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Correlation of colloidal microstructure, drug release and permeation through excised human skin

I.L. Wilisch and C.C. Müller-Goymann

Institut für Pharmazeutische Technologie, TU Braunschweig, Braunschweig (Germany)

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Summary

For lamellar vehicles composed of lecithin, isopropyl myristate (IPM) and water, a phase transition into reversed hexagonal systems was observed on the addition of fenoprofen acid. Furthermore, the solubilization capacity for IPM was increased. An excess of oil resulted in reversed micellar solutions. The colloidal microstructure was characterized by polarized light microscopy, small-angle X-ray diffraction and transmission electron microscopy. Drug release from the systems increased with increasing content of IPM. The same trend was observed for drug permeation through excised human skin.

Introduction

Several investigations have been performed on percutaneous absorption of drugs using in vitro models with artificial or biological membranes (Flynn and Roseman, 1971; Barry and El Eini, 1976; Washitake et al., 1980; Bronaugh et al., 1982; Wild, 1989). The drug may be applied as a thin film (finite dose technique) or dissolved or suspended in a vehicle (infinite dose technique). The infinite dose technique provides insight into vehicle effects on the permeation of the drug. In order to increase the permeability of the skin several enhancing agents such as azone, dimethylsulfoxide or oleic acid which are supposed to fluidize the intercellular lipids of the stratum corneum can be added to the vehicle. The aim of the present work was to investigate the effect of an enhancer incorporated into a liquid crystalline vehicle on the microstructure of the formulation and on the permeation of drug through human skin. As enhancing agent isopropylmyristate (IPM) was chosen. The model vehicle was composed of lecithin and water. Fenoprofen acid, a non-steroidal anti-inflammatory agent was employed as the drug. The formulations were characterized with regard to their colloidal structure and drug release. Further investigations deal with percutaneous absorption of fenoprofen acid.

Correspondence to: C.C. Müller-Goymann, Institut für Pharmazeutische Technologie, TU Braunschweig, Braunschweig, Germany.

Materials and Methods

Materials

Fenoprofen acid was prepared from fenoprofen calcium dihydrate (Eli Lilly, Bad Homburg, Germany). The lecithin used was Phospholipon® (Rhone-Poulenc Rorer, Köln, Germany). Phospholipon® 90 consists of 95% of purified phosphatidylcholine, and less than 5% of lysolecithin with 1.5% of residual humidity. IPM (Caelo, Hilden, Germany) of a quality conforming to the German Pharmacopeia DAB 9 was used. IPM is a wax of low viscosity and good spreading properties. In spite of its generally good compatibility with skin and tissue, sensitization effects have also been reported (Komatsu et al., 1979).

Percutaneous absorption was investigated in vitro by using human full thickness skin. The samples were obtained by plastic surgery of female breasts. The tissue was pathologically negative. Fresh excised skin was cooled immediately after excision. Subepidermal tissue was removed mechanically. The samples were spread on aluminium foil and frozen in liquid nitrogen. They were stored for up to 6 weeks at −28°C.

Methods

Polarized light microscopy (PLM) All formulations were examined under a photomicroscope (Zeiss, Type III, Oberkochen, Germany) with crossed polarizers and a λ plate.

Small angle X-ray diffraction (SAXD) Small angle X-ray diffraction analysis was performed according to Kiessig (1942) using a local sensitive detector (Braun, Munich, Germany). The semisolid samples were exposed to the X-ray beam in a sample holder which was constructed by the Institut für Pharmazeutische Technologie der TU Braunschweig, Germany. The samples were pressed between Capton foils (Krempel, Vaihingen, Germany) to a thickness of 1 mm avoiding air bubbles. X-rays were produced by a PW-1730 generator (Philips, Kassel, Germany) with a copper anode (anode current, 40 mA; accelerating voltage, 80 kV). The exposure time was 300 s.

Transmission electron microscopy (TEM) 'Sandwiched' samples were shock-frozen in melting nitrogen at 63 K with a cooling rate of about 10^4–10^5 K/min. The samples were fractured in a high vacuum of less than 5×10^{-5} bar at 173 K. The fracture plane was replicated by shadowing with platinum/carbon (Pt/C) at an angle of 45°, followed by carbon-shadowing to improve the mechanical stability of the replica. The cleaned replicas were examined under an EM-300 transmission electron microscope (Philips, Kassel, Germany).

High-pressure liquid chromatography Reversed-phase HPLC was performed isocratically using a column of Hypersil® ODS 5 μm, 125 × 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 Beckman model 126 pump and model 165 UV detector (Beckman, Munich), with peak identification and integration on a Shimadzu C-R 3 A (Kyoto, Japan).

Diffusion experiments Release of drug from the vehicle and permeation of drug through human skin were investigated using a modified Franz cell (Franz, 1975) as diffusion apparatus. The acceptor compartment contained 80 ml of isotonic phosphate buffer solution of pH 7.4 at a temperature of 37°C. At appropriate intervals, samples were withdrawn from the acceptor, replacing the volume withdrawn with buffer. The drug concentration in the samples was monitored by HPLC.

For release experiments, a Spectropore 1 dialysis membrane (MWC 6000–8000; Spectrum Medical Industries, Los Angeles, CA) was used. The effective diffusion coefficient was calculated following Eqn 1 (Higuchi, 1960):

$$D_{\text{eff}} = \frac{Q\pi}{4tA^2C_0^2} \tag{1}$$

where D_{eff} is the effective diffusion coefficient, Q the cumulative mass released, t the time, A the effective membrane area and C_0 the drug concentration at $t = 0$ in the donor compartment. The effective membrane area was calculated according to Mueller-Goymann and Frank (1986).

For permeation experiments skin was placed between the donor and acceptor compartment,

dermal side to the acceptor. Before application of about 500 mg of the formulations upon the stratum corneum, the skin was allowed to hydrate for 2 h. The penetrability of the drug was evaluated the permeability coefficient P:

$$P = DK_p/h \quad (2)$$

where D is the diffusion coefficient, K_p the skin/vehicle partition coefficient and h the thickness of the stratum corneum. The permeability coefficient was determined from the plot of cumulative mass permeated (M) vs time according to Eqn 3.

$$P = M/tC_0 \quad (3)$$

Preparation of the formulations For the formulations, the vehicle components lecithin, water and IPM were melted together and cooled to room temperature while being stirred. After 72 h of equilibration, 10% of fenoprofen acid was added.

Results and Discussion

Colloidal structure of the formulations

Lamellar liquid crystals of lecithin and water are able to solubilize IPM up to a certain content without the occurrence of a phase transition. The investigated system of lecithin/water in a ratio of 62.8:37.2 solubilizes 14% IPM. Further increase in the concentration of IPM results in oily vesicle dispersions or in reversed micellar solutions.

Apolar molecules with a saturated hydrocarbon chain such as IPM can be incorporated only to a small extent into the bilayer between the apolar parts of the lecithin molecules as a result of weak van der Waals-London binding forces. Most of the IPM appears as bulk liquid. The excess of IPM forms the outer phase of the vesicle dispersions or the reversed micellar solutions.

On addition of fenoprofen acid to the lamellar systems, a phase transition into reversed hexagonal liquid crystals is observed. Even biphasic vesicle dispersions undergo transformation into reversed hexagonal systems. These systems are not very stable. During 4 weeks of storage they undergo transformation into reversed micellar solutions.

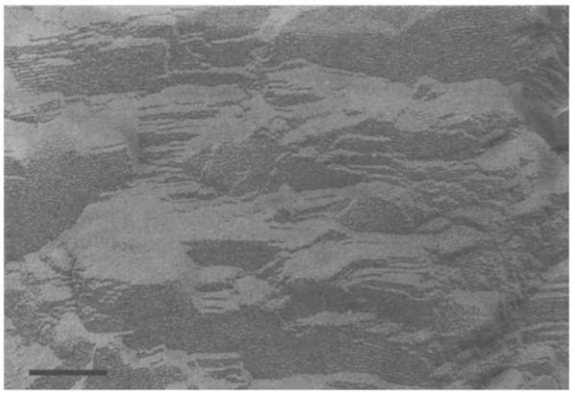

Fig. 1. Transmission electron micrograph of the reversed hexagonal structure containing 5.0% of IPM, 31.6% of water, 53.4% of lecithin and 10.0% of fenoprofen acid; bar 100 nm.

Under close investigation are systems containing lecithin and water in a ratio of 62.8:32.7 (w/w), 10.0% of fenoprofen acid and 5.0, 12.7, 25.0 and 36.0 % of IPM, respectively. Except for the micellar formulation containing 36.0% of IPM all systems are semisolid reversed hexagonal liquid crystals. Identification of the microstructure has been achieved by PLM, TEM and SAXD. PLM shows characteristic double refraction for the hexagonal systems, micellar solutions appearing isotropic without birefrigence. The micelles cannot be detected by TEM. For hexagonal systems aggregates shaped like tubes can be seen (Fig. 1). X-ray investigations reveal increasing diameters of the tubes of the hexagonal systems with increasing amount of IPM in the formulation (Fig. 2). The sharpness of the reflections diminishes simultaneously, indicating a loss of order in the microstructure. The micellar formulation with 36% IPM shows no periodicity under X-ray investigation but a scattering curve. The loss of order is obvious.

Lecithin bilayers may host amphiphilic molecules such as fenoprofen which is inserted into the polar region of the bilayers. The form of the new associates is determined by the geometry of the components which can be described by the

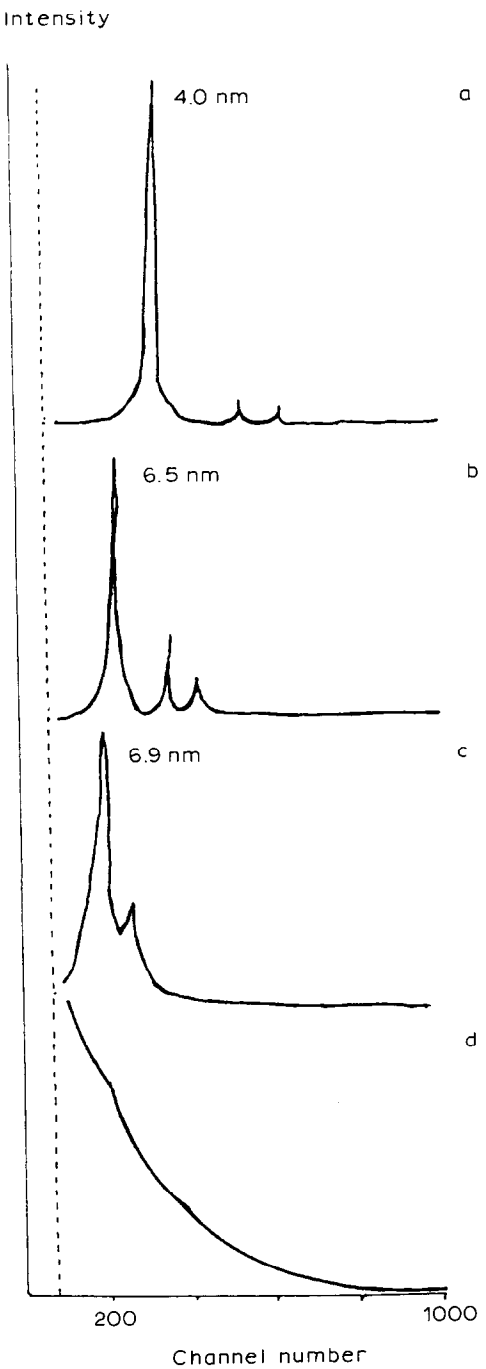

Fig. 2. SAXD pattern of the quaternary formulations (IPM, water, lecithin, fenoprofen acid) with increasing content of IPM: (a) 5.0%, (b) 12.7%, (c) 25.0% and (d) 36.0%. The interlayer spacing (in nm) of the first peak is indicated in the graph.

packing ratio (Tanford, 1980; Israelachvili et al., 1976, 1977, 1980). Hydrated lecithin molecules, shaped like a cylinder (packing ratio = 1), tend to form lamellar structures. By addition of fenoprofen acid a conical element is integrated. The overall packing ratio increases and according to Silver (1985) a reversed hexagonal order arises. Because of the shortness of the drug molecule compared to lecithin molecules, 'gaps' would arise, located in the apolar region of the bilayer. These gaps may be filled by hydrocarbon chains of the lecithin with simultaneous reduction of the interlayer spacing (Hamann, 1990) or by IPM molecules increasing the solubilizing capacity of the system for IPM. Phase transformation into micellar solutions which is observed on storage for 4 weeks is due to the weak binding forces between lecithin and IPM molecules. Simultaneously, dissolution of drug within the lipophilic bulk phase competes with the incorporation of fenoprofen into the bilayer. Decreasing drug content within the bilayer results in a decreased solubilization capacity for IPM, favouring the phase transition into the micellar solution.

Release experiments

Drug release increases with increasing IPM content of the formulation. The effective diffusion coefficients are given in Table 1. This observation can be explained by the increased mobility of the drug, especially within the micellar system of low viscosity.

Permeability through excised human skin

For all investigated systems, the skin is rate limiting for the permeation of drug as expressed

TABLE 1

Effective diffusion coefficients D_{eff} depending on the concentration of IPM

Content of IPM (%)	D_{eff} (cm^2/s)
5.0	3.2×10^{-9}
12.7	5.3×10^{-9}
25.0	6.9×10^{-9}
36.0	14.4×10^{-9}

TABLE 2

Permeability coefficients (P) depending on the concentration of IPM

Concent of IPM (%)	Mean permeability coefficient P (cm/s) ($n = 3$)
5.0	0.6×10^{-7}
12.7	0.8×10^{-7}
25.0	0.9×10^{-7}
36.0	2.5×10^{-7}

n, number of probands.

via the linear correlation between permeated amount of drug and time. Increasing the content of IPM results in increased permeability. Due to great inter-individual variation, comparative investigations must be performed with skin of the same donor. It is worth mentioning that there are small differences between the different hexagonal systems. Changing the colloidal structure results in a drastic increase in permeation (see permeability coefficients Table 2). By increasing the amount of IPM while diminishing the content of water, the formulation becomes more lipophilic. Hence, the partition coefficient of the drug between skin and vehicle should decrease. This assumption is correct unless the skin is altered by the formulation. However, enhancing properties have been discussed for IPM (Loth, 1991). IPM molecules penetrate into the intercellular lipid bilayer of the stratum corneum and thus increase the lipophilicity of the skin. Therefore, the skin/vehicle partition coefficient should change during permeation.

Enhancement of drug permeation into the skin requires sufficient penetration of the enhancer itself. Therefore, the thermodynamic activity of IPM molecules within the formulation is important. With respect to the microstructure, it is obvious that bound IPM molecules are less active than those of the bulk phase. Increasing the IPM content until a phase transition occurs increases the number of thermodynamically active molecules, so that the considerable increase in permeation enhancement for the micellar system can be explained.

Conclusion

Addition of fenoprofen to the vehicles alters the microstructure of the formulation. Fenoprofen acid promotes a state of high order. In contrast, an increase in the content of IPM results in a gain in mobility. Thus, drug release must be considered in context with the microstructure of the formulation. The effectiveness of a penetration enhancer relates not only to its concentration in the vehicle but also to its integration into the microstructure. Strongly bound enhancer molecules result in a smaller promoting effect on drug permeation.

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References

Barry, B.W. and El Eini, D.D., Influence of nonionic surfactants on permeation of hydrocortisone, dexamethasone, testosterone and progesterone across celluloseacetate membranes. *J. Pharm. Pharmacol.*, 28 (1976) 219–227.

Bronaugh, R.L., Stewart, R.F. and Congdon, E.R., Methods for in vitro percutaneous absorption studies: II. Animal models for human skin. *Toxicol. Appl. Pharmacol.*, 62 (1982) 481–488.

Flynn, G.L. and Roseman, T.J., Membrane diffusion: II. Influence of physical adsorption on molecular flux through heterogeneous dimethyl-polysiloxane barriers. *J. Pharm. Sci.*, 60 (1971) 1788–1796.

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

Hamann, H.-J., Wechselwirkungen mesogener Arzneistoffe mit kolloidalen Lecithinassoziaten am Beispiel des Fenoprofens, Dissertation, Braunschweig, 1990.

Higuchi, T.J., Physical chemical analysis of percutaneous absorption process from creams and ointments. *Soc. Cosm. Chem.*, 11 (1960) 85–97.

Israelachvili, J.N., Marcelja, S. and Horn, R.G., Physical properties of membrane organization. *Q. Rev. Biophys.*, 13 (1980) 121–200.

Israelachvili, J.N., Mitchell, D.J. and Ninham, B.W., Theory of self-assembly of hydrocarbon amphiphiles into micelles

and bilayers. *J. Chem. Soc. Far. Trans. II*, 72 (1976) 1525–1568.

Israelachvilli, J.N.., Mitchell, D.J. and Ninham, B.W., Theory of self-assembly of lipid bilayers and vesicles. *Biochim. Biophys. Acta*, 470 (1977) 185–201.

Kiessig, H., Roentgenuntersuchung grosser Netzebenenabstaende und Untersuchung stroemender Loesungen. *Kolloid Z.*, 98 (1942) 213–221.

Komatsu, H., Asaba, K. and Suzuki, M., Some biochemical effects of isopropylmyristate and squalene on rabbit skin. *J. Soc. Cosm. Chem.*, 30 (1979) 263–278.

Loth, H., Vehicular influence on transdermal penetration. *Int. J. Pharm.*, 68 (1991) 1–10.

Mueller-Goymann, C.C. and Frank, S.G., Interaction of lidocaine and lidocaine·HCl with liquid crystal structure of topical preparation. *Int. J. Pharm.*, 29 (1986) 147–159.

Tanford, C., *The Hydrophobic Effect*, Wiley, New York 1980, Chap. 6, 11.

Silver, B., *The Physical Chemistry of Membranes*, Allen & Unwin/Salomon, Winchester, MA, 1985, p. 75.

Washitake, M., Takashima, Y., Tanaka, S., Anmo, T. and Tanaka, I., Drug permeation through egg shell membranes. *Chem. Pharm. Bull.*, 28 (1980) 2855–2861.

Wild, T., Einfluss der physikochemischen Eigenschaften von Arzneistoffen und Vehikeln auf die Permeabilitaet der menschlichen Hornschicht, Dissertation, Saarbruecken, 1989.