them an ideal choice for dermal and transdermal drug delivery. All formulations were

formed spontaneously, their appearance being of a transparent homogenous yellow liquid.

3.2 Characterisation of the microemulsions

All investigated formulations showed black isotropic textures under polarized light microscope. This indicates a microemulsion system and no liquid crystalline phase, which appears under the microscope with characteristic coloured textures[15]. In the first part of the work the influence of different soybean lecithins on the physicochemical properties of the microemulsions was analysed. Droplet diameter in stable microemulsions is usually within the range of 10-100 nm, which means that the term “microemulsion” maybe misleading and these systems are actually nano-sized systems [16]. As shown in Table 3, all investigated microemulsions are characterised by a very small droplet size. The mean particle size was less then 10 nm and the polydispersity index (PDI) obtained values under 0.2 in each formulation, which indicates a close size distribution providing good stability [17]. A smaller content of PC, but higher content of phosphatidylethanolamine (PE) led to an increase in the mean particle size, which might be a consequence of the different microstructures caused by the different soybean lecithins used in the systems. In addition, the incorporated drug can take part in the microstructure of the system, thus influencing the microemulsion arrangement and properties via molecular interaction, as seen in Table 2 [2]. In order to investigate the influence of the different lecithin composition the viscosity of the placebo formulations was measured. All investigated formulations showed ideal newton`sche flow curves (data not shown) [15]. As expected, the viscosity depended on the lecithin composition and was 11.05 ±4.4 mPas (A), 16.35 ±2.7 mPas (B) and 17.06 ±1.7 mPas (C).

Table 3 Mean particle size (d.nm) and polydispersity index (PDI) of the tested microemulsions (*significant different to

Microemulsion	Placebo	Flufenamic acid	Flumethasone pivalate	Flutamide	Fluconazole
	Size d.nm[a]	Size d.nm[a]	Size d.nm[a]	Size d.nm[a]	Size d.nm[a]
A	1.34±0.02	1.56±0.16	1.12±0.02	1.06±0.01	0.77±0.03
B	1.26±0.03	1.39±0.04	1.12±0.02	1.07±0.04	1.40±0.06
C	2.25±0.14*	3.72±0.31*	1.71±0.10*	1.62±0.05*	2.19±0.05*
	PDI[b]	PDI[b]	PDI[b]	PDI[b]	PDI[b]
A	0.05-0.30	0.05-0.21	0.08-0.33	0.07-045	0.21-0.5
B	0.09-0.33	0.03-0.24	0.10-0.46	0.11-0.4	0.06-0.22
C	0.13-0.33	0.04-0.36	0.12-0.5	0.01-0.21	0.05-0.34

[a] average over 10 weeks of measurements, n=30

[b] average over 10 weeks of measurements, n=30

3.3 NMR

A multinuclear NMR approach was chosen for analysing the phospholipid-based microemulsions alone as well as the drug loaded formulations. First the variations in the different lipoids were studied by ^{31}P NMR. The main phosphorous containing components give characteristic resonance lines in the ^{31}P NMR spectra (Fig. 1). The assignment is based on the literature [18], or on ^{1}H coupled ^{31}P NMR spectra (not shown). Due to the ^{1}H-^{31}P scalar coupling small amounts of phosphatidic acid can be identified by a triplet splitting, were all the other components show quintet signals. Using the same approach, phosphatidylinositol, another common constituent of soybean lecithins, can be excluded, which should show a quartet splitting. The amounts of the main components within the different soybean lecithins are listed in Table 1. The first row presents the % (w/w) of the phospholipids within the total lecithin preparations as given by the supplier, the second the relative mol percentages calculated from the integrated ^{31}P NMR spectra setting the phosphatidylcholine (PC) to 100%, respectively. This information is more feasible for the present

study delivering the relative proportion of the most prominent phospholipids in the individual lecithins. Going in the series from lipoid S100 to S45, the overall amount of PC is reduced, increasing at the same time the phosphatidylethanolamine (PE) as well as the lyso-form of PE. Whereas lipoid S100 is almost quantitatively PC, the molar ratio of PC to PE is about 9 to 1 in lipoid S75, going to 3 to 1 in lipoid S45.

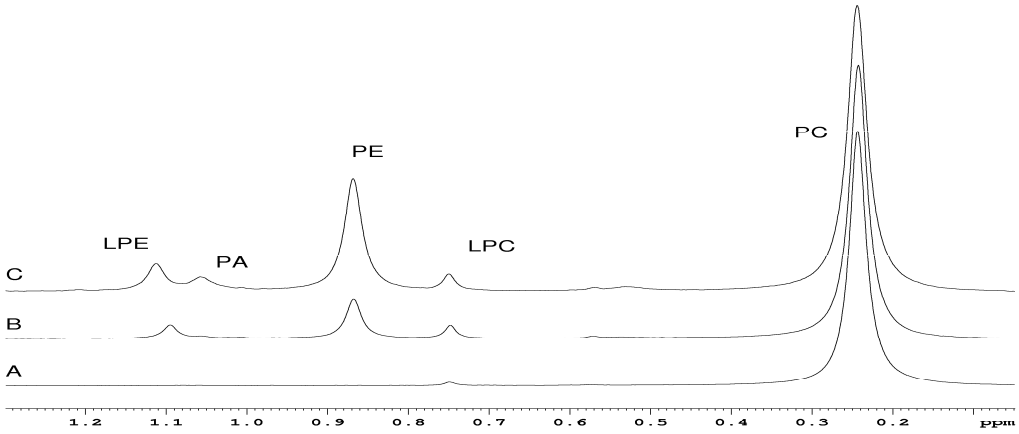


Figure 1 ^{31}P NMR of microemulsions A, B, and C (PC phosphatidylcholine, PE phosphatidylethanolamine, LPC lysophosphatidylcholine, LPE lysophosphatidylethanolamine, PA phosphatidic acid)

The next step was the characterisation of the microemulsion carrier systems by NMR self-diffusion experiments. The discrimination between the individual components is possible based on characteristic resonance lines in the well resolved ^{1}H NMR spectra (Fig. 2, top spectrum), namely the HDO signal (4.97 ppm) for the water, the CH (4.19 ppm) or CH_3 (1.39 ppm) signal for isopropanol, the alcohol CH (5.18 ppm) or CH_3 (1.42 ppm) or the CH_2 (2.43 ppm) adjacent to the ester group for isopropylmyristate (IPM), and mainly the trimethylamino resonance (3.48 ppm) of phosphatidylcholine for the lecithins. The diffusion ordered spectrum (DOSY) of microemulsion A is shown in Fig. 2. In this representation the series of the Fourier transformed stimulated echo spectra were fitted independently resulting in a two dimensional data matrix with the full spectral information for every compound on one axis separated by the corresponding diffusion coefficient on the other. As can be seen in Fig. 2, the individual components are spread over the diffusion axis giving well resolved

single rows for water, oil and surfactant as well as for the co-surfactant at the appropriate diffusion constants. The ability of resolving multi-exponential decays in this representation should not be overestimated as can be seen especially in the region of the overlapping aliphatic chains of PC and IPM. Nevertheless discrete self-diffusion coefficients for all components can be calculated from undisturbed resonances using a linear least-squares approach.

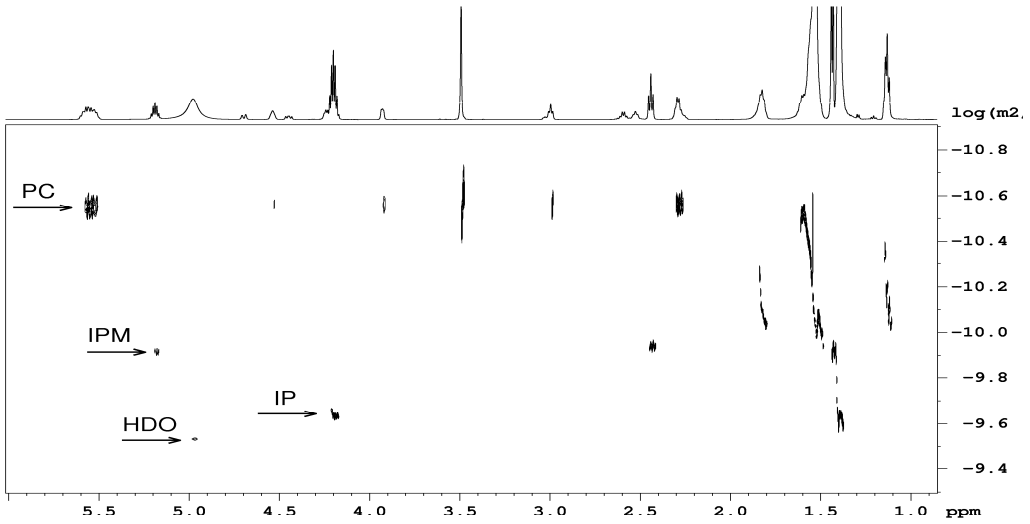


Figure 2 DOSY (^{1}H Diffusion Ordered Spectroscopy) of microemulsion A with ^{1}H NMR spectrum as top trace (IP isopropanol, IPM isopropylmyristate, PC phosphatidylcholine)

In Table 4 these diffusion coefficients D in the three formulations are summarized, together with the values for the neat solvents D_0. In the case of water, the observed experimental values have to be corrected due to exchange contributions mainly with the co-surfactant isopropanol [19]. The diffusion behaviour of water, oil, and surfactant system looks very similar in the three different formulations, overall only a small slowdown for all constituents is observed going from microemulsion A over B to C. Thus the NMR diffusion data are in good agreement with photon correlation spectroscopy in this formulation series (Table 3) showing an increased particle size as well as an increased polydispersity index following the same order of the vehicles.

Table 4 NMR Self-diffusion coefficients for the individual components neat and in the three formulations from ^{1}H or ^{31}P NMR (for PC and PE) experiments

	neat	A		B		C	
	D_0 [m^2s^{-1}][b]	D [m^2s^{-1}]	D/D_0	D [m^2s^{-1}]	D/D_0	D [m^2s^{-1}]	D/D_0
Water	$1.90x10^{-9}$	$3.00x10^{-10}$	0.16	$2.50x10^{-10}$	0.13	2.38x10-^{10}	0.12
IP[a]	$5.40x10^{-10}$	$2.30x10^{-10}$	0.42	$2.16x10^{-10}$	0.40	$2.15x10^{-10}$	0.40
IPM	$2.11x10^{-10}$	$1.15x10^{-10}$	0.54	$1.09x10^{-10}$	0.51	$1.08x10^{-10}$	0.51
PC (^{1}H)[1]	$3.43x10^{-10}$	$2.70x10^{-11}$		$2.22x10^{-11}$		$2.18x10^{-11}$	
PC (^{31}P)		$2.71x10^{-11}$		$2.25x10^{-11}$		$2.26x10^{-11}$	
PE (^{31}P)		-		$2.26x10^{-11}$		$2.20x10^{-11}$	

[a] Abbreviations: IP = isopropanol, IPM = isopropylmyristate, PC = phosphatidylcholine, PE = phosphatidylethanolamine; [b] D_0 for PC 0.3 % (w/w) in MeOH-d_4

To gain further insight into the diffusion of the phospholipids, additional NMR diffusion experiments were performed on the phosphorous frequency to discriminate between the phosphatidylcholine and phosphatidylethanolamine signals. However, the measured self-diffusion coefficients are within the experimental error almost the same for PC and PE and comparable with the values derived from ^{1}H NMR. This behaviour could be expected due to their similar molecular weight, but can on the other hand not be taken for granted within a high concentrated multi component system. So both phospholipids seem to be incorporated in a similar structural surrounding within the microemulsions. The lyso-forms of PC and PE could not be analysed by ^{31}P NMR diffusion spectra, as the signal to noise ratio for these low concentrated constituents is not sufficient for a reliable fitting of their diffusion decays.

The detailed analysis of the NMR derived self-diffusion coefficients (Table 4) for water and oil results in high values for both, which are in the same order of magnitude, thus indicating a non discrete particle system. The diffusion of lecithin is found to be slow, far below the molecular level, which was measured as reference for solutions of the three lipoids in deuterated methanol below the critical micelle concentration [20]. Taking these results together, on the one hand the fast diffusion of water and oil and on the other the one order of magnitude lower surfactant diffusion, a bicontinuous microemulsion can be concluded [22].

Calculating relative or normalized diffusion coefficients D_{rel}, the microstructure of the three formulations can be further evaluated. They are given by the fractions D/D_0, thereby D_0 being the diffusion coefficient of the neat and D of the component within the microemulsion, respectively. For isopropylmyristate D_{rel} with 0.54 to 0.51 in the three formulations seems to be suitable for layered structures. With a hindered diffusion in one and free in the two other directions D_{rel} should be 2/3 in an ideal layering. The observed values are somewhat reduced for IPM, but very much lowered for water with D_{rel} 0.16 for formulation A to 0.12 for C. Thus other factors than microstructure have to be taken into account resulting in a pronounced reduction of the diffusion coefficients, namely solvation and hydration. Both become very significant at high surfactant concentrations [21]. In addition the investigated microemulsions contain high amounts of the co-surfactant isopropanol, defining together with water the polar domain. The high alcohol content decreases the polarity of the polar medium significantly. With a D_{rel} around 0.41 the diffusion is significantly different compared to pure IP, so it seems be incorporated to a certain extend in the lipid layers. The interactions of water with the surfactant mixture, as for hydration, the strong polar headgroup coupling to the phospholipids, and as for the cosurfactant, the high solubility of water in isopropanol, can be summarized as an explanation for the rather low observed diffusion of water in the microemulsion systems.

The drug loaded formulations were also investigated by NMR self-diffusion experiments. They contain 1 % (w/w) of flufenamic acid, flumethasone pivalate, flutamide, or fluconazole in the same above described carrier systems. As can be seen from the diffusion data of drug loaded microemulsion A (Table 5), all diffusion coefficients are reduced, most pronounced for the isopropylmyristate. The same reduction is found in a similar manner for the two other formulations B and C. This observation may be explained by small irregularities in the structure induced by the addition of the drugs, thereby reducing the D values significantly [22]. D_{rel} = 0.34 for IPM in the drug loaded versus 0.54 in the unloaded system would be consistent with proposed models in the literature, e.g. a changeover from a layered to a more tubular type bicontinuous microemulsion [21].

Table 5 NMR Self-diffusion coefficients for the individual components in the drug loaded microemulsion A

	Flufenamic acid		Flumethasone pivalate		Flutamide		Fluconazole	
	D [m^2s^{-1}]	D/D_0	D [m^2s^{-1}]	D/D_0	D [m^2s^{-1}]	D/D_0	D [m^2s^{-1}]	D/D_0
Water	$2.00x10^{-10}$	0.11	$2.10x10^{-10}$	0.11	$2.04x10^{-10}$	0.11	$2.08x10^{-10}$	0.11
IP	$1.50x10^{-10}$	0.28	$1.51x10^{-10}$	0.28	$1.54x10^{-10}$	0.29	$1.51x10^{-10}$	0.28
IPM	$7.20x10^{-11}$	0.34	$7.26x10^{-11}$	0.34	$7.39x10^{-11}$	0.35	$7.24x10^{-11}$	0.34
PC	$1.65x10^{-11}$		$1.80x10^{-11}$		$1.76x10^{-11}$		$1.71x10^{-11}$	

The last missing parameter is the diffusion of the drugs in the vehicles. In this case, ^{1}H NMR self-diffusion experiments are very unfavourable, as the concentration of the drug is only 1% w/w, resulting in dynamic range problems of the receiver and poor signal to noise ratios for the drug signals. Nevertheless it has been successfully demonstrated recently for steroid hormones in eucalyptus oil based microemulsions [23]. In the present study, fluorine containing compounds were chosen having with the ^{19}F isotope a very sensitive NMR probe suitable for self-diffusion studies. This approach is very promising, as already about 20% of the pharmaceuticals on the market contain fluorine [24]. ^{19}F NMR alleviates most of the problems encountered with ^{1}H observation, e.g. signal overlap or dynamic range problems. Additionally, the ^{19}F nucleus has 100% natural abundance and a magnetogyric ratio almost like ^{1}H. Performing stimulated echo experiments on the fluorine frequency by altering the gradient amplitude results in signal decays with a high signal to noise ratio, very well suitable for fitting the self-diffusion coefficients (Fig. 3). Flufenamic acid and flutamide exhibit a CF_3 group with a chemical shift of -64.68 ppm and -62.10 ppm, respectively. The two other compounds contain two fluorine atoms, both can be used for the diffusion studies. Fluconazole has a difluoro-substituted aromatic ring (-109.52 ppm, -112.66 ppm), flumethasone pivalate has two fluorines in the steroidal positions 6 and 8 (-189.01 ppm, -166.19 ppm).

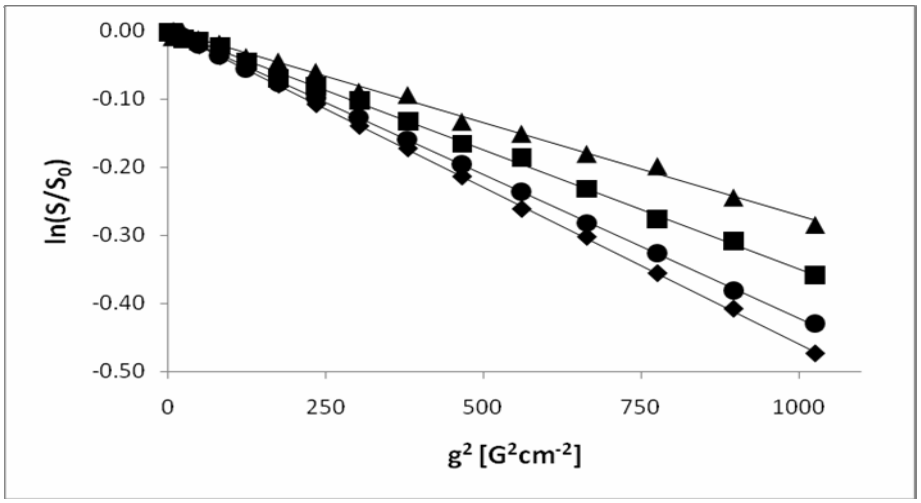


Figure 3 Experimental echo decay for the fluorinated drugs in microemulsion A as measured by ^{19}F diffusion NMR (▲, flumethasone pivalate; ■, fluconazole; ● flufenamic acid; ◆, flutamide)

The ^{19}F NMR derived self-diffusion coefficients in the three different microemulsions are given in Table 6, together with values measured in 0.1% w/w solutions in deuterated methanol as reference.

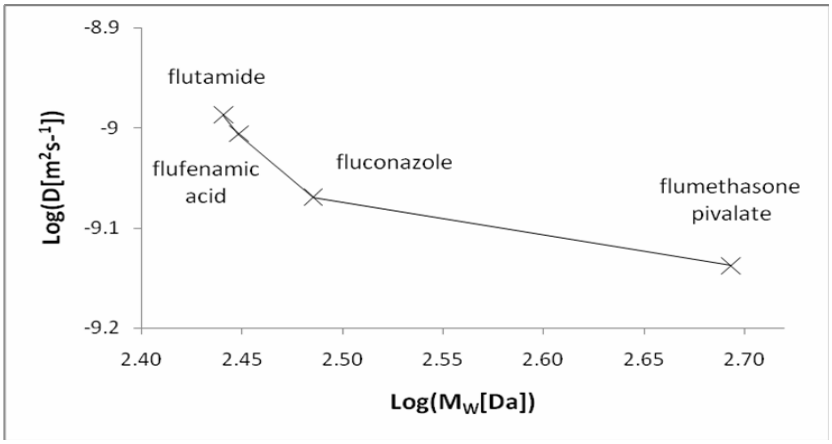
Table 6 ^{19}F NMR Self-diffusion coefficients of the drugs in the three microemulsions and in 0.1% (w/w) solution of MeOH-d_4

	Flufenamic acid D [m^2s^{-1}]	Flumethasone pivalate D [m^2s^{-1}]	Flutamide D [m^2s^{-1}]	Fluconazole D [m^2s^{-1}]
A	$6.65x10^{-11}$	$4.26x10^{-11}$	$7.23x10^{-11}$	$5.51x10^{-11}$
B	$6.59x10^{-11}$	$3.88x10^{-11}$	$7.22x10^{-11}$	$5.46x10^{-11}$
C	$6.79x10^{-11}$	$2.20x10^{-11}$	$7.16x10^{-11}$	$4.99x10^{-11}$
MeOH-d_4	$9.86x10^{-10}$	$7.28x10^{-10}$	$1.03x10^{-9}$	$8.52x10^{-10}$

In Figure 4A the diffusion of the four drugs in methanol is plotted against their molecular weight. For ideal spherical molecules a reciprocal cube-root dependence of the diffusion coefficient on molecular weight is expected at infinite low concentration [26]. Obviously Fig. 4A displays deviations from the proposed model. The diffusion of fluconazole seems to be slowed down compared to the three other drugs. If the reason is a non-spherical shape, this

behaviour would be more expected for flumethasone pivalate due to the extended steroide skeleton. Another explanation for the reduced D value of flucanozole could be some kind of molecular association. As for the drug loaded microemulsions, the diffusion of the fluorinated compounds in the three vehicles is significantly lowered by one order of magnitude compared to the molecular diffusion. The derived D values of the drugs are in the diffusion range between isopropylmyristate and lecithin. The line width of ^{19}F NMR signals in the formulations is only slightly increased relative to in methanol, upmost by a factor of 1.5 in the case of flumethasone pivalate. So the rotational correlation time of the drugs seems to be nearly unaffected in the microemulsions, but the translational motion is spatially hindered in the bicontinuos structural network formed by the lecithins. The individual picture within the drug series looks similar compared to the methanolic solutions (Fig. 4B), although on another scale. Again, the diffusion of fluconazole is lowered relative to the three others. But the most pronounced absolute reduction of the diffusion coefficient can be seen for flumethasone pivalate in the phosphatidylethanolamine enriched vehicles. For this spatially enlarged structure the molecular displacement becomes more hindered in microemulsion B and especially in C, which would be consistent with a shift in microstructure to a more tubular type.

A



B

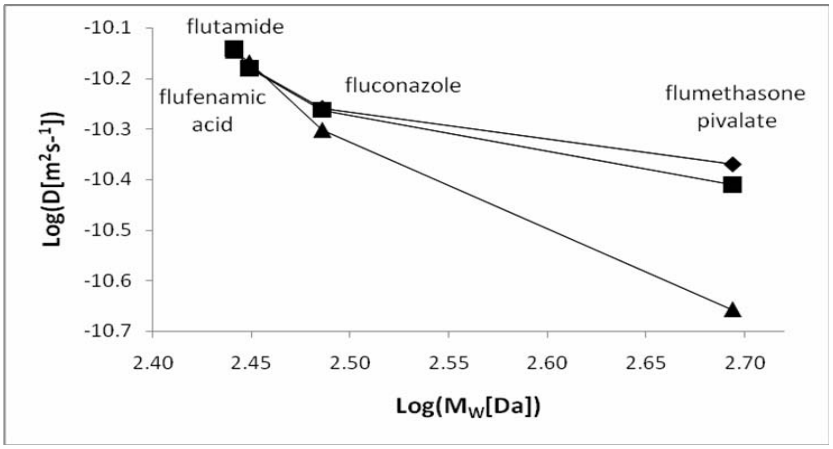


Figure 4 A, B

A: ^{19}F NMR self-diffusion of the fluorinated drugs in deuterated methanol as a function of the molecular weight (M_W)

B: ^{19}F NMR self-diffusion of the fluorinated drugs in the three microemulsions (◆, A; ■, B; ▲, C) as a function of the molecular weight (M_W)

Overall, the incorporation of the drugs in the surfactant structure of the microemulsions as proven by ^{19}F NMR self-diffusion studies can be an explanation for their good skin permeation within these lecithin based formulations, which will be described in the next part.

3.4 In vitro permeation studies

Permeation studies were performed using microemulsion systems consisting of different commercially available lecithin surfactants (Table 1) in order to evaluate the cumulative amount permeated of selected fluorinated drugs. Lecithins can influence the permeability of the skin due to their high affinity for epidermal tissue. They are able to mix with lipid components of skin and induce a change in skin lipid fluidity, thus leading to an enhanced percutaneous absorption of drugs [2]. In addition, the lecithin-mediated improvement of percutaneous drug absorption can also be elicited by the increased hydration of the stratum corneum, determined by various lecithin-based formulations [25]. The

cumulative amounts permeated of the incorporated drugs after 8 h of diffusion time are presented in Table 7. The results show good skin permeation for all tested formulations. Drug release appears to be more dependent on the structure and interactions of the incorporated drugs than on the lecithin composition, as confirmed by the ^{19}F NMR self-diffusion data (Table 6). Though, the skin permeation rate seems to be slightly dependent on the lecithin composition, but no significance was calculated (Table 7). This might be due to hydrophobic interactions between skin and phosphatidylethanolamine, which is in good agreement with already published data [9]. Though, it was not possible to find a direct correlation between the cumulative amount permeated and the self-diffusion coefficient of the drugs in the vehicle.

Table 7 Comparison of the cumulative drug amount (µg/cm²) of the fluorinated drugs permeated after 8 h of diffusion time through porcine skin, n=4

	Cumulative drug amount permeated [µg/cm²]			
Microemulsion	Flufenamic acid	Flumethasone pivalate	Flutamide	Fluconazole
A	9.58±6.48	16.78±4.31	2.22±0.84	334.06±40.29
B	31.17±8.15	29.29±9.00	3.46±0.41	548.3±113.16
C	27.47±6.37	14.80±2.46	2.70±0.46	299.54±37.05

4. Conclusion

These studies demonstrated a direct relationship between droplet size and NMR self-diffusion coefficients of the individual components going from microemulsion A over B to C. An increase of LPC, PE and LPE in the phospholipid mixtures increased the droplet sizes and PDI. The NMR self-diffusion coefficients indicated a bicontinuous structure. In addition, the chemical structure of the incorporated drugs had an impact on the diffusion in the microemulsions and an influence on skin diffusion. However, a direct correlation was not seen. Due to the slow diffusion of the drugs, as obtained by

^{19}F NMR diffusion studies, they were incorporated in lipid bilayers. The insight in the physicochemical structure is of great importance to understand the complex interactions within the vehicle as well as between skin and vehicle.

5. References

1 Paul BK, Moulik SP. 2001. Uses and applications of microemulsions. Curr Sci **80**: 990-1001.

2 Paolino D, Ventura CA, Nistico S, Puglisi G, FrestaPaolino M. 2002. Lecithin microemulsions for the topical administration of ketoprofen: percutaneous adsorption through human skin and in vivo skin tolerability. Int J Pharm **244**: 21-31.

3 Spernath A, Aserin A, Garti N. 2006. Fully dilutable microemulsions embedded with phospholipids and stabilized by short-chain organic acids and polyols. J Colloid and Interface Sci **299**: 900-909.

4 Fiume Z. 2001. Final report on the safety assessment of lecithin and hydrogenated lecithin. Int J Toxicology **20**: 21-45.

5 Shinoda K, Kaneko T. 1989. Characteristic properties of lecithin. J Dispersion Sci Technol **9**: 555-559.

6 Aboofazeli R, Lawrence MJ, 1995. Investigation into the formation and characterization of phospholipid microemulsions. IV. Pseudo-ternary phase diagrams of systems containing water-lecithin-oil and co-surfactant. Int J Pharm **125**: 107-116.

7 Fa N, Babak VG, Stebe M, 2004. The release of caffeine from hydrogenated and fluorinated gel emulsions and cubic phases. Colloids Surf A **243**:117-125.

8 Spernath A, Aserin A, Ziserman L, Danino D, Garti N. 2007. Phosphatidylcholine embedded microemulsions: Physical properties and improved caco-2 cell permeability. J Contr Rel **119**: 279-290.

9 Hoeller S, Klang V, Valenta C. 2008. Skin compatible lecithin drug delivery systems for fluconazole: effect of phosphatidylethanolamine and oleic acid on skin permeation. J Pharm Pharmacol **60(5)**: 587-91.

10 Wu DH, Chen AD, Johnson CS. 1995. An improved diffusion-ordered spectroscopy experiment incorporating bipolar-gradient pulses. J Magn Reson Series A **115**: 260-264.

11 Esturau N, Sanchez-Ferrando F, Gavin JA, Roumestand C, Delsuc MA,

Parella T. 2001. The use of sample rotation for minimizing convection effects in self-diffusion NMR measurements. J Magn Reson **153** :48-55.

12 Kählig H, Valenta C, Dampfhart U, Auner B.G. 2005. Rheology and NMR self- diffusion experiments as well as skin permeation of diclofenac-sodium and cyproterone acetate of new gel preparations. J Pharm Sci **94**: 288-296.

13 Hoeller S, Valenta C. 2007. Effect of selected fluorinated drugs in a "ringing" gel on Rheological behaviour and skin permeation. Eur J Pharm Biopharm **66**: 120-126

14 Park KM, Lee MK, Hwang KJ, Kim CK. 1999. Phospholipid-based microemulsions of flurbiprofen by the spontaneous emulsification process. Int J Pharm **183(2)**: 145-154.

15 Krause SA. 2001.Entwicklung und Charakterisierung von Mikroemulsionen zur dermalen Applikation von Arzneistoffen, Martin-Luther Universität Halle Wittenberg

16 Chen H. Chang X, Du D, Li J, Xu H, Yang X. 2006. Microemulsion-based hydrogel formulation of ibuprofen for topical delivery. Int J Pharm **315**: 52-58.

17 Yilmaz E, Borchert HH. 2005. Design of a phytosphingosine-containing, positively-charge nanoemulsion as a colloidal carrier system for dermal application of ceramides. Eur J Pharm Biopharm **60:** 91-98.

18 Bosco M, Culeddu N, Toffanin R, Pollesello P. 1997. Organic solvent systems for ^{31}P Nuclear Magnetic Resonance analysis of lecithin phospholipids: applications to two-dimensional gradient-enhanced ^{1}H-detected heteronuclear multiple quantum coherence experiments. Anal Biochem **245**: 38-47.

19 Nilsson PG, Lindman B. 1983. Water self-diffusion in nonionic surfactant solutions: Hydration and obstruction effects. J Phys Chem **87**: 4756-4761.

20 Rizwanul H, Tinsley IJ, Schmedding D. 1971. Nuclear magnetic resonance studies of phospholipid micelles. J Bio Chem **247:** 157-161.

21 Lindman B, Olsson U, Söderman O. 1999. Characterization of microemulsions by NMR. In: Kumar P. Mittal KL, editors. Handbook of

Microemulsion Science and technology.New York:Marcel Decker pp 309-356.

22 Shinoda K, Araki M, Sadaghiani A, Khan A, Lindman B. 1991. Lecithin-based microemulsions: phase behaviour and microstructure. J Phys Chem **95:** 989-993.

23 Biruss B, Kählig H, Valenta C. 2007. Evaluation of an eucalyptus oil containing topical drug delivery system for selected steroid hormones. Int J Pharm **328(2)**: 142-51.

24 Thayer MA, 2006. Fabulous fluorine. Chem Eng News **84(23)**: 15-24.

25 Valenta C, Wanka M, Heidlas J. 2002. Evaluation of novel soya-lecithin formulations for dermal use containing ketoprofen as a model drug. J Contr Rel **63:**165-173.

26 Waldeck AR, Kuchel PW, Lennon AJ, Chapman BE. 1997. NMR diffusion measurements to characterise membrane transport and solute binding. Prog Nucl Magn Reson **30**: 39-68.

4.5 Interactions of the anti-inflammatory phytosphingosine and N-palmitolyethanolamine with DPPC model membranes

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Abstract

The effect of the anti-inflammatory components phytosphingosine (PS) and N-palmitoylethanolamine (PEA) on the phase transition and phase properties of 1,2- diplamitoylphosphatidylcholine (DPPC) as model membrane has been investigated. The technique employed to monitor these processes was micro-differential scanning calorimetry (microDSC) that appears to be particularly suitable to follow interactions of active agents with biomembrane models. The formulations have been characterised by means of photon-correlation-spectroscopy after swelling the liposomes with different molar ratios of PS and PEA. The mean particle size and polydispersity index (PDI) increased by increasing the concentration of PS and PEA, respectively. Moreover, both compounds studied altered the main transition temperature of the gel to liquid-crystalline phase transition and the enthalpy values in the same manner. A slight increase of the main phase transition and enthalpies could be observed indicating the perturbation of the membrane structure and fluidity. Overall, the results obtained give indications of interactions with the model membrane. Since skin is also a complex bilayer system, these interactions might be important for the use of these naturally occurring and anti-inflammatory ingredients in drug delivery systems.

Keywords: DPPC-liposomes, Phytosphingosine, N-palmitoylethanolamine, DSC

1. Introduction

Dipalmitoylphosphatidylcholine (DPPC) is a typical phospholipid with regard to its role in determining the physical-chemical and biological properties of cellular membranes. When dispersed in water multilamellar liposomes are spontaneously formed having a similarity to biological membranes and are therefore used very often as such simple model membranes (Gardikis et al 2006; Zhao et al 2007).

The formed phases from these model membranes with increasing temperature have already been described (El Maghraby et al 2005; Gardikis et al 2006; Panicker 2008). The main transition from gel-to-liquid crystalline (Pß´→Lα) phases is of physiological importance, as in physiological conditions the lipids are in their liquid crystalline state in most of the bio-membranes. Therefore any alterations in these properties are indicative of the perturbation of the membrane structure and fluidity. The interaction of the lipid with exogenous molecules in the bilayer, depends on its chemical-physical behaviour and concentration (Panicker 2008).

The free sphingoid base phytosphingosine is naturally found in the human body and is present at high levels in the stratum corneum. In recently performed studies PS had anti-microbial properties against probionibacterium acnes and staphylococcus aureus and therefore effectively reduced the signs of acne. Furthermore, additional studies confirmed that PS reduced redness and inflamed skin (Yilmaz & Borchert 2005; Yilmaz & Borchert 2006; Kang et al 2007). Additionally we documented a positive effect on the skin permeation of fludrocortisone acetate in nanoemulsion formulations (Hoeller & Valenta 2008; Hoeller & Valenta 2008). N-palmitoylethanolamine (PEA) is also a naturally occurring lipid found in the human body and isolated from soybean lecithins and egg yolk (Lambert et al 2002). Recently cannabinoid receptors have been identified on cutaneous sensory nerve fibers binding to endogenous cannabinoids such as N-palmitoylethanolamine offering a rational therapeutic option for treatment of pruritus (Ständer et al 2006). In a small clinical study (22 patients) it has been proven that a cream containing PEA was successful in treatment of chronical itch (Ständer et al 2006). Moreover another big clinical

study (2456 patients) showed substantial relief of objective and subjective symptoms of atopic eczema after regular skin care with a PEA cream (Eberlein et al 2007). PEA is also of considerable interest because of its properties as anti-inflammatory and anti-viral agent (Swamy et al 2003).

Phytosphingosine and N-palmitoylethanolamine both are anti-inflammatory ingredients with positive effects on skin. Other anti-inflammatory substances such as non-steroidal anti-inflammatory drugs have been found to show a complex interaction with DPPC liposomes inducing perturbations in the lipid-crystalline phase suggesting a major change in the hydration behaviour of DPPC (Lucio et al 2008). In the current study micro-differential scanning calorimetry measurements (microDSC) were applied for the thermoanalysis of the phase behaviour of DPPC liposomes and the ability of the naturally occurring anti-inflammatory phytosphingosine (PS) and N-palmitoylethanolamine (PEA) to bind or to penetrate into layers of the surface active phospholipids.

2. Materials and Methods

2.1. Materials

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) was purchased from Lipoid (Switzerland). The product was Lipoid PC 16:0/16:0. According to its specification the content of phosphatidylcholine related to the dry weight was at least 99 %. Phytosphingosine (PS) was kindly provided by Degussa (Cosmoferm BV, NL) and N-palmitoylethanolamine (PEA) was obtained from Sanova Pharma GesmbH (Austria). All other chemicals used in this study were of analytical reagent grade and were used as received without any further purification.

The chemical structures of phytosphingosine and N-palmitoylethanolamine are presented in Fig. 1.

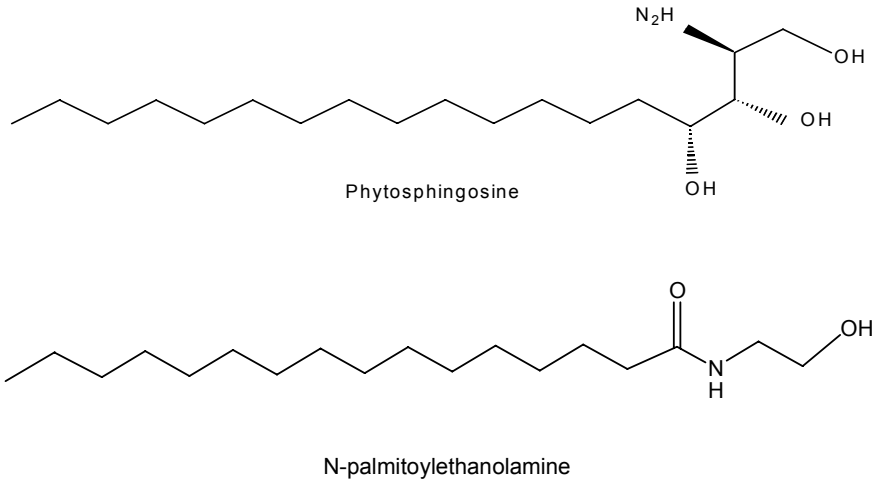


Fig. 1 Chemical structure of phytosphingosine and N-palmitoylethanolamine

2.2. Preparation of Liposomes

DPPC liposomes were prepared according to a previous described method (Thompson et al 2006). In brief, the phospholipid powders were dispersed in deionised water at 60°C and magnetically stirred until the powder was completely dissolved and thoroughly mixed using an ultra-Turrax (Omni 500) to produce a dispersion with a final phospholipid concentration of 2,5 % (w/w). Afterwards the dispersion was homogenised with a high-pressure homogeniser (EmulsiFlex-C3, Avestin) for sixteen times at ~ 1100 bar.
For interaction studies, phytosphingosine and N-palmitoylethanolamine (from 2, 5 to 10 mol%) were incorporated, respectively, in the liposome structure by adding to the liposome dispersion and mechanical swelling for 24 h at 60°C.

2.3 Particle size measurement of liposomes

The mean particle size and size distribution were determined by photon correlation spectroscopy with a Zetasizer Nano ZS (Malvern, UK) at 25°C. The liposomes dispersion was diluted to the appropriate concentration with deionised water after 24 h of preparation. The size distribution was represented

by the polydispersity index (PDI) values. The measurements were performed using a He-Ne laser at 633 nm.

2.4. Micro-Differential scanning calorimetry (microDSC) studies

Thermal scans were performed using a Setaram III micro-calorimetry (n=3 per liposome preparation). 0.45 ml of the liposomal dispersion containing an average of 11.25 mg DPPC was sealed in a batch cell. The samples were analysed by heating at a scanning rate of 1 °C/min over the temperature range 15-65°C, using deionised water as reference.

2.5. Data analysis

Results are expressed as the means of at least three parallel experiments (± S.D.). Thermal transitions were analysed using Setsoft 2000 software. After baseline subtraction, raw power data were converted to molar heat capacity data. Baselines were fitted to the pre-transition and main transition regions using a linear baseline function so that transition temperatures and enthalpies of reaction could be calculated for each lipid concentration.

3. Results and Discussion

New anti-inflammatory drugs for safe dermal applications are of great interest, because the number of people with atopic eczema and intensely pruritic deseases are increasing. Phytosphingosine and N-palmitoylethanolamine might be promising candidates for safe application. In case of PS beside the anti-inflammative an additional penetration enhancing effect on fludrocortisone acetate skin permeation was measured (Hoeller & Valenta 2008). Therefore, this multifunctional PS could have the benefit of reducing topical corticosteroids and acting himself anti-inflammatory. However, the mechanism of action is not clearly understood. PEA, another anti-inflammatory agent has already been included in one atopic product and has been tested to reduce symptoms of atopic eczema after regular skin care (Eberlein et al 2007).

Therefore, the question was in which manner the two compounds interacts with the model membrane in order to obtain a better understanding of the mechanism of action.

DPPC liposomes were prepared with increasing PS and PEA content and their size and size distribution was monitored. The results of the mean particle sizes are presented in Table 1 for the incorporation of phytosphingosine and for N-palmitoylethanolamine in Table 2. As seen mean particle size of the liposomes and PDI-values increased with increasing concentration of PS and PEA suggesting that the molecules are adsorbing on the surface of the particles.

Table 1 The effect of incorporation of phytosphingosine into DPPC liposomes on mean particle size, polydispersity index (PDI) and pH-values.

Mol% PS	Particle size (d.nm)	PDI	pH
0	81 ± 6	0.24-0.25	6.3 ±0.2
2,5	152 ± 7	0.29-0.41	8.4 ±0.0
5	166 ±10	0.26-0.48	8.3 ±0.1
10	178 ±7	0.34-0.50	8.6 ±0.0

Table 2 The effect of N-palmitoylethanolamine into DPPC liposomes on mean particle size, polydispersity index (PDI) and pH-values

Mol% PEA	Particle size (d.nm)	PDI	pH
0	81 ±6	0.24-0.25	6.3 ±0.2
2,5	86 ±2	0.23-0.20	7.3 ±0.2
5	90 ±4	0.26-0.33	7.5 ±0.0
10	93 ±4	0.31-0.36	7.3 ±0.0

Representative micro-DSC thermograms of DPPC liposomes containing various concentrations of PS and PEA are shown in Fig. 2 a-b. Furthermore effects of PS and PEA on pre- and main transition temperatures and enthalpies of reaction are presented in Table 3 and Table 4. The changes of the enthalpy are depicted in Fig. 3.

Table 3 Transition temperature and enthalpy values of DPPC liposomes containing phytosphingosine (PS).

Mol %	T_{max} (°C)	Linear Onset (°C)	Enthalpy (J/M)
0	34.4 ±0.3 pre-transition	33.5 ±0.6 pre-transition	30 ±1 pre-transition
0	41.4 ±0.1 main transition	40.1 ±0.1 main transition	30600 ±430main transition
2,5	41.7 ±0.1	39.8 ±0.0	44000 ±2000
5	41.8 ±0.0	40.3 ±0.3	40000 ±15000
10	42.4 ±0.1	40.0 ±0.2	88000 ±16000

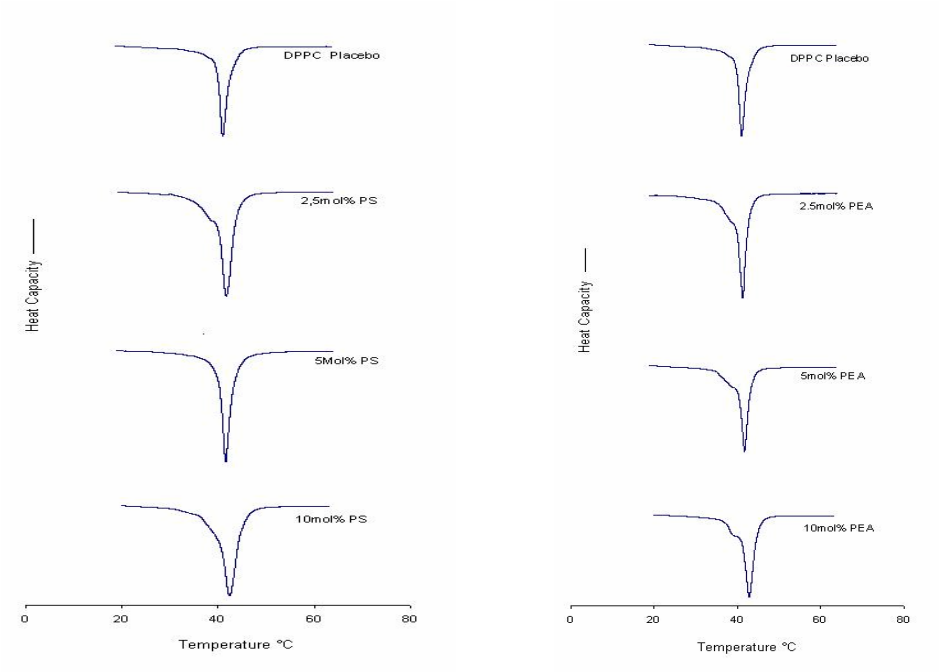
Table 4 Transition temperature and enthalpy values of DPPC liposomes containing N-palmitoylethanolamine (PEA).

Mol %	T_{max} (°C)	Linear Onset (°C)	Enthalpy (J/M)
0	34.4 ±0.3 pre-transition	33.5 ±0.6 pre-transition	30 ±1 pre-transition
0	41.4 ±0.1main transition	40.1 ±0.1 main transition	30600 ±430main transition
2,5	42.0 ±0.0	40.0 ±0.1	40700 ±1700
5	42.0 ±0.2	40.2 ±0.8	45200 ±1080
10	43.0 ±0.0	41.0 ±0.2	63000 ±2400

Pure DPPC liposomes display the well-known sharp main transition near 41.4 ±0.05 °C, which belongs to the gel-to-liquid crystalline (Pß´→Lα) phase transition and a smaller pre-transition, which appears near 34.4 ±0.25°C reflecting the lamellar gel to rippled gel (Lß´ →Pß´) transition in the gel phase. These data are in good agreement with literature values (Wolka et al 2004; Auner et al 2005; El Maghraby et al 2005; Krivanek et al 2008; Panicker 2008). The pre-transition have been generally attributed on the surface structure of the membrane and is related to rotations of the phospholipid head groups or transformation in the lamellar structure and changes in the hydrocarbon chain packing (Auner et al 2005). The main transition reflected the acyl chain "melting", trans-gauche isomerisation took place in the acyl chain conformation of lipid molecules, effecting increasing the fluidity of the phospholipid bilayer (Wolka et al 2004; El Maghraby et al 2005).

Incorporation of the two lipophilic compounds phytosphingosine (LogP 5.18)

and N-palmitoylethanolamine (LogP 5.82) are expected to preferentially partition into the lipid domains of the bilayer structure. The pre-transition disappeared with addition of PS and PEA for all tested concentrations (Fig. 2 a-b).



a) b)

Fig. 2a-b Representative DSC scans of DPPC liposomes containing 0-10 mol% PS (a) and 0-10 mol% PEA (b)

The disappearance of the pre-transition suggests that the compounds interacted with the polar region of the phospholipid bilayer and interfered with the tilting of phospholipid acyl chains (Auner et al 2005; Panicker 2008). The main endotherm transition peak was increased with increasing molecular ratio of PS and PEA, respectively, and the peak becoming broader. The slight increase in the main transition peak suggests an interaction with the surface of the phospholipids. The results obtained could also be confirmed by measurements using ultrasonic velocity (data not shown). PS and PEA probably were able to fit into the bilayer with their head level with the head groups and the aliphatic chain parallel with the acyl chains of the phospholipid. Therefore, they were able to add bulkiness to the acyl chains and thus compensated for the bulkiness of the head groups. Thus chains will no longer tilt which can explain the absence of the pre-transition. This may add more structure to the

bilayer and can explain the increase T_m and the apparent increase in the enthalpy of the main transition seen in Fig. 3 (El Maghraby et al 2005). Main and pre-transition enthalpies in control liposomes were 30.7 KJ/mol and 0.03 KJ/mol, respectively, which is in good agreement with literature data (Wolka et al 2004; Auner et al 2005). An additional transition was evident incorporating PEA into DPPC liposomes. The presence of two separate peaks suggests different modes of interaction of PEA with DPPC resulting in the formation of two-phase region of enhancer-phospholipid complex (Wolka et al 2004; Krivanek et al 2008).

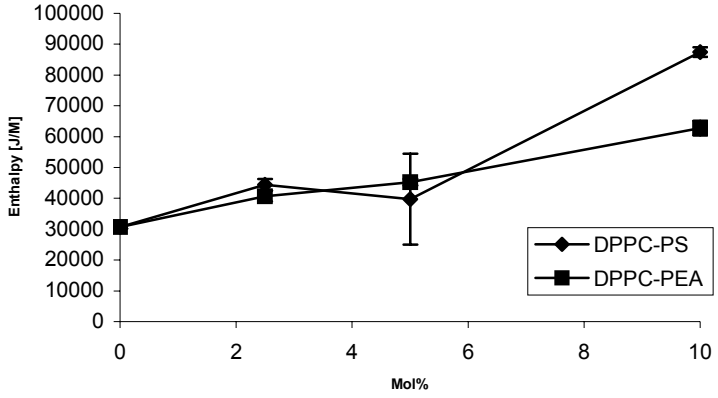


Fig. 3 Effect of phytosphingosine (♦) and N-palmitoylethanolamine (■) on the transition enthalpies of DPPC liposomes.

4. Conclusion

The anti-inflammatory PS and PEA interacts with the lipid bilayers and induce variations in the temperature associated with the lipid main phase transition. Results indicate hydrophobic interactions in the liposome matrix and at the particle surface. Therefore, the observed effects on the model membrane may be part on the mechanism by which PS attenuate the hydrophobic properties of the stratum corneum lipids with the consequence increase in the skin diffusion. Consequently, the anti-inflammatory PS and PEA might be promising additives in drug delivery systems on skin.

5. References

Auner, B.G., O´ Neill, M.A.A., Valenta, C., Hadgraft, J. (2005) Interaction of phloretin and 6-ketocholestanol with DPPC-liposomes as phospholipid model membranes. Int. J. Pharm. **294**: 149-155.

Eberlein, B., Eicke, C., Reinhardt, H-W., Ring, J. (2007) Adjuvant treatment of atopic eczema: assessment of an emollient containing N-palmitoylethanolamine (ATOPA Study). J. Eur. Academy Derm. Ven. **22**: 73-82.

El Maghraby, G. M. M., Campbell, M., Finnin, B. C. (2005) Mechanisms of action of novel skin penetration enhancers: Phospholipid versus skin lipid liposomes. Int. J. Pharm. **305**: 90-104.

Gardikis, K., Hatziantoniou, S., Viras, K., Wagner, M., Demetzos, C. (2006) A DSC and Raman spectroscopy study on the effect of PAMAM dendrimer on DPPC model lipid membranes. Int. J. Pharm **318(1-2)**: 118-123.

Hoeller, S., Valenta, C. (2008) Poster: Positively charged nanoemulsions for enhanced topical delivery of fludrocortisone-acetate. 9th skin forum meeting, London, UK, http://www.skin-forum.eu/gallery.html.

Hoeller, S., Valenta, C. (2008) Poster: Nanoemulsion based on lecithin and sugar surfactant: Effect of Phytosphingosine on in vitro permeation of fludrocortisone-acetate. 6th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Barcelona

Kang, B.Y., Kim, S.J., Kim H.G., Hwang, J.S.,Kang, H.H. (2007) External preparation containing phytosphingosine as active component for skin. Application: KR
KR, (Amorepacific Corp., S. Korea). 21pp.

Krivanek, R., Okoro, L., Winter, R. (2008) Effect of cholesterol and ergosterol on

the compressibility and volume fluctuations of phospholipid-sterol bilayers in the critical point region: a molecular acoustic and calorimetric study. Biophysical Journal **94**: 3538-3548.

Lambert, D.M., Vandevoorde, S., Jonsson, K-O., Fowler, C.J. (2002) The palmitoylethanolamide family: A new class of anti-inflammatory agents?. Curr. Med. Chem. **9(6)**: 663-674.

Lucio, M., Bringezu, F., Reis, S., Lima, J.L.F.C., Brezesinski, G. (2008) Binding of nonsteriodal anti-inflammatory drugs to DPPC: Structure and Thermodynamic Aspects. Langmuir **24**: 4132-4139.

Panicker L. (2008) Interaction of propyl paraben with dipalmitoyl phosphatidylcholine bilayer: A differential scanning calorimetry and nuclear resonance study. Colloids and Surfaces B: Biointerfaces **61**: 145-152.

Ständer, S., Reinhardt, H.W., Luger, T.A. (2006) Topische Cannabinoidagonisten: Eine effektive, neue Möglichkeit zur Behandlung von chronischen Pruritus. Hautarzt **57:** 801-807.

Swamy, M. J., Ramakrishnan, M., Marsh, D., Wurz, U. (2003) Miscibility and phase behaviour of binary mixtures of N-palmitoylethanolamine and dipalmitoylphosphatidylcholine. Biochim. Biophys. Acta, Biomembr. **1616(2)**:174-183.

Thompson, A.K., Hindmarsh, J.P., Haisman, D., Rades, T., Singh, H. (2006) Comparison of the structure and properties of liposomes prepared from milk fat globule membrane and soy phospholipid. J. Agric. Food Chem. **54**: 3704-3711.

Wolka A.M., Rytting J.H., Reed, B.L., Finnin, B.C. (2004) the interaction of the penetration enhancer DDAIP with a phospholipid model membrane. Int. J. Pharm. **271**: 5-10.

Yilmaz, E., Borchert, H-H. (2005) Design of a phytosphingosine-containing, positively-charged nanoemulsion as a colloidal carrier system for dermal application of ceramides. Eur. J. Pharm. Biopharm. **60(1)**: 91-98.

Yilmaz, E., Borchert, H-H. (2006) Effect of lipid-containing, positively charged nanoemulsions on skin hydration, elasticity and erythema-An in vivo study. Int. J. Pharm. **307(2)**: 232-238.

Zhao L., Feng S-S., Kocherginsky N., Kostetski I. (2007) DSC and EPR investigations on effects of cholesterol component on molecular interactions between paclitaxel and phospholipid within lipid bilayer membrane. Int. J. Pharm. **338(1-2)**: 258-266.

5 Conclusion

In the first part of this work changes in liquid crystalline microstructure of a transparent “ringing” gel have been investigated by incorporating different selected fluorinated drugs. The rheological properties and inner structure of cubic liquid crystal change hugely depended on temperature as well as on chemical structure of the model drug. Furthermore, the viscoelastic properties were dependent on the chemical structure of the drugs and can probably be related to the microstructure of the cubic system. Beside, the cubic gel showed excellent release profiles for the incorporated drugs promising a new candidate for drug delivery systems. Depending on the physiochemical properties of the drug, it may reside in the lipid bilayer or the aqueous channels of the gel and the location of the drug in the gel may influence its release [20]. However, also the mechanism by which cubic gels enhance the release and percutaneous absorption has to been taken into consideration. Therefore the combined effect of both the lipophilic and hydrophilic domains may be responsible for its enhancing activity over other common vehicles [55]. But not only the excellent vehicle properties of the cubic gel might be an advantage, also the high chemical stability of the incorporated drugs favour such colloidal systems as drug delivery vehicles. High chemical stability could be obtained over an observation period of 6 months (data shown for 10 weeks).

In the second part of the work it was possible to create skin-compatible lecithin microemulsions. The phase behaviour of the microemulsions was influenced by the composition of the different soybean lecithins, particularly regarding the isotropic areas. The components phosphatidylethanolamine and lysophosphatidylcholine may be responsible for this probably by influencing the effective critical packing parameter in a different manner [23]. The biggest area achieved the microemulsion containing lipoid S75. Beside, the study demonstrated an impact on the release profiles of the model drug fluconazole using different commercially available soybean lecithins. The phosphatidylethanolamine content seems to play a major role in increasing the skin permeability, probably by inducing hydrophobic interactions between the

skin and the outwardly facing acyl chains of the inverted hexagonal phase adapted by PE [56].

Moreover, the penetration enhancing effect of oleic acid and isopropyl myristate was analysed in different microemulsion systems. Oleic acid exhibited higher skin permeation profiles compared to IPM independent of the hydrophilicity of the lecithin based microemulsions. These results seem compatible with the main mechanism suggested for the action of oleic acid as a permeation enhancer increasing the fluidity of the skin lipids and therefore increasing stratum corneum permeability [47]. Additionally, it has been shown that oleic acid is more effective with highly hydrophilic compounds, which could be confirmed in our studies. The penetration enhancing effect of oleic acid was more pronounced in the hydrophilic microemulsion system.

The insight in the physicochemical structure is of great importance to understand the complex interactions within the vehicle as well as between skin and vehicle. Therefore, in a next step the influence of different commercially available soybean lecithins on the microstructure of the colloidal systems could be demonstrated using different techniques. An increase of LPC, PE and LPE in the phospholipid mixtures increased the particle sizes and PDI. Moreover, a direct relationship between droplet size and NMR self-diffusion coefficients of the individual components within the different microemulsion systems could be demonstrated. The NMR self-diffusion coefficients indicated a bicontinuous structure. Furthermore, reasonable permeation of the incorporated lipophilic drugs of all microemulsions systems was confirmed in standard diffusion studies. This could be due to the incorporation of the drugs in the surfactant structure of the lecithin based bicontinous micro textures, as proven by ^{19}F NMR self-diffusion studies.

In the third part of this work we succeeded in creating nanoemulsions with the skin friendly mild surfactant sucrose laureate and polysorbate 80. The nanoemulsions exhibited a good stability, although the formulation with the conventional polyoxyethylene derivatives exhibited a prolonged physicochemical stability probably due to the steric stabilising effect. Moreover,

the good stability was due to the close droplet size distribution, preventing Ostwald ripening. In addition, the positive charge of the nanoemulsion particles, which was induced by phytosphingosine at physiological pH, was able to increase skin permeation of the tested model drug. However once again, the nanoemulsions containing polysorbate 80 exhibited higher skin permeation data. This could be due to the different penetration routes across skin of the different surfactants. Overall, PS is an interesting multifunctional additive for drug delivery systems due to its enhancement effect on skin permeation and above all its hydrating and anti-inflammatory power.

In last part of this study liposomal membrane interaction studies were performed. Results indicate that phytosphingosine and N-palmitoylethanolamine undergoes hydrophobic and other types of interactions either in the liposome matrix or at the particle surface, or both, as indicated by changes in the pre- and main transition. Furthermore, phytosphingosine as well as N-palmitoylethanolamine, destabilises both gel and liquid crystal phases within the DPPC bilayer as indicated by changes in the enthalpy.

6 References

1. Valenta C. and Schultz K., Influence of carragenan on the rheology and skin permeation of microemulsions formulations. J. Contr. Rel. (2004) 95: 257-265.
2. Kählig H., Valenta C., Dampfhart U., Auner B.G, Rheology and NMR self-diffusion experiments as well as skin permeation of diclofenac-sodium and cyproterone acetate of new gel preparations. J. Pharm. Sci. (2005) 94:288-296.
3. Spernath A., Aserin A., and Garti N., Fully dilutable microemulsions embedded with phospholipids and stabilized by short-chain organic acids and polyols. J. Coll. Interface Sci. (2006) 299: 900-909.
4. Piemi M.P.Y., Korner D., Benita S.,Marty J-P., Positively and negatively charged submicron emulsions for enhanced topical delivery of antifungal drugs. J. Contr. Rel. (1999) 58:177-187.
5. Yilmaz E. and Borchert H.-H., Design of a phytosphingosine-containing, positively-charged nanoemulsion as a colloidal carrier system for dermal application of ceramides. Eur. J. Pharm. Biopharm. (2005) 60:91-98
6. Roberts M.S. and Walters K.A., Dermal Absorption and Toxicity Assessment, Marcel Dekker, INC. 91:1-43 (1998).
7. Neubert R.H and Wepf R., Stratum corneum: Struktur und Morphologie einer Barriere, (http://www.pharmazeutische-zeitung.de/index.:) access date: September 2008.
8. Hadgraft J., Skin, the final frontier. Int. J. Pharm. (2001) 224:1-18.
9. Wertz P.W. and Downing D.T., Stratum corneum: Biological and Biochemical considerations, In: Transdermal Drug Delivery, Development Issues and research Initiatives, pp:1-22, ed. J. Hadgraft and R.H. Guy., New York: Marcel Dekker (1989).
10. Elias P.M. and G.K. Menon, Structural and biochemical correlates of the epidermal permeability barrier. In: P.M. Elias, Editor, Advances in Lipid Research. pp. 1-26. Academic Press, New York (1991),
11. Schaefer H. and Lademann J., The role of follicular penetration. A differential view. Skin Pharmacol. Appl. Skin Physiol. (2001) 14 (1): 23-7.

12. Lademann J., Otberg N., Richter H., Jacobi U., Schaefer H., Blume Peytavi U., Sterry W., Follicular penetration. An important pathway for topically applied substances. Hautarzt (2003) 54(4):321-3.
13. Benson H.A.E., Transdermal drug delivery: Penetration enhancement techniques. Curr. Drug Del. (2005) 2: 23-33.
14. Williams A.C. and Barry B.W., Penetration enhancer. Adv. Drug Deliv. Rev. (2004) 56(5):603-18.
15. Peltola S., Saarinen-Savolainen P., Kiesvaara J., Suhonen T.M., Urtti A., Microemulsions for topical delivery of estradiol. Int. J. Pharm. (2003) 254: 99-107
16. Sapra B., Jain S. and Tiwary A.K., Percutaneous permeation enhancement by terpenes: mechanistic view. AAPS J. (2008) 10(1):120-32.
17. Graaff De A.M., Li G.L., Van Aelst A.C., Bouwstra J.A., Combined chemical and electrical enhancement modulates stratum corneum structure. J. Contr. Rel. (2003) 90(1): 49-58.
18. Barratt G.M., Therapeutic applications of colloidal drug carriers. Pharm. Sci. Technol. Today (2000) 3: 163-169.
19. Jores, K., Lipid nanodispersions as drug carrier systems- a physichochemical characterization, Dissertation. Martin-Luther-University Halle-Wittenberg (2004)
20. Shah J., Sadhale Y. and Chilukuri D., Cubic Phase gels as drug delivery systems. Adv. Drug Del. Rev. (2001) 47: 229-250
21. Daniels R., Neuartige Gelsysteme, (2002) http://www.dermotopics.de/german/ausgabe_2_02_d/daniels; access date: October 2008.
22. Valenta C., Auner B. and Loibl I., Skin permeation and stability studies of 5-aminolevulinic acid in a new gel and patch preparation. J. Contr. Rel. (2005) 107(3): 495-501.
23. Aboofazeli R. and Lawrence M.J., Investigations into the formation and characterization of phospholipid microemulsions.II.Pseudo-ternary phase diagrams of systems containing water-lecithin-alcohol-isopropyl myristate and alcohol: influence of purity of lecithin. Int. J. Pharm. (1994)106:51-

61.

24. Paul B.K. and Moulik S.P., Uses and applications of microemulsions. Curr. Sci. (2001) 80(8): 990-1001.
25. Chen H., Chang X., Du D., Li J., Xu H., Yang X., Microemulsion-based hydrogel formulation of ibuprofen for topical delivery. Int. J. Pharm. (2006) 315: 52-58.
26. Wolf G., Diffusionsuntersuchungen an (polymer-modifizierten) Mikroemulsionen mittels Feldgratiendenimpuls-NMR-Spektroskopie, Dissertation. University Potsdam, 2005.
27. Aboofazeli R. and Lawrence M.J., Investigations into the formation and characterization of phospholipid microemulsions. IV. Pseudo-ternary phase diagrams of systems containing water-lecithin-alcohol-and oil; the influence of oil. Int. J. Pharm. (1995) 125: 107-116.
28. Aboofazeli R. and Lawrence M.J., Investigations into the formation and characterization of phosopholipid microemulsions. I. Pseudo-ternary phase diagrams of systems containing water-lecithin-alcohol-isopropyl myristate. Int. J. Pharm. (1993) 93: 161-175.
29. Kreilgaard M., Pedersen E.J., and Jaroszewski J.W., NMR Characterisation and transdermal drug delivery potential of microemulsion systems. J. Contr. Rel. (2000) 69: 421-433.
30. Podlogar F., Bester Rogac M., and Gasperlin M., The effect of internal structure of selected water-Tween 40- Imwitor-IPM microemulsions on ketoprofene release. Int. J. Pharm. (2005) 302: 68-77.
31. Paolino D., Ventura C.A., Nistico S., Puglisi G., Fresta M., Lecithin microemulsions for the topical administration of ketoprofen: percutaneous adsorption through human skin and in vivo human skin tolerability. Int. J. Pharm. (2002) 244: 21-31.
32. Taha Mutasem O., Abdel-Halim H., Al-Ghazawi M., Khalil E., QSPR modeling of pseudoternary microemulsions formulated employing lecithin surfactants: application of data mining, molecular and statistical modelling. Int. J. Pharm. (2005) 295(1-2):135-55.

33. Mason T.G., Wilking J.N., Meleson K., Chang C.B., Graves S. M., Nanoemulsions: formation, structure and physical properties. J. Phys.: Condens. Matter (2006) 18: 635-666.
34. Sonneville-Aubrun O., Simonnet J.T., and L`Àlloret F., Nanoemulsions: a new vehicle for skincare products. Adv. Coll. Interface Sci. (2004) 108-109: 145-149.
35. Gutierrez J. M., Gonzalez C., Maestro A., Sole I., Pey C. M., Nolla J., Nano-emulsions: New applications and optimization of their preparation. Curr. Opin. Coll. Interface Sci. (2008) 13(4): 245-251.
36. Yilmaz, E., Entwicklung phytosphingosinhaltiger, positiv geladener Nanoemulsionen zur dermalen Applikation, Dissertation. University Berlin. (2005)
37. Tadros T., Izquierdo P., Esquena J. Solans C., Formation and stability of nano-emulsions. Adv. Coll. Interface Sci. (2004) 108-109: 303-318.
38. Solans C., Izquierdo P., Nolla J., Azemar N., Garcia-Celma M.J., Nano-emulsions. Curr. Opin. Coll. Interface Sci. (2005) 10(3,4): 102-110.
39. Gutierrez J. M., Gonzalez C., Maestro A., Sole I., Pey C. M., Nolla J., Nano-emulsions: New applications and optimization of their preparation. Curr. Opin. Coll. Interface Sci. (2008) 13(4): 245-251.
40. Klang V., Einfluss von Öl- und Tensidgehalt auf das Diffusionsverhalten von Fluconazol in ausgewählten Mikroemulsionen, Diploma Thesis, University Vienna (2007)
41. Fiume Z., Final report on the safety assessment of lecithin and hydrogenated lecithin. Int. J. Toxic. (2001) 20: 21-45.
42. Van Nieuwenhuyzen W. and Szuhaj B.F., Effects of lecithins and proteins on the stability of emulsions Fett/lipid (1998) 7: 282-291.
43. Claesson P.M. and Mikael Kjellin U.R., Sugar Surfactants, in Hubbard, A.T. (Ed.) Encyclopaedia of Surface and Colloid Science. Marcel Dekker, New York. pp. 4909-4925 (2002)
44. Ayala-Bravo Hilda A., Quintanar-Guerrero D. , Naik A., Kalia Yogeshvar N. Cornejo-Bravo Jose M. , Ganem-Quintanar A., Effects of sucrose oleate and sucrose laureate on in vivo human stratum corneum permeability. Pharm. Res. (2003) 20(8): 1267-73.

45. Calderilla-Fajardo S.B., Cazares-Delgadillo J., Villalobos-Garcia R., Quintanar Guerrero D., Ganem-Quintanar A., Influence of sucrose esters on the in vivo percutaneous penetration of octyl methoxycinnamate formulated in nanocapsules, nanoemulsions and emulsion. Drug Dev. Ind. Pharm. (2006) 32: p. 107-113

46. Ongpipattanakul B., Burnette R.R., Potts R.O., Francoeur M.L., Evidence that oleic acid exists in a separate phase within stratum corneum lipids. Pharm. Res. (1991) 8: 350 - 354.

47. Touitou E., Godin B., Karl Y., Bujanover S., Becker Y., Oleic acid, a skin penetration enhancer, affects Langerhans cells and corneocytes. J. Contr. Rel. (2002) 80: 1-7.

48. Pierre M.B., Ricci E.J., Tedesco A.C., Bentley M.V., Oleic acid as optimizer of the skin delivery of 5-aminolevulinic acid in photodynamic therapy. Pharm. Res. (2006) 23: 360 -366.

49. Goldberg-Cettina M., Liu P., Nightingale J., Kurihara-Bergstrom T., Enhanced transdermal delivery of estradiol in vitro using binary vehicles of isopropyl myristate and short-chain alkanols. Int. J. Pharm. (1995) 114(2): 237-45.

50. Brinkmann I. and Müller-Goymann C.C., Role of Isopropyl Myristate, Isopropyl Alcohol and a Combination of Both in Hydrocortisone Permeation across the Human Stratum corneum. Skin Pharm. Applied Skin Phys. (2003) 16: 393-404.

51. Farwick M. , Watson R.E.B., Rawlings A.V., Wollenweber U,Lersch P., Bowden J.J., Bastrilles J.Y., Griffiths C.E.M., Salicyloyl-phytosphingosine: a novel agent for the repair of photoaged skin. Int. J. Cosmet. Sci. (2007) 29(4): 319-329.

52. Lambert D.M., Vandevoorde S., Kent-Olov J., Fowler C.J., The palmitoylethanolamide family: A new class of anti-inflammatory agents? Curr. Med. Chem. (2002) 9(6): 663-674.

53. Swamy M.J., Ramakrishnan M., Marsh D., Wurz U., Miscibility and phase behaviour of binary mixtures of N-palmitoylethanolamine and dipalmitoylphosphatidylcholine. Biochim. Biophys. Acta Biomembr. (2003) 1616(2): 174-183.

54. Ständer S., Reinhardt H.W. and Luger T.A., Topische Cannabinoidagonisten: Eine effektive, neue Möglichkeit zur Behandlung von chronischem Pruritus. Hautarzt (2006) 57: 801-807.

55. El Laithy H.M. and El-Shaboury K.M.F., The development of Cutina Lipogels and Gel Microemulsions for Topical Administration of Fluconazole. AAPS Pharm. Sci. Tech. (2002) 3(4): 1-9.

56. Sameershing G.R. and Hope M.J., The effect of bilayer and hexagonal H (II) phase lipid films on transepidermal water loss. Exp. Derm. (2006) 15(7): 493-500.

7 List of scientific publications, within the present work

7.1 Publications

1. Effect of selected fluorinated drugs in a “ringing” gel on rheological behaviour and skin permeation.
   Hoeller S. and Valenta C., Eur. J. Pharm. Biopharm. 66 (2007) 120-126

2. Skin compatible lecithin drug delivery systems for fluconazole: effect of phosphatidylethanolamine and oleic acid on skin permeation.
   Hoeller S., Klang V. and Valenta C., J. Pharm. Pharmacol. 60 (2008) 587-591

3. Multinucelar NMR characterisation and dermal delivery of fluorinated drugs in soybean-microemulsion systems.
   Hoeller S., Kählig H. and Valenta C., J. Pharm. Sci., in press

4. Lecithin based nanoemulsions: A comparative study of the influence of non-ionic surfactants and the cationic phytosphingosine on physicochemical behaviour and skin permeation
   Hoeller S., Sperger A. and Valenta C., Int. J. Pharm., under review

5. Interactions between phytosphingosine and N-palmitoylethanolamine and DPPC liposomes as phospholipids model membranes.
   Hoeller S. and Valenta C., J. Pharm. Pharmacol., under review

7.2 Poster presentations

1. Skin permeation, rheology and stability studies of flufenamic acid and fluconazole incorporated in a cubic gel preparation.
   Hoeller S. and Valenta C., 33rd Annual Meeting of the Controlled Release Society, Vienna, July 2006

2. The influence of fluorinated drugs on the microstructure of a “ringing” gel.
   Hoeller S. and Valenta C., Innano, Innsbruck, October 2006

3. The influence of different phosphatidylcholine-molecules in microemulsions on the skin permeation of fluconazole.
   Hoeller S., Biruss B. and Valenta C., Pharmaceutical Science World Congress, Amsterdam, April 2007

4. Nanoemulsion based on lecithin and sugar surfactant: Effect of Phytosphingosine on in vitro permeation of fludrocortisone-acetate.
   Hoeller S. and Valenta C., 6th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Barcelona, April 2008

5. Positively charged nanoemulsions for enhanced topical delivery of fludrocortisone-acetate.
   Hoeller S. and Valenta C., 9th Annual Skin Forum Meeting, London, June 2008

6. NMR self diffusion and skin permeation of selected fluorinated drugs: Influence of phospholipids composition.
   Valenta C., Hoeller S., Kählig H., 35th Annual Meeting of the Controlled Release Society, New York, July 2008

7.3 Oral presentations

1. Effect of selected fluorinated drugs in a “ringing” gel on rheological behaviour and skin permeation.
   Sonja Hoeller, Department of Pharmaceutical Technology and Biopharmaceutics, University Vienna, February 2007

2. Lecithin basierte Nanoemulsionen mit Zuckertensiden: Effekt von Phytosphingosin,
   Claudia Valenta, Sonja Hoeller, Forum Cosmeticum, 9.-11.4.2008 in Wien, Austria Trend Hotel Savoy Vienna

8 List of Scientific publications, beyond the present work

8.1 Publications

1. Chitosan glycolic acid a new matrix for progesterone delivery to skin, drug development
   Kählig H., Hasanovic A., Biruss B., Höller S., Grim J., Valenta C., Ind. Pharm. Drug. Dev. Ind. Pharm., under Review

8.2 Poster presentation

1. Chitosan-EDTA a promising stabilizer for topically applied liposomes.
   Biruss B., Hoeller S. and Valenta C., Pharmaceutical Science World Congress, Amsterdam, April 2007

2. Chitosan glycolic acid as possible matrix for progesterone and 17ß-Estradiol.
   Hasanovic A., Hoeller S. and Valenta C., 6th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Barcelona, April 2008

9 Abbreviations

DPPC	Dipalmitoylphosphatidylcholine
GRAS	Generally Regarded as Safe
GMO	Glyceryl monooleate
HLB	Hydrophilic Lipophilic Balance
IPM	Isopropyl myristate
LPC	Lysophosphatidylcholine
OA	Oleic acid
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PEA	N-palmitoylethanolamine
PS	Phytosphingosine
PDI	Polydispersity Index
SC	Stratum corneum
SE	Sucrose fatty acid ester

10 CURRICULUM VITAE

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