ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 46 (1998) 191–200

European Journal of Pharmaceutics and Biopharmaceutics

Research paper

Transformation of a liposomal dispersion containing ibuprofen lysinate and phospholipids into mixed micelles – physico-chemical characterization and influence on drug permeation through excised human stratum corneum

Inken Stoye, Katrin Schröder, Christel C. Müller-Goymann*

Institut für Pharmazeutische Technologie der TU Braunschweig, Braunschweig, Germany

Received 1 September 1997; accepted 12 January 1998

Abstract

Our study is meant as a contribution to the investigation of molecular association of phospholipids and amphiphilic substances like, e.g. non-steroidal anti-inflammatory drugs. Our research focussed on physico-chemical characterization of ternary systems containing ibuprofen lysinate, lecithin and water. The influence of the resulting microstructures on drug release and permeation through excised human stratum corneum was also investigated. Depending on the mixing ratio different physical states (lamellar and hexagonal liquid crystals, micellar solutions, liposomal dispersions and biphasic systems) were determined by gross, polarizing microscopic and small angle X-ray analysis. A special emphasis was laid upon the storage-induced transformation of liposomal dispersions into mixed micellar solutions and its influence on drug release and permeation. A model for the transformation process is presented. Drug release of the liposomal dispersion ($D_a = 1.1 \times 10^{-5}$ cm^2/s) and of the identically composed mixed micellar solution ($D_a = 2.9 \times 10^{-5}$ cm^2/s) was delayed compared with the data of the aqueous drug solution ($D_a = 4.7 \times 10^{-5}$ cm^2/s). Because of the association of ibuprofen lysinate molecules with phospholipid molecules within the liposomal and the mixed micellar system the share of free ibuprofen lysinate monomers, which can pass through the dialysis membrane in the release experiment, is markedly reduced. The results of permeation experiments of these systems, however, did not correspond to the release data. The permeability of the mixed micellar solution, which arose after 6 weeks of storage ($P = 4.2 \times 10^{-8}$ cm/s) almost reached the permeation of the aqueous solution ($P = 3.9 \times 10^{-8}$ cm/s), whereas the permeation of the liposomal dispersion was very slow ($P = 1.5 \times 10^{-8}$ cm/s). The different colloidal microstructures of the formulations obviously resulted in divergent interactions with the permeation barrier - the stratum corneum - and subsequently in different permeation behaviour.

Keywords: Ibuprofen lysinate; Lecithin; Physico-chemical characterization; Liposomal dispersion; Mixed micelles; Transformation; Drug release; Drug permeation; Stratum corneum

1. Introduction

The addition of a polar amphiphile to a suspension of less polar phospholipids normally involves the generation of complex aggregates. Phase transformations as well as phase separations may occur, sometimes in time-dependent manner.

In this report, the physical states of different ibuprofen lysinate-lecithin-water systems are defined and the molecular structures of their aggregates are determined.

Physico-chemical characterization was carried out by polarizing microscopy, transmission electron microscopy and also by small angle X-ray diffraction.

The results permitted the construction of a triangular

* Corresponding author. Institut für Pharmazeutische Technologie, TU Braunschweig, Mendelssohnstrasse 1, D-38106 Braunschweig, Germany. Tel.: +49 531 3915650; fax: +49 531 3918108;
e-mail: c.mueller-goymann@tu-bs.de

0939-6411/98/$19.00
PII S0939-6411(98)00023-X

phase diagram defining the regions of two liquid crystalline phases (lamellar and hexagonal), mixed micellar solutions and liposomal dispersions as well as further regions of phase separations.

After 6 weeks of storage at room temperature, some of the liposomal dispersions containing therapeutically relevant doses of ibuprofen lysinate for dermal application (2–10%) turned into a yellowish mixed micellar solution. The phase transformation and the microstructure of these systems were examined with the long-term view of developing an antirheumatic spray formulation with good penetration and minimized irritation potential.

Fast drug release as well as good penetration into the skin are two imperative prerequisites for drug permeation. The colloidal structure of the dermally applied formulation, as well as possible phase transformations, may have an influence on drug release and permeation through the barrier of the topmost skin layer, the stratum corneum [1,2]. For this reason, in vitro drug release experiments and in vitro permeation studies through excised human stratum corneum were carried out after the physico-chemical characterization of the chosen systems.

In previous studies an increase in permeation could be observed after administration of liposomal formulations [3, 4]. In contrast to these results other research groups did not discover any permeation enhancing effect after dermal application of liposomal formulations [1,5]. Even a reduction of the permeation rate was shown [6].

As a contribution to the discussion of phospholipids as potential permeation enhancers for topical application, emphasis was laid on the influence of the colloidal microstructure of the identically composed liposomal and mixed micellar formulations on release and permeation behaviour.

2. Materials and methods

2.1. Materials

Ibuprofen lysinate was a gift from Merckle (Blaubeuren, Germany). Phospholipon 90G® is a highly purified soybean lecithin with a content of at least 90% phosphatidylcholine and a maximum content of 6% lyso-phosphatidylcholine. It was donated by Nattermann (Köln, Germany). 1,6-diphenyl-1,3,5-hexatriene (DPH) as well as 1-anilinonaphthalene-8-sulfonic acid (ANS) were purchased from Sigma (Dreisenhofen, Germany). For dialysis and release experiments a Spectra/Por molecular porous membrane (MWCO 6-8000) (Spectrum Medical Industries, Los Angeles, CA) was used. Bidistilled water was used.

2.2. Methods

2.2.1. Preparation of the ternary systems

All three components (ibuprofen lysinate, phospholipids and water) were mixed together and heated at 60°C for a period of 20 min while being stirred with a Teflon coated magnetic stirring bar (350 rounds/min). After cooling down to room temperature, lost water was substituted and the systems were stirred again.

2.2.2. Polarizing microscopy (PLM)

All formulations were examined with a Zeiss type III photomicroscope (Oberkochen, Germany) using crossed polarizers and a λ-sheet.

2.2.3. Transmission electron microscopy (TEM)

'Sandwiched' samples were shock-frozen in melting nitrogen at –210°C. Formulations with a high water content were frozen using liquid propane and a Cryo Jet JFD/030 (Balzers, Wiesbaden, Germany). The samples were fractured in a high vacuum of less than 5×10^{-6} bar at –100°C with a BAF 400 freeze fracture apparatus (Balzers, Wiesbaden, Germany). The fracture surface was replicated by shadowing with platinum/carbon (Pt/C) at an angle of 45° (layer thickness 2 nm). For mechanical stability the samples were consecutively shadowed with carbon at an angle of 90° (layer thickness 2 nm). After cleaning with chloroform/methanol (1:1 (v/v)) and water, the replica were examined with an EM-300 transmission electron microscope (Philips, Kassel, Germany).

2.2.4. Small angle X-ray diffraction (SAXD)

Small angle X-ray diffraction studies were performed according to Kiessig (1942) using a local sensitive detector (Braun, München, Germany). Semisolid and liquid samples were exposed to the X-ray beam using a sample holder and camera, which were manufactured by the Institut für Pharmazeutische Technologie der TU Braunschweig. The samples were pressed between two capton foils (Krempel, Vaihingen, Germany) to a thickness of 1 mm avoiding air bubbles. In order to minimize the effect of gas molecules, analysis was performed at a pressure of 4 mbar. X-rays were produced by a Müller Micro III generator (Philips, Kassel, Germany) with a PW 2213/20 X-ray tube (equipped with a copper anode, anode current 30 mA, $\lambda = 0.154$ nm, accelerating voltage 45 kV). Exposure time was 300 s.

2.2.5. Phosphor-NMR

All formulations for Phosphor-NMR studies were prepared with D_2O (99.9% deuterium oxide, Aldrich, Steinheim, Germany) and investigated using a Bruker AC 200 Mc/s (200 MHz) NMR apparatus (Bruker Analytische Meßtechnik GmbH, Rheinstetten, Germany). The shift was measured in ppm in relation to an external signal of phosphoric acid. D_2O served as a lock and was used as a substitute for water in binary and ternary systems.

2.2.6. Fluorescence studies

Drug binding to the lipophilic and/or hydrophilic sites of phospholipid liposomes was investigated by displacement of either DPH as lipophilic or ANS as hydrophilic substrate [7].

DPH: DPH (44 μmol/l) as well as phospholipids (20 mmol/l) were dissolved in tetrahydrofurane (THF). THF was eliminated using a Rotavapor. A liposomal dispersion was achieved by dispersing the remaining film with water. After dilution (1:1) of the liposomal dispersion with aqueous solutions of ibuprofen lysinate (resulting drug concentrations ranged between 0.25 and 2.0 mmol/l), the samples were stirred at 50°C. After exactly 1 h of stirring, the fluorescence intensity was determined with an F-4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) ($\lambda_{ex.}$ = 368 nm; $\lambda_{em.}$ = 445 nm).

ANS: A liposomal dispersion was achieved by mixing phospholipids (1 mmol/l), ANS (10 μmol/l), ibuprofen lysinate (0.125-1.0 mmol/l) and water and stirring at 50°C. After exactly 1 h of stirring, the fluorescence intensity was determined with an F-4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) ($\lambda_{ex.}$ = 380 nm; $\lambda_{em.}$ = 480 nm).

2.2.7. Equilibrium dialysis

In order to determine the fraction of unbound ibuprofen lysinate in liposomal dispersions, equilibrium dialysis was performed. The Spectra/Por dialysis membrane was filled with 2.00 ml of the formulation. At room temperature, this sample was dialyzed against 98 ml of water over a period of 12 h. The drug content of the aqueous phase was determined by high pressure liquid chromatography (HPLC) (see Section 2.2.11).

2.2.8. Preparation of excised stratum corneum

Pathologically negative skin samples of the abdominal region were obtained from surgical corrections. Isolated stratum corneum was prepared by trypsination using the method of Christophers and Kligman [8]. For cleaning the stratum corneum, it was rinsed with a solution of trypsin inhibitor (0.001% (m/m)) (Sigma, Dreisenhofen, Germany) and with water.

2.2.9. In vitro drug release

Drug release experiments were carried out at 20°C using a modified Franz cell [9] (surface area: 0.50–0.57 cm^2, amount of donor: 0.5 g, acceptor medium: 4.5–5.5 ml of isotonic phosphate buffered saline, pH 7.4). A siliconized (1.2 mg silicone/cm^2) Spectra/Por membrane was used to separate donor and acceptor compartment [10] and to forestall water influx into the donor compartment. Aliquots (250 μl) were withdrawn from the acceptor compartment at appropriate intervals and analyzed for ibuprofen concentration (see Section 2.2.11). Corresponding volumes were replaced by acceptor medium, immediately.

2.2.10. In vitro permeation studies

The modified Franz cells (see Section 2.2.9) were also used for permeation studies through excised human stratum corneum at 37°C. To provide mechanical strength to the fragile tissue, the stratum corneum was placed on a polycarbonate membrane TMTP with a pore size of 5 μm (Millipore, Eschborn, Germany). It was then located between the donor and acceptor compartment. Before the donor was placed into the donor compartment, the stratum corneum was allowed to hydrate for 30 min. Aliquots (250 μl) withdrawn from the acceptor compartment at appropriate intervals were analyzed for ibuprofen concentration (see Section 2.2.11). Corresponding volumes were replaced by acceptor medium, respectively.

2.2.11. High pressure liquid chromatography (HPLC)

Ibuprofen analysis was performed isocratically by reversed phase chromatography [11] using a column of Hypersil®ODS 5 μm, 125 × 4 mm with precolumn 10 × 4 mm (Grom, Herrenberg, Germany), a flow rate of 1.7 ml/min, a mobile phase consisting of acetonitrile:water:acetic acid (40:60:2), a Kratos Spectroflow 400 pump (Weiterstadt, Germany) and a Beckman System Gold Detector Module 166 (λ = 264 nm) (München, Germany). Peak identification and integration was carried out by Beckman System Gold Chromatography Software Version 6.01 (München, Germany). Calibration was performed within a range of 10-500 μg/ml with a correlation coefficient > 0.999.

3. Results and discussion

3.1. Association behavior in binary systems

In order to understand aggregation in ternary mixtures, the aggregation of ibuprofen lysinate as well as phospholipon 90G® in binary systems in water will be discussed very briefly.

3.1.1. Aggregation in binary phospholipid-water-mixtures

At room temperature phospholipon 90G®, which served as the phospholipid, is of liquid crystalline state [12]. Above a water concentration of 7%, a lamellar liquid crystal exists. When the water content exceeds 40%, the amount of lipid is not sufficient to form a coherent lamellar phase anymore. Hence, a dispersion of lamellar vesicles – so-called liposomes –is formed with rising water concentrations. A formation of phospholipid micelles is not possible.

For fully hydrated phospholipon 90G® water content (30%) a cylindrical molecular geometry with a critical packing parameter P~1 can be assumed (Fig. 1a).

For further detailed information refer to Ref. [13].

3.1.2. Aggregation in binary ibuprofen lysinate-water-mixtures

Ibuprofen lysinate exhibits a concentration-dependent aggregation to either micelles (cmc: 2.67–3.06%) [14] or a hexagonal liquid crystalline phase (concentrations >49%) (Fig. 2). If the drug content exceeds 50%, the drug will recrystallize from the hexagonal phase upon storage [15].

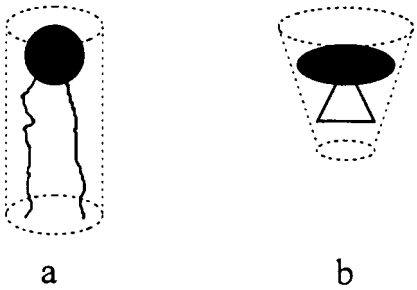

Fig. 1. Assumed molecular geometry of hydrated phospholipon 90G® (a) and ibuprofen lysinate (b).

Because of the formation of highly curved aggregates such as micelles and hexagonal systems, a coned geometry with a critical packing parameter of $P \leq 0.5$ is assumed for ibuprofen lysinate (Fig. 1b).

3.2. Association behavior in ternary systems

The different molecular geometry determines the association behaviour of both phospholipids and ibuprofen lysinate and will also modify aggregation in ternary systems depending on the mixing ratio employed.

In fact, positions of the different individual phases shift. Moreover, phase transformations as well as phase separations can be detected.

Fig. 3 represents the triangular phase diagram obtained from gross, microscopic and X-ray analysis of numerous mixtures immediately after preparation and cooling. All three components are expressed in wt.% of the total weight. On the connecting line between two components all corresponding binary systems are represented. Ternary mixtures are represented inside the triangular diagram.

If the 1:1 ratio of ibuprofen lysinate/lecithin is exceeded, drug crystals embedded in a lamellar or hexagonal gel appear with increasing ibuprofen lysinate content. No further characterization of these systems will be given.

Above a concentration of 60%, lecithin tends to form extended bilayers in the presence of water. As shown by polarizing microscopy and X-ray analysis, these bilayers are of lamellar structure.

By addition of small amounts of ibuprofen lysinate, a shift of the lamellar phase position can be observed. In the presence of 5% ibuprofen lysinate, 20% lecithin is sufficient to form a coherent lamellar bilayer. Furthermore, the consistency of the lamellar gel increases after addition of the drug.

An increase of the total amount of drug above 15% leads to phase separations. Liposomal dispersions, micellar solutions above a paracrystalline gel as well as cloudy gels consisting of lamellar and hexagonal fractions can be found.

In mixtures with a further increased drug concentration (20–40%) and a reduced phospholipid concentration (≤20%), the molecular geometry of ibuprofen lysinate dominates aggregation behaviour. Highly curved aggregates such as mixed micelles and hexagonal liquid crystalline phases are formed.

3.2.1. Lamellar gel

Lamellar gels are formed over a wide concentration range. The X-ray data reveal a decrease in interlayer spacing from 5.05 nm (phospholipid bilayer without drug) to 4.68 nm (drug content ≥6%).

This change in phase characteristics indicates interactions between ibuprofen lysinate and phospholipid molecules. As shown in Fig. 1, there is a distinct difference in the mole-

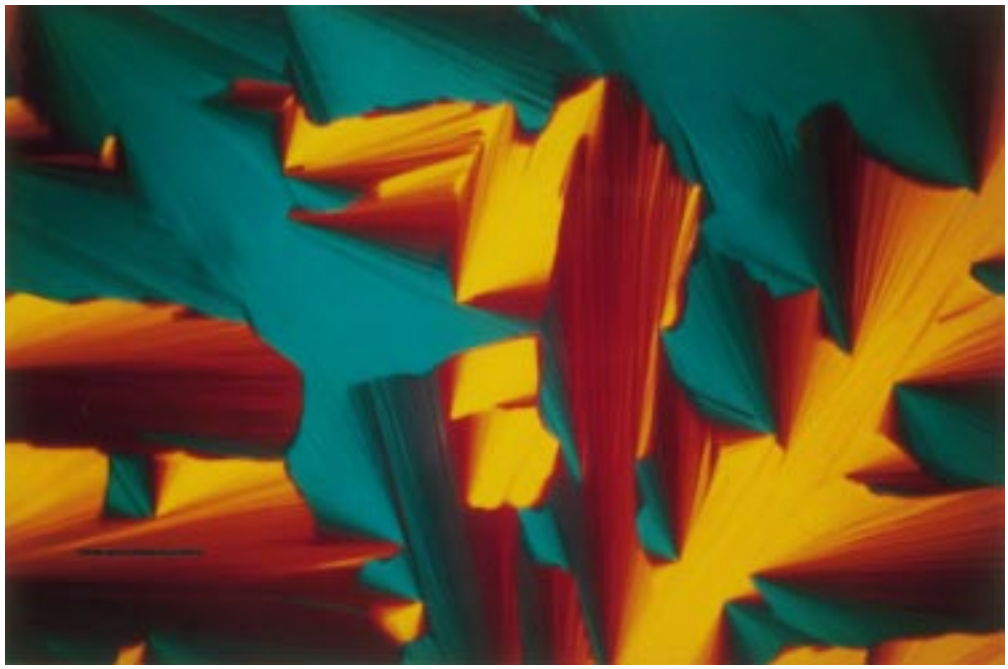

Fig. 2. Polarizing microscopy of the hexagonal liquid crystal (50% ibuprofen lysinate, 50% water). Scale bar, 100 μm.

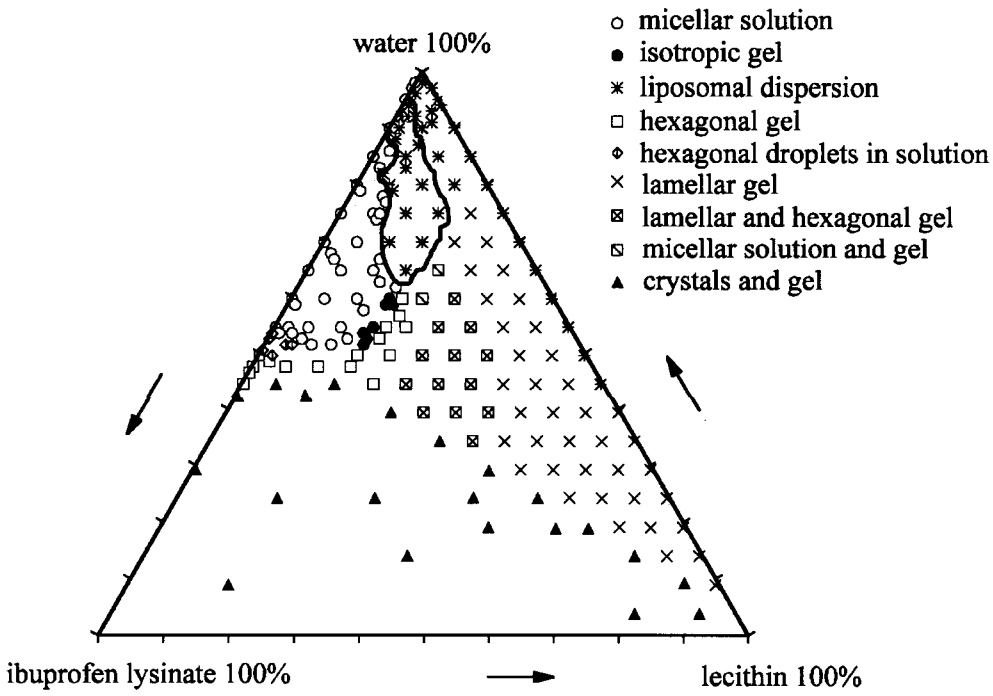

Fig. 3. Triangular diagram of ibuprofen lysinate-lecithin-water.

cular geometry of the two components leading to divergent packing ratios. Lecithin bilayers may host amphiphile molecules such as fenoprofen [2] and diclofenac diethylamine [1]. Also the polar amphiphile ibuprofen lysinate with its conic shape is expected to be located between the more polar regions of the phospholipid molecules forming the bilayer. Because of the localization and shortness of the drug molecule compared with the lecithin molecules, 'gaps' would arise in the non-polar region of the lamellar bilayer. These 'gaps' may be filled by bent hydrocarbon chains of the lecithin molecules, which would also explain the reduction in interlayer spacing [13].

3.2.2. Hexagonal gel

With increasing ibuprofen lysinate concentration, the curvature of the lamellar bilayers will increase. First hexagonal fractions can be detected at drug concentrations ≥15%; pure hexagonal gels can be found at drug concentrations ≥20%. Up to 20% lecithin can be integrated into the hexagonal structure. The mixtures, which separate into a solution and a gel, turn into the one-phase hexagonal gel after 6 weeks of storage, the same way the isotropic gels do. A cubic structure of the latter cannot be analyzed. The interferences by small angle X-ray diffraction indicate a hexagonal structure already after preparation.

All hexagonal gels are clear and firm. The X-ray interferences confirm the hexagonal structure. In case of a constant content of 50% water, the bragg spacings increase with higher content of lecithin. The diameter of the hexagonal tubes increases from 3.9 up to 4.5 nm as phospholipid molecules are integrated into the coat of the tubes. Spacings of this order fit well to data of other hexagonal phases [16].

However, polarizing microscopy reveals that textures, typical for hexagonal liquid crystals [17], seem to be disturbed. Oily streaks, typical for lamellar structures, appear (Fig. 4a). Because of the high consistency of the gels, this disruption may be caused by the technique of preparation.

The region of the hexagonal gels with small amounts of phospholipids borders on a region of isotropic solutions. In the crossing area of both phases we detected viscous solutions. By microscopic examination, particles of a hexagonal phase appear as 'fanlike units' floating in the isotropic solution (Fig. 4b).

The colloidal aggregates of the solution, assumed as mixed micelles, associate to higher ordered systems like hexagonal tubes. In conclusion, the molecular structure of the mixed micelles can be derived from the fine structure of the hexagonal gels with a high concentration of ibuprofen lysinate.

3.2.3. Micellar solution

Within a concentration range of about 3% (cmc)–49% ibuprofen lysinate micelles are formed in binary systems with water. For steric reasons, lecithin cannot form micelles in aqueous systems. In the presence of at least 10% ibuprofen lysinate up to 15% of lecithin may be solubilized by the formation of mixed micelles. The solubilizing capacity rises with increasing drug concentration.

The mixed micellar systems are yellowish, clear, isotropic formulations. The existence of particles of colloidal magnitude was shown by laser light scattering [15]. Data concerning particle size are not available by photon correlation spectroscopy, because dilution of the mixed micellar systems led to a phase transformation into a liposomal dispersion. Analog

transformations of mixed micelles into liposomes are described in the literature. For a summary refer to Ref. [13].

The viscosity of the micellar solutions increases with rising concentration. Probably, the mixed micelles become more and more anisometric with increasing drug concentration. The viscous mixed micellar solutions represent the area of transition into hexagonal liquid crystalline structures as already mentioned in Section 3.2.2.

3.2.4. Liposomes

The addition of lecithin to low concentration aqueous solutions of ibuprofen lysinate provokes a phase separation. Besides the already existing micelles, phospholipid liposomes are formed.

Because of the amphiphilic structure of ibuprofen lysinate incorporation of the drug into the liposomes is very likely to occur. By means of equilibrium dialysis (Section 3.4.) and ^{31}P-NMR spectroscopy, it was shown that the drug is in fact incorporated into the liposomes. For ^{31}P-NMR measurements unilamellar vesicle formulations were used, which were generated by sonification of the liposomal dispersions prepared as in Section 2.2.1. Comparing the

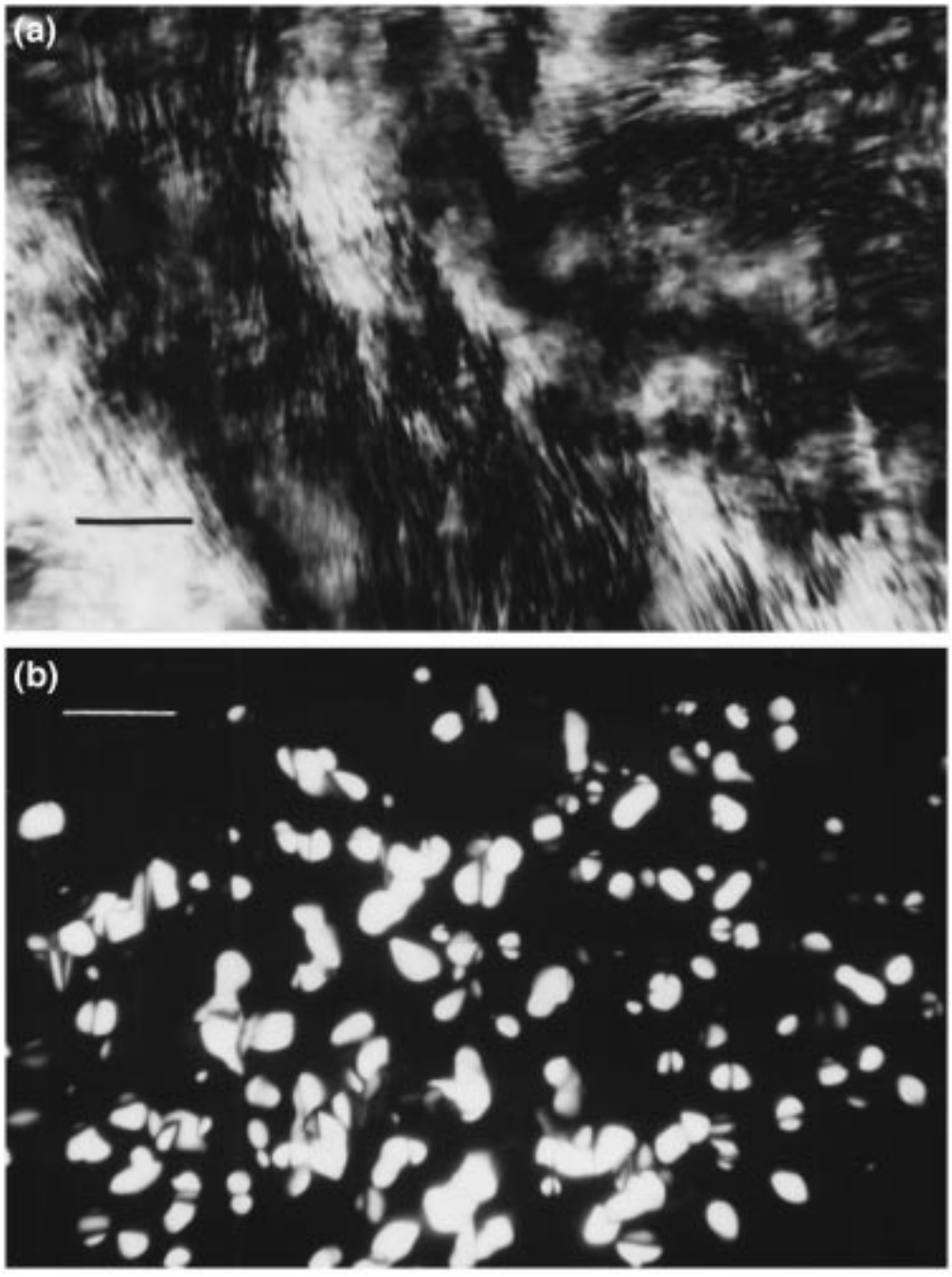

Fig. 4. Polarizing microscopy of (a) hexagonal phase with texture typical for hexagonal and lamellar liquid crystals and (b) micellar solution with floating 'fanlike units'. Scale bar, 100 μm.

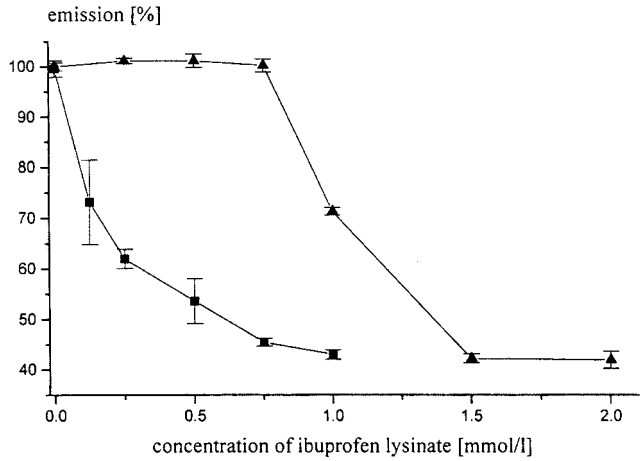

Fig. 5. Displacement of DPH (▲) and ANS (■) by ibuprofen lysinate.

^{31}P-NMR spectrum of a binary liposomal formulation (6% lecithin, 94% water) with the spectrum of the corresponding ternary dispersion (6% lecithin, 4% ibuprofen lysinate, 90% water), a narrow symmetrical signal was obtained in both cases. Such signals are typical for lipid structures allowing isotropic motional averaging, for example small vesicles [18]. The drug containing system, however, showed a low-field shift from –0.31 to –0.05 ppm which indicated an interaction of drug molecules with the phosphorous atoms of the lecithin headgroups from both sides of the bilayer. If drug molecules were only adsorbed to the surface of liposomes, a separation into two signals should occur.

In order to detect the localization of the drug within the liposomes, we employed a fluorescence technique to study hydrophobic and hydrophilic interactions of ibuprofen lysinate with phospholipid molecules. This technique is based on the displacement of a hydrophobic (DPH) and/or a hydrophilic (ANS) substrate incorporated into the liposomes by ibuprofen lysinate. The lipid-bound substrates show intense fluorescence, while the free substrates do not exhibit fluorescence. Therefore, a decrease in fluorescence intensity in the presence of the drug is assumed to be due to the binding of the drug to the liposomes.

Due to its amphiphilic structure, ibuprofen lysinate is able to bind to the hydrophobic as well as to the hydrophilic sites of phospholipid molecules (Fig. 5).

Hence, the already supposed lateral intercalation of ibuprofen lysinate between the more polar regions of the phospholipid molecules – which means in the region of the polar headgroup and the neighbouring alkyl chain regions – is confirmed.

3.3. Transformation of liposomes into mixed micelles

After 6 weeks of storage, some of the liposomal dispersions turned into an isotropic, yellowish clear solution (see marked area in Fig. 3). Due to HPLC as well as thin-layer chromatographic investigations, decomposition of the components could be excluded as reason for the transformation process. Also no water loss could be detected by Karl-Fischer titration. Neither small angle X-ray scattering nor wide angle X-ray scattering studies revealed any interferences. By laser light scattering the existence of particles of colloidal magnitude - mixed micelles - was detected within the arisen systems [15]. Again, the determination of micelle size was not possible, because upon the necessary dilution the systems would transform into liposomal dispersions. This is an indirect proof of the existence of mixed micelles, because the transformation corresponds to the preparation

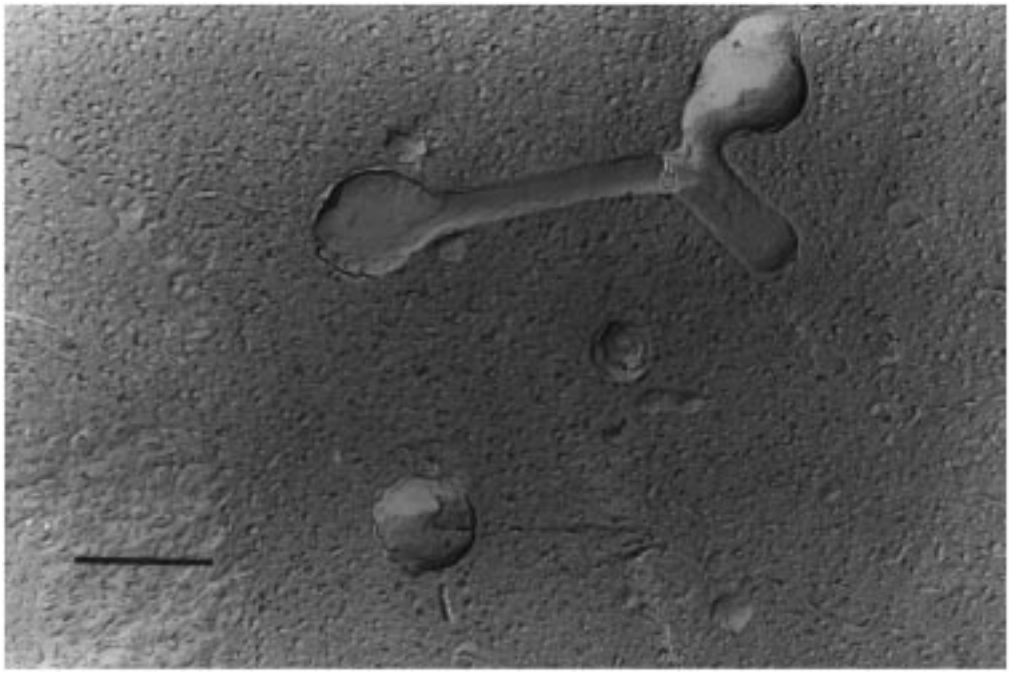

Fig. 6. Transmission electron microscopy of myelin figures. Scale bar, 400 nm.

of liposomes by removal of detergents from phospholipid/detergents mixed micelles [19].

At the beginning of the transformation process, polarizing microscopy revealed an increase in myelin-like structures. These myelin figures were also detected by transmission electron microscopy (Fig. 6).

After an initial rise in the number of myelin figures, the number of vesicles decreased and disappeared totally 6 weeks after preparation of the formulations.

No acceleration of the transformation process could be reached by storage at elevated temperature or any other variation of the preparation such as sonification of the liposomal dispersions or dilution of more concentrated mixed micellar solutions.

The transformation is a dynamic process and seems to be governed by entropy gain.

Our interpretation of the transformation process is as follows. Due to the possible interactions of ibuprofen lysinate with phospholipids, more and more ibuprofen lysinate will enter the bilayer region of the already ibuprofen lysinate containing liposomes (Fig. 7a). As the packing parameter of ibuprofen lysinate is assumed to be slightly smaller than 0.5, further incorporation of the drug will lead to curvature effects and to the externalization of bilayer regions with high drug content (Fig. 7b). If the curvature of a bilayer region exceeds a certain value, mixed micelles will be formed (Fig. 7c).

The better the water solubility of the drug the smaller the tendency of drug molecules to enter phospholipid bilayers [20]. As ibuprofen lysinate has a very good solubility in water, the time until the first mixed micelles are formed should be relatively long.

The formation of the first mixed micelles will lead to a loss of integrity of the phospholipid bilayers and will facilitate the further influx of ibuprofen lysinate, which will promote the transformation process. After a period of 6 weeks the transformation process is assumed to be complete (Fig. 7d).

3.4. Release of ibuprofen lysinate from liposomal and mixed micellar systems

Ibuprofen lysinate is well known for its extremely high water solubility. Pharmaceutically it is mainly used for oral

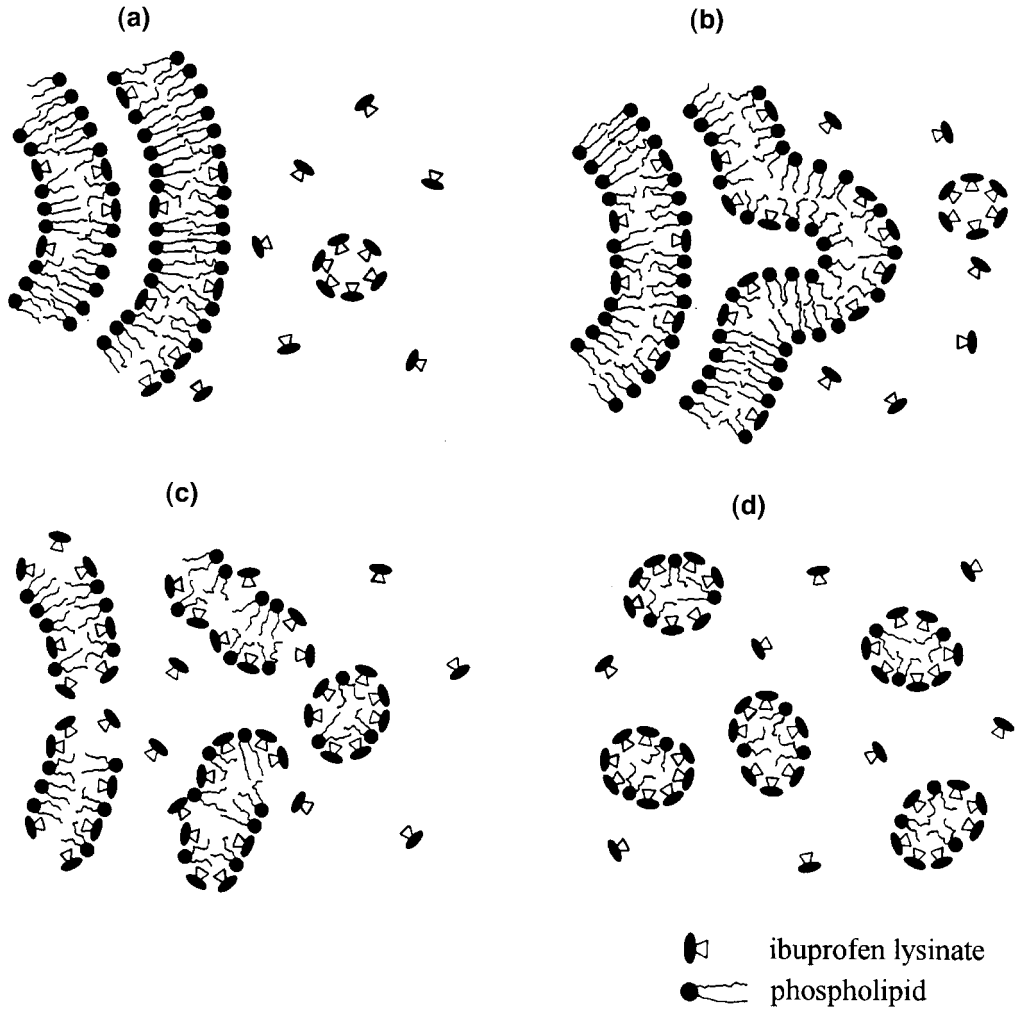

Fig. 7. Model for the transformation of the liposomal dispersion into mixed micelles.

dosage forms designed for a fast antiphlogistic effect and pain relief. For dermal preparations ibuprofen acid is normally used. The use of ibuprofen lysinate for dermal administration is restricted to three gel formulations available in Switzerland and Italy.

The permeation process through the skin is influenced by the colloidal microstructure of the vehicle and by interactions of the drug and/or vehicle with stratum corneum structures. Before entering the stratum corneum, which represents the main barrier for dermal absorption, the drug has to be released from the vehicle.

The apparent diffusion coefficient D_a is calculated according to Eq. (1) [21], where Q is the cumulative mass of drug released, A represents the release area, c_0 is the donor drug concentration at $t = 0$ and t is the time. The term Q^2/t can be calculated from the linear gradient, which is reached after a lag time of 170 min, by linear regression (correlation coefficient >0.99).

$$D_a = Q^2 \cdot \pi / (4A^2 \cdot c_0^2 \cdot t) \quad (1)$$

The tendency of the drug to leave the vehicle depends mainly on the microstructure of the vehicle. Strong interactions of drug and excipient molecules, for example phospholipids, usually lead to a retarded release.

In the liposomal as well as in the mixed micellar systems association of phopholipids and ibuprofen lysinate occurred. Release experiments showed, that the different microstructures lead to a different release behaviour (Fig. 8). Compared with an aqueous solution of 5.5% ibuprofen lysinate ($D_a = 4.7 \times 10^{-5}$ cm^2/s), release is distinctly retarded by a liposomal dispersion (5.5% ibuprofen lysinate, 6.0% phospholipids, 88.5% water; 1 day after preparation) ($D_a = 1.1 \times 10^{-5}$ cm^2/s). After 6 weeks of storage the formulation turned into a mixed micellar solution, which still shows a retarded release behaviour ($D_a = 2.9 \times 10^{-5}$ cm^2/s)

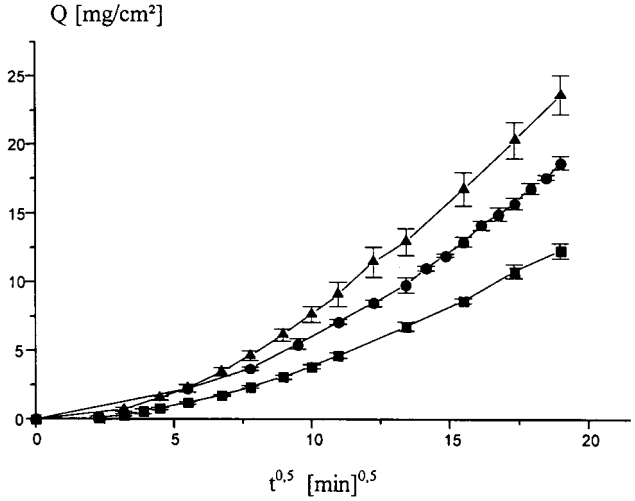

Fig. 8. Release of ibuprofen lysinate (5.5%) from different vehicles, (●) mixed micellar system (5.5% ibuprofen lysinate, 6.0% phospholipids, 88.5% water), (▲) aqueous solution (5.5% ibuprofen lysinate), (■) liposomal dispersion (5.5% ibuprofen lysinate, 6.0% phospholipids, 88.5% water), $n = 3$.

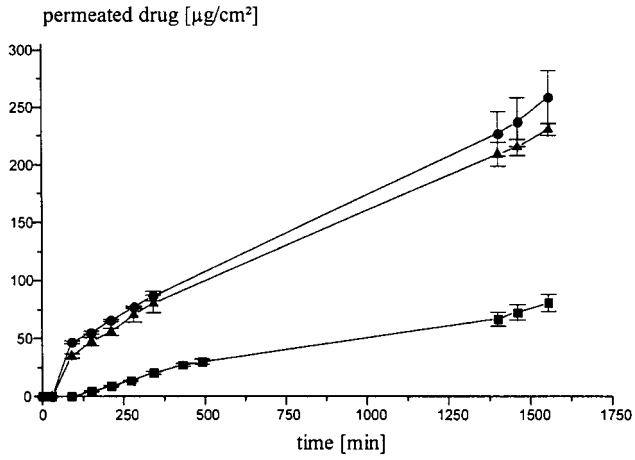

Fig. 9. Permeation of ibuprofen lysinate (5.5%) from different vehicles, (●) mixed micellar system (5.5% ibuprofen lysinate, 6.0% phospholipids, 88.5% water), (▲) aqueous solution (5.5% ibuprofen lysinate), (■) liposomal dispersion (5.5% ibuprofen lysinate, 6.0% phospholipids, 88.5% water), $n = 3$; donor: female, abdomen, 32 years.

compared with the aqueous solution of ibuprofen lysinate. Indeed, different associations of ibuprofen lysinate with phospholipids at a molecular level led to different drug release from the vehicles although the drug concentration is the same in both formulations.

Only molecules with a molecular weight up to 6000-8000 Da may permeate the siliconized dialysis membrane. This means, that only monomers or oligomers but neither mixed micelles nor liposomes are able to pass through the membrane. From the aqueous solution with almost unhindered mobility of ibuprofen lysinate, the drug is quickly released. Although the mobility of ibuprofen lysinate is reduced by the formation of mixed micelles and liposomes, the release behaviour of a mixed micellar system is still better than that of a liposomal dispersion. One reason might be, that the lifetime of the micelles is very much shorter than that of liposomes [22], resulting in a larger number of drug monomers able to permeate the siliconized membrane at a certain time.

The release behaviour of the liposomal dispersion corresponds to that of an aqueous solution of 3.72% ibuprofen lysinate. Indeed, dialysis experiments with the liposomal dispersion revealed that 1.77% ibuprofen lysinate is incorporated into the liposomes whereas 3.73% of drug is still unbound. Drug release is hence determined only by the amount of free drug.

3.5. Permeation of ibuprofen lysinate from liposomes and mixed micelles

As drug release is a prerequisite of skin permeation, different release behaviour might provoke different permeation behaviour.

The permeability coefficient P of ibuprofen lysinate was determined from the linear gradient from the plot of cumulative mass permeated versus time (dM/dt) reached after a

certain lag time and the donor concentration (c_0) according to Eq. (2).

$$P = \mathrm{d}M / \mathrm{d}t \cdot c_0 \quad (2)$$

It is remarkable, that the differences in release behaviour do not correspond to drug permeation (Fig. 9). Best permeation is observed from the mixed micellar system obtained after 6 weeks of storage ($P = 4.2 \times 10^{-8}$ cm/s). The permeation from the aqueous solution ($P = 3.9 \times 10^{-8}$ cm/s) almost reaches the permeation from the mixed micellar formulation whereas permeation from the liposomal dispersion is much slower ($P = 1.5 \times 10^{-8}$ cm/s). The results indicate, that parameters other than drug release also influence the permeation behaviour. Presumably, different interactions with the permeation barrier - the stratum corneum - occur. Obviously, not only the existence of phospholipids within a formulation lead to a permeation enhancing effect as it is often assumed [23]. Instead the resulting microstructure after the addition of phospholipids to a formulation has to be taken into account. Further studies should therefore concentrate on the effect of the different colloidal microstructures of the formulations on stratum corneum structures. Such studies are already being carried out and confirm our assumption of strong interactions of mixed micelles with stratum corneum structures. The results will be published in the near future.

4. Conclusion

Knowledge of the molecular geometry of drug and excipient molecules enables us to produce formulations with certain colloidal microstructures, which may distinctly modify for example drug permeation.

The mixed micellar solution of ibuprofen lysinate and lecithin improves the skin permeation compared with the identically composed liposomal formulation and the aqueous solution. Therefore the permeation process is influenced by the colloidal microstructure of the vehicle and by interactions of the drug and/or vehicle with stratum corneum structures. Further studies will be published.

Acknowledgements

We wish to thank Dr. Flory of the Städtisches Krankenhaus Holwedestr., Braunschweig, for the donation of the skin samples and acknowledge the gifts of ibuprofen lysinate by Merckle and of phospholipids by Nattermann Phospholipid GmbH.

References

[1] K. Kriwet, Diclofenac-Diethylamin und seine Assoziate mit Phospholipiden: Charakterisierung der Systeme und Einfluß auf Struktur und Permeabilität humanen Stratum corneums, Dissertation Technische Universität Braunschweig, 1994.

[2] I.L. Wilisch, Veränderungen der kolloidalen Struktur von Lecithinassoziaten bzw. Stratum corneum durch unterschiedliche Ölkomponenten und Fenoprofen im Hinblick auf Arzneistofffreigabe und-permeation durch exzidierte Humanhaut, Dissertation Technische Universität Braunschweig, 1994.

[3] W. Wohlrab, J. Lasch, K.-H. Taube, K.-D. Wozniak, Hautpermeation von liposomal inkorporiertem Hydrocortison, Pharmazie 44 (1988) 333–335.

[4] A. Gesztes, M. Mezei, Topical anaesthesia of the skin by liposome-encapsulated tetracain, Anaesthes. Analges. 67 (1988) 1079–1081.

[5] K. Kriwet, C.C. Müller-Goymann, Diclofenac release from phospholipid drug systems and permeation through excised human stratum corneum, Int. J. Pharm. 125 (1995) 231–242.

[6] D. Imbert, G.B. Kasting, R.R. Wickett, Influence of liposomal encapsulation on the penetration of retinoic acid through human skin in vitro, J. Soc. Cosmet. Chem. 45 (1994) 119–134.

[7] U.M. Joshi, P.R.S. Kodavanti, V.G. Lockard, H.M. Mehendale, Fluorescence studies on binding of amphiphilic drugs to isolated lamellar bodies: relevance to phospholipidosis, Biochim. Biophys. Acta 1004 (1989) 309–320.

[8] A.M. Kligman, E. Christophers, Preparation of isolated sheets of human stratum corneum, Arch. Dermatol. 88 (1964) 702–705.

[9] T.J. Franz, Percutaneous absorption on the relevance of in vitro data, J. Invest. Dermatol. 64 (1975) 190–195.

[10] H.L.G.M. Tiemessen, Non-ionic surfactant systems for transdermal drug delivery, Dissertation Universität Leiden, 1989.

[11] B. Levine, Y.H. Caplan, Simultaneous liquid-chromatographic determination of five non-steroidal anti-inflammatory drugs in plasma or blood, Clin. Chem. 31 (2) (1985) 346–347.

[12] K. Westesen, Beitrag zur Strukturaufklärung eines arzneistoffhaltigen hochkonzentrierten Tensidsystems und ausgewählter Verdünnungen, Dissertation Universität Braunschweig, 1988.

[13] H.J. Hamann, Wechselwirkungen mesogener Arzneistoffe mit kolloidalen Lecithinassoziaten am Beispiel des Fenoprofens, Dissertation Technische Universität Braunschweig, 1990.

[14] I. Stoye, Permeabilitätsveränderung von humanen Stratum corneum nach Applikation nicht-steroidaler Antirheumatika in verschiedenen kolloidalen Trägersystemen, Dissertation Universität Braunschweig, 1997.

[15] K. Schreiber, Erstellung eines Dreikomponentendiagramms aus Ibuprofen-Lysinat, Lecithin und Wasser sowie physiko-chemische Charakterisierung ausgewählter Systeme, Diplomarbeit Martin-Luther-Universität Halle, 1996.

[16] V. Luzatti, H. Mustacchi, A. Skoulios, F. Husson, La structure des colloïdes d'association. I. Les phases liquide-cristallines des systèmes amphiphile-eau, Acta Cryst. 13 (1960) 660–667.

[17] F.B. Rosevear, Microscopy of liquid crystalline neat and middle phases of soaps and synthetic detergents, J. Am. Oil Chem. Soc. 31 (1954) 628–639.

[18] G. Lindblom, L. Rilfors, Cubic phases and isotropic structures formed by membrane lipids - possible biological relevance, Biochim. Biophys. Acta 988 (1989) 221–256.

[19] H. Schreier, Liposomen - ein neuartiger Arzneistoffträger, Pharmazie in unserer Zeit 4 (1982) 97–108.

[20] C. Washington, Drug release from microdisperse systems: a critical view, Int. J. Pharm. 58 (1990) 1–12.

[21] W. Higuchi, Analysis of data on the medicament release from ointments, J. Pharm. Sci. 51 (8) (1962) 802–804.

[22] J.N. Israelachvili, J.D. Mitchell, B.W. Ninham, Biochim. Biophys. Acta 470 (1977) 185–201.

[23] M. Ghyczy, Arzneimittel mit Phosphatidylcholin und Liposomen: Entwicklung, Bewertung, Perspektiven, in: R.H. Müller, G.E. Hildebrand (Eds.), Pharmazeutische Technologie: Moderne Arzneiformen, Wissenschaftliche Vertragsgesellschaft mbH, Stuttgart, Germany, 1997, pp. 155–166.