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Diclofenac release from phospholipid drug systems and permeation through excised human stratum corneum

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Abstract

This study deals with the relationship between the colloidal structure of a topical formulation and the drug release in vitro as well as the influence of the microstructure on the stratum corneum drug permeability. The nonsteroidal anti-inflammatory drug diclofenac diethylamine was chosen as model drug. The vehicles consist of phospholipids, diclofenac diethylamine and water. Depending on the ratio of the three components, these systems have various colloidal structures from liposomal dispersions via microemulsions to lamellar liquid crystals. The drug participates in the microstructure of the resulting systems. A dialysis membrane impregnated with silicone polymer was used for the in vitro release studies. The effective diffusion coefficient of diclofenac diethylamine changes rapidly with a phase transformation of the vehicle. Drug transport across the stratum corneum from aqueous solution and from vehicles with a high effective diffusion coefficient is controlled by the stratum corneum. In contrast to this observation the flux from the phospholipid drug systems with a low effective diffusion coefficient is controlled by drug release from the vehicle. The diffusional resistance inside these vehicles is higher than that in the stratum corneum. The drug release from liposomes is too slow, so that there is no stratum corneum permeation of diclofenac diethylamine from liposomes at all, either from large multilamellar vesicles or from small unilamellar vesicles. Fluoromicrography of cryosections of human skin shows that intact liposomes cannot penetrate deep into the skin. The fluorescence is limited to the cell layers of the stratum corneum.

Keywords: Topical formulation; Diclofenac diethylamine; Phospholipid; Liposome; Microemulsion; In vitro release; Stratum corneum permeability

1. Introduction

The ability of a drug in a topical formulation to permeate the skin and to exert its effect is dependent on two consecutive events. The drug must first diffuse out of the vehicle to the skin surface and then it must permeate this barrier en route to the site of action. Both steps are dependent upon the physicochemical properties of the drug, vehicle and barrier (Nishihata et al., 1988). The stratum corneum provides the principal barrier to the percutaneous permeation of topically applied substances (Barry, 1983). For controlled transdermal drug delivery the diffusional resistance inside the vehicle should be higher than that in the stratum corneum (Tiemessen, 1989).

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The aim of this study was to investigate the relationship between colloidal vehicle structure and the kinetics of drug release in vitro, and to investigate the influence of the colloidal structure on the stratum corneum permeability. Systems consisting of phospholipids, diclofenac diethylamine and water were chosen as model vehicles to study drug release. Diclofenac, which is a strong nonsteroidal anti-inflammatory drug, can be absorbed by transdermal application (Takahashi et al., 1991). Diclofenac diethylamine is an amphiphilic molecule, hence interactions with phospholipids are very distinct. The addition of diclofenac diethylamine to phospholipid water systems leads to phase transitions. Drug molecules participate in the microstructure of the resulting systems. Depending on the ratio of drug to phospholipids and water, these systems have various colloidal structures (multilamellar vesicles, lamellar crystals, microemulsions and mixed phases of these structures) (Kriwet and Müller-Goymann, 1994).

The aqueous phospholid drug systems tend to exchange water with an aqueous acceptor phase during in vitro studies of drug release. The water uptake induces phase transition of the systems, so that no reliable information about the diffusional behaviour of the drug can be obtained. Therefore, it was necessary to use a water impermeable membrane for the drug release studies that separates the vehicle from the aqueous acceptor phase. In order to make sure that the membrane did not control the drug release rate, a membrane with a high drug permeability was selected. A dialysis membrane impregnated with a silicone polymer (Tiemessen, 1989) was used.

Since the stratum corneum is the major permeability barrier of the skin, the drug transport from vehicles with different colloidal structures through isolated human stratum corneum was also investigated. The kinetics of the drug release in vitro were compared to the drug permeation through the stratum corneum barrier. On account of the numerous studies and the discussion about liposomes as a drug carrier in the skin (Mezei and Vijeyalakshmi, 1982; Lasch et al., 1991; Hofland, 1992), emphasis was laid on the vehicles that contained liposomes. Fluoromicrography of cryosections of skin was employed to investigate the penetration of liposomes into the skin.

2. Materials and methods

2.1. Materials

Diclofenac diethylamine was a gift from Ciba Geigy, Basel, Switzerland. Phospholipon 90 G® is a highly purified soybean lecithin with a mass content of phosphatidylcholine of up to 90% (donated by Nattermann, Köln, Germany). Triamteren was purchased from Bayer AG (Leverkusen, Germany). Double-distilled water was used throughout the study. All chemicals were employed without further purification.

2.1.1. Silicone impregnated membrane

The silicone impregnated membrane for the release experiments was produced by pouring out a solution of 2% w/v silicone polymer (Wacker Chemie, München, Germany) in diethyl ether on a dialysis membrane (Spectrapor, MWCO 600–8000, Spectum Medical Industries, Los Angeles, CA), so that the membrane contained 1.2 mg silicone per cm^2.

2.2. Methods

2.2.1. Preparation of the ternary systems

The three components (diclofenac diethylamine, phospholipids and water) were heated and mixed together at 60°C for 15 min. Then the mixtures were cooled to room temperature while being stirred with a teflon coated magnet. Weight loss of water was replaced at room temperature and the systems were stirred again.

2.2.2. Preparation of small unilamellar vesicles (SUVs)

The SUVs were formed by sonication of the three components with a Soniprep 150 MSE Ultraintegrator (MSE Scientific Instruments, Crawley, UK) for 10 min at intervals of 60 s. Thereafter the liposomes were filtered through a cellulose acetate membrane of pore size 0.2 μm (Seitz, Bad Kreuznach, Germany). By photon correlation spectroscopy and transmission electron mi-

croscopy of freeze-fractured samples it was determined that the SUVs had a size diameter between 20 and 30 nm.

2.2.3. Viscosity

A Haake Rotovisco viscometer RV 100 with measuring system CV 100 and measuring cup and bob ME 15 (Haake, Karlsruhe, Germany) was used at 20°C.

2.2.4. In vitro drug release

The in vitro drug release experiments were performed at a temperature of 20°C by using a modified Franz cell (2.25–2.55 cm^2 surface area, 75–82 ml acceptor medium, 0.5 g vehicle on the donor side). The acceptor medium was isotonic phosphate buffer pH 7.4 with a content of 10% methanol (v/v) to maintain sink conditions. Water content of the vehicles changed less 3% during 10 h. The drug concentration in the acceptor phase was determined continuously by pumping it through a flow-through cuvette of spectrophotometer (Shimadzu UV 210 A, Duisburg, Germany). The absorbance at 280 nm was recorded (x,t recorder Pm 8222, Philips, Kassel, Germany).

2.2.5. Permeability study

Permeation experiments with a modified Franz cell were carried out at 37°C (0.7–0.8 cm^2 surface area, 0.2 g vehicle on the donor side, 5.5–6.1 ml acceptor medium of isotonic phosphate buffer pH 7.4). The stratum corneum was prepared by trypsination (Tiemessen, 1989) of excised human skin, obtained from plastic surgery of healthy female breasts. Prior to the experiment, the stratum corneum was hydrated in buffer solution for 30 min. Stratum corneum was laid onto a polycarbonate membrane (5 μm pore size, TMTP Millipore, Eschborn, Germany), which provided mechanical strength for the thin and fragile horny layer. Diclofenac diethylamine permeates from the vehicle through completely hydrated stratum corneum into the aqueous acceptor. Samples of the acceptor solution were collected over a period of 24 h and the drug concentration was analysed by HPLC (Kriwet and Müller-Goymann, 1993).

2.3. Preparation of liposomes and microemulsions with the fluorescence probe triamteren

Triamteren is a lipophilic fluorescence probe with very low solubility in water. 1 g of diclofenac diethylamine (for microemulsions) or 200 mg of diclofenac diethylamine (for liposomes), 1.2 g phospholipids and 10 mg triamteren were dissolved in 50 ml methanol. After evaporation of methanol, the resulting film was resuspended in 20 ml water. Depending on the ratio of drug to phospholipids, liposomes (6% phospholipids and 1% diclofenac diethylamine) or microemulsion (6% phospholipids and 5% diclofenac diethylamine) were formed.

2.4. Fluoromicrography of cryosections of human skin

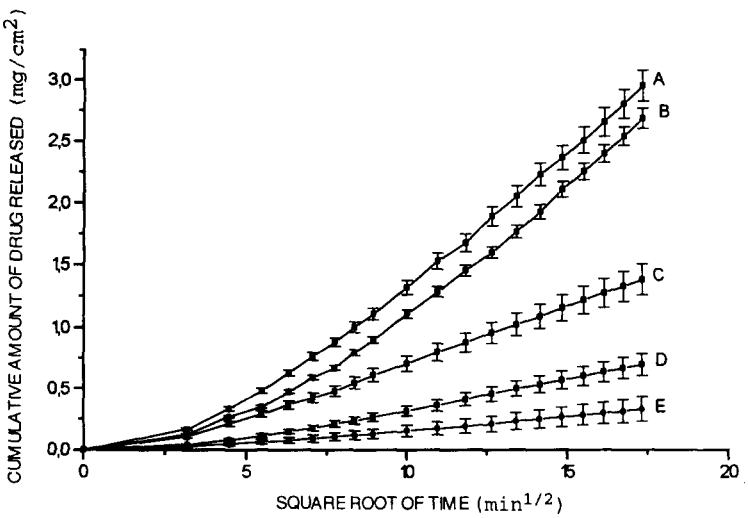
Systems with triamteren were applied to the excised human skin. A volume of 0.5 ml of the liposomes or the microemulsions was pipetted onto 2.5 cm^2 skin. After 24 h the skin was cryofixed in liquid nitrogen and cut with a cryomicrotome at thickness of 7 μm (Kryostat, Watter Dittes, Heidelberg, Germany). The slices were examined with the fluorescence microscope (Forschungsmikroskop Zeiss, Oberkochen, Germany). Fluorescence excitation was at 365 nm and a 410 nm Zeiss filter was used.

3. Results and discussion

3.1. Suitability of the membrane for the in vitro release studies

The diffusion profile of diclofenac diethylamine from aqueous solution through the silicone impregnated membrane is recorded. After an initial time lag which takes 16 min, linearity is achieved when the amount of drug diffused is plotted vs the square root of time. The flux rate through the membrane increases linearly with an increase of the concentration of drug dissolved (Kriwet, 1994). This observation indicates that the membrane does not control the drug diffu-

a)



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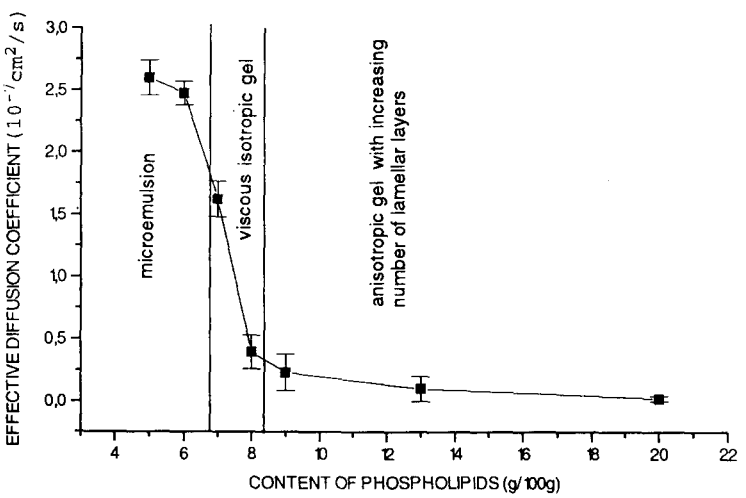

Fig. 1. (a) Release of diclofenac diethylamine from vehicle with 5% diclofenac diethylamine and various concentrations of phospholipids at 20°C. (A,B) Microemulsion with (A) 5% phospholipids and (B) 6% phospholipids; (C–E) anisotropic gel with (C) 9.3%, (D) 13.8% and (E) 20% phospholipids, respectively. (b) Effective diffusion coefficient of diclofenac diethylamine from vehicle with 5% diclofenac diethylamine vs content of phospholipids. Data represent mean and standard deviation of three duplicate determinations.

sion rate. Only within the first 16 min does membrane control occur.

3.2. *In vitro diclofenac diethylamine release from the vehicles*

The drug release of vehicles with 5% diclofenac diethylamine and various concentration of phospholipids are investigated. When the amount of drug released is plotted vs the square of time, a linear relationship is obtained for each vehicle after an initial lag time (Fig. 1a). The slope of the plots in the time interval 60–360 min are determined by using linear regression. A correlation coefficient higher than 0.999 is obtained in each experiment. Values of the effective diffusion coefficient D are calculated following the Higuchi equation (Higuchi, 1962) (Eq. 1):

$$Q = 2 \cdot A \cdot C_0 \cdot (D \cdot t \cdot \pi^{-1})^{1/2}$$

where Q is the cumulative amount of drug released (mg), A denotes the membrane arear (cm^2), C_0 is the initial concentration of drug in the vehicle (mg cm^{-3}), D represents the effective diffusion coefficient (cm^2 s^{-1}) and t is time (s).

In Fig. 1b the calculated effective diffusion coefficients are plotted against the content of phospholipids in the vehicles. The content of diclofenac diethylamine is kept constant in all systems. Every given effective diffusion coefficient is a mean value of three separate release experiments. The effective diffusion coefficients decrease with an increasing of the content of phospholipids. The decrease is rapid in the concentration range from 6 to 9% phospholipids. The maximum difference between the coefficients amounts up to a factor of 100.

The microstructure of the systems influences the diffusion behaviour of diclofenac diethylamine. The systems with a content of 6% phospholipids and less are microemulsions with low viscosity (Kriwet and Müller-Goymann, 1994). The release of the drug from the microemulsion is fast. The addition of phospholipids leads to phase transformation into isotropic gels. The microstructure of the resulting systems consists of

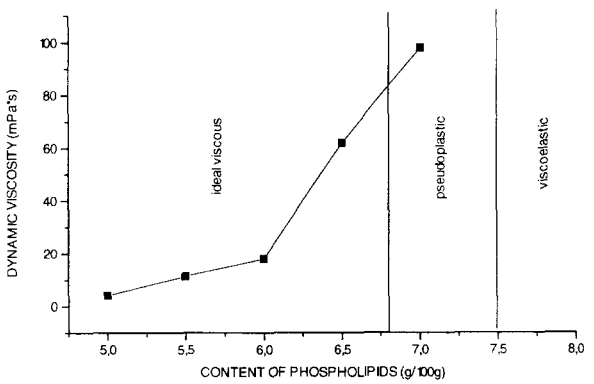

Fig. 2. Dynamic viscosity of aqueous systems with 5% diclofenac diethylamine and various concentrations of phospholipids at 20°C determined with a rotational viscometer. The viscosities of the systems with viscoelastic properties are not detectable with the rotational viscometer.

droplets with few lamellar layers surrounding the droplets (Kriwet and Müller-Goymann, 1994). The phase transition from microemulsion into viscous gels is detectable at the diffusion behaviour (cf. Fig. 1b). The differences in the effective diffusion coefficients can be explained by the change of the viscosity of the systems (Fig. 2). A rapid increase of the viscosity leads to a strong decrease in the effective diffusion coefficient.

The number of lamellar layers in the microstructure grows with further addition of phospholipids and the flow characteristics of the systems change from pseudoplastic flow into viscoelastic properties (Fig. 2). The effective diffusion coefficient of diclofenac diethylamine decreases gradually. The release of the drug from these systems is very slow. Only a fraction of the drug content of the vehicle is released while the time of experiment (for example E of Fig. 1a (20% phospholipids and 5% diclofenac diethylamine) 3% of the drug content of the system is delivered during 360 min). Since the water content of these systems is 75% and more, the volume fraction of the hydrophilic domains in these systems is large. The diffusion of the drug molecules may take place either via these water domains or via the lamellar layers (Tiemessen, 1989). Diclofenac diethylamine has a low solubility in water, the part of diclofenac diethylamine

which is dissolved in the hydrophilic domains is small. Hence the diffusion through the water domains should not play a significant role. The drug molecules are located within the bilayer of the lamellae. Diclofenac diethylamine is not simply dissolved in the lipophilic region of the phospholipids (between the alkyl chains), but the amphiphilic drug molecules align with the appropriate regions of phospholipid molecules forming the bilayer (polar-polar, nonpolar-nonpolar) (Kriwet and Müller-Goymann, 1994). This incorporation seems to be rigid and hinders the drug molecules from diffusion. The effective diffusion coefficient does not increase with an increasing of the number of the lamellar layers, which would occur if diclofenac diethylamine is able to diffuse easily through the lamellae. The diffusion of the drug out of its position within the bilayer into the hydrophilic domains or into the lipophilic part of the lamellae may be the rate limiting step of the drug release.

The influence of drug content within the vehicle on the effective diffusion coefficient is also investigated. Fig. 3 shows the calculated coefficients plotted against the content of diclofenac diethylamine. Up to a content of 4.5% diclofenac diethylamine there is a gradual increase of the effective diffusion coefficient. Systems with more than 4.5% diclofenac diethylamine show a rapid

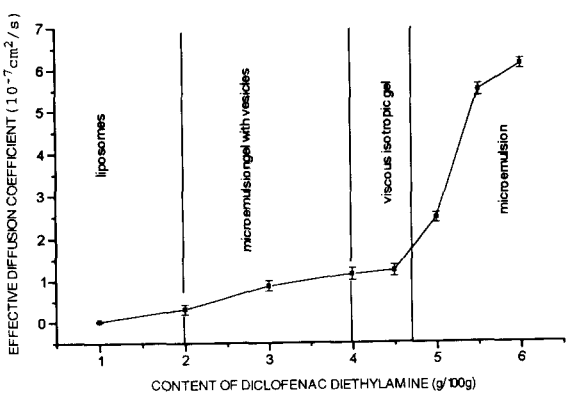

Fig. 3. Effective diffusion coefficient of diclofenac diethylamine in systems with 6% phospholipids vs content of diclofenac diethylamine. Data represent mean and standard deviation of three duplicate determinations.

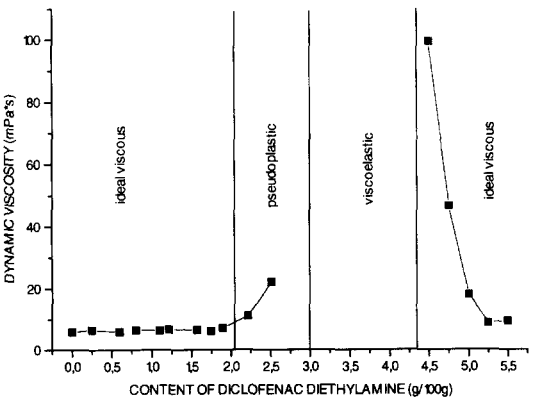

Fig. 4. Dynamic viscosity of aqueous systems with 6% phospholipids and various concentrations of diclofenac diethylamine at 20°C determined with a rotation viscometer. The viscosities of the systems with viscoelastic properties are not detectable with the rotational viscometer.

increase of their coefficients. This increase is in accordance with the phase transition into microemulsions that occurs with addition of diclofenac diethylamine. Drug phospholipid systems up to a content of 4.5% diclofenac diethylamine form either liposomal dispersion, dispersions of multilamellar vesicles in a microemulsion gel or an isotropic gel (Kriwet and Müller-Goymann, 1994). The viscosity of these systems is high, even viscoelastic properties occur (Fig. 4). Therefore the release of the drug is slow. Further addition of diclofenac diethylamine initials microemulsion systems with low viscosity. The rate of drug delivery increases with the decrease of the viscosity.

The differences in the dynamic viscosity cannot completely explain the diffusion bearing of the drug because within the region of microemulsion gel with vesicles the systems show strong differences in viscosity. These differences in viscosity are not detectable in the diffusion behaviour. A system with 6% phospholipids and 2.5% diclofenac diethylamine has a clearly lower viscosity than a system with 6% phospholipids and 4% diclofenac diethylamine. Nevertheless, the higher effective diffusion coefficient is observed in the system with the high viscosity. A possible explanation for the observed phe-

nomenon may be based upon the following reasoning: in these systems there are vesicles with multilamellar layers. The number of vesicles decreases with the addition of diclofenac diethylamine before the systems transform into the pure microemulsion. Drug molecules encapsu-

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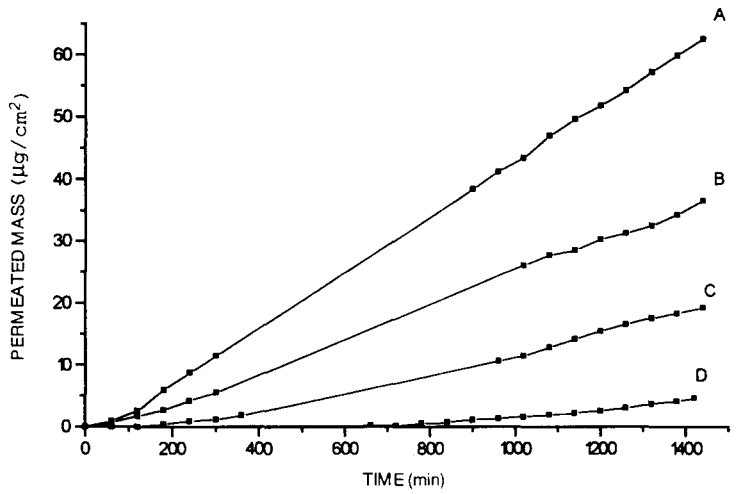

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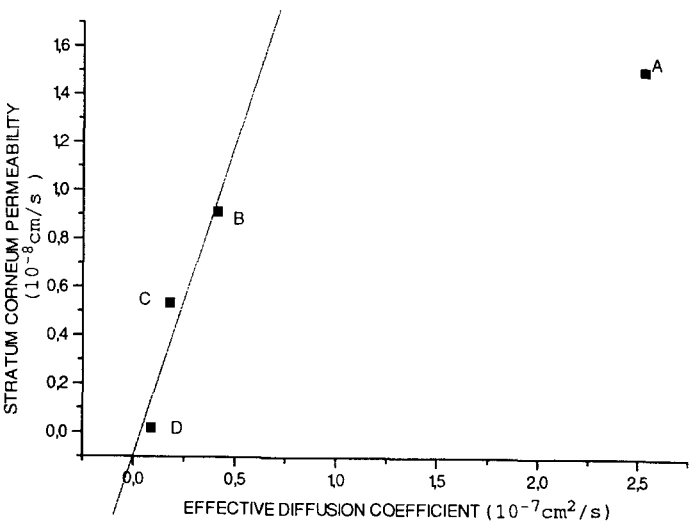

Fig. 5. Diclofenac diethylamine permeation through stratum corneum (source A) from vehicles with 5% diclofenac diethylamine and various concentration of phospholipids. (A) Microemulsion with 5% phospholipids and (B–D) anisotropic gel with (B) 9.3%, (C) 13.8% and (D) 20% phospholipids respectively. (a) Typical permeation curves. (b) Stratum corneum permeability vs effective diffusion coefficient from the vehicles with 5% diclofenac diethylamine.

Table 1
Permeation flux through stratum corneum from 0.5 or 1% aqueous solution of diclofenac diethylamine and microemulsions

Source	Type of system	Flux ($\times 10^{-10}$) (g/cm^2 per s)
A	aqueous solution	
	0.5% drug	5.20
	microemulsion	
	5% PL+5% drug	7.49
	microemulsion	
	4% PL+4% drug	9.47
B	aqueous solution	
	0.5% drug	5.30
	aqueous solution	
	1% drug	5.42
	microemulsion	
	6% PL+5% drug	5.94
C	aqueous solution	
	0.5% drug	6.14
	aqueous solution	
	1% drug	6.28
	microemulsion	
	5% PL+5% drug	7.70
D	aqueous solution	
	0.5% drug	5.20 ± 0.60 [a]
E	aqueous solution	
	0.5% drug	9.12
F	aqueous solution	
	0.5% PL+5% drug	9.40 ± 0.70 [a]

PL, phospholipids.
[a] Mean and standard deviation ($n = 3$).

lated within the vesicles are hindered to diffuse. Therefore the drug release increases with the decrease of the number of vesicles.

3.3. *In vitro drug permeation through human stratum corneum*

The diclofenac diethylamine can permeate isolated human stratum corneum from aqueous solutions (Table 1). There are inter- and intra-individual differences between the skin samples due to the biological variability (Provost et al., 1986). Since variations between stratum corneum of different individuals are far more important in our experiment than variations between samples of the same skin, a series of experiments was carried out on the stratum corneum of one individual. The flux from aqueous solution is nearly identical at dissolved drug concentrations of 0.5 and 1%. Since the solubility of diclofenac diethylamine in water is 1.3% (Kriwet and Müller-Goymann, 1993) a 2-fold difference in the flux was expected. This is obviously not the case. Due this observation it can be concluded that the slow permeation is membrane controlled.

Fig. 5a shows the transport profiles of diclofenac diethylamine through stratum corneum from phospholipid drug systems with 5% drug and various concentrations of phospholipids. A flux of diclofenac diethylamine from microemulsion (Fig. 5a, A) can be detected beyond a short lag-time. Permeability coefficients decrease with an increase of the concentration of phospholipids (Fig. 5a, B–D). The permeability coefficients are calculated by using Eq. 2 (Flynn et al., 1974):

$$J = P \cdot \Delta C$$

where J is the steady-state flux per unit area (g cm^{-2} s^{-1}), P denotes the permeability coefficient (cm s^{-1}) and ΔC is the concentration difference across the stratum corneum (g cm^{-3}).

The calculated permeability coefficients and the lag-times are listed in Table 2. If these results are compared to the in vitro release studies, a linear relationship between the release rate of diclofenac diethylamine out of the vehicle through the silicone impregnated membrane and the rate

Table 2
Permeability and lag-time of diclofenac diethylamine permeation through stratum corneum (source A) from systems with 5% drug and various concentration of phospholipids

Type of system	Content of phospholipids (%)	Permeability coefficient ($\times 10^{-9}$) (cm/s)	Lag-time (min)
Microemulsion	5	15.00	64
Anisotropic gel	9.3	9.14	100
Anisotropic gel	13.8	5.36	230
Anisotropic gel	20	1.90	730

of drug permeability across stratum corneum is observed up to an effective diffusion coefficient of 4×10^{-8} cm^2 s^{-1} (Fig. 5b). Diffusional resistance inside the vehicles with a low effective diffusion coefficient is higher than that in the stratum corneum. The rate limiting step for the

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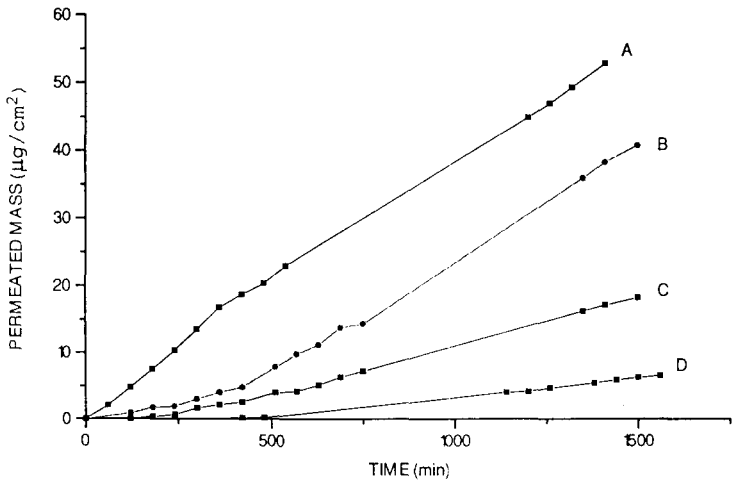

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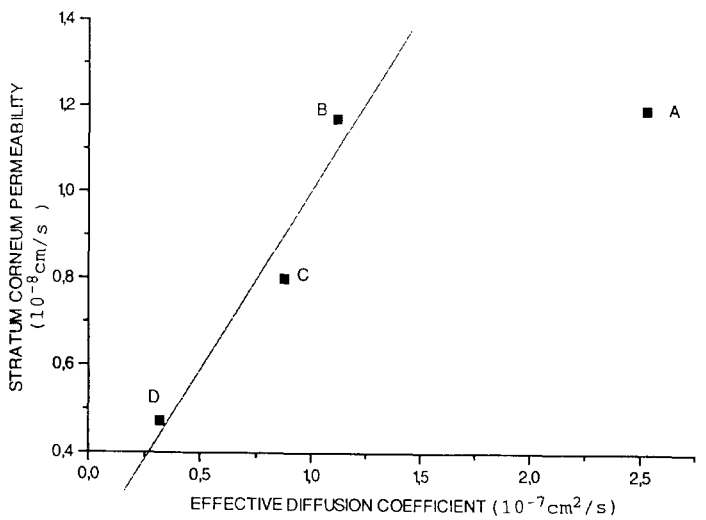

Fig. 6. Diclofenac diethylamine permeation through stratum corneum (source B) from vehicles with 6% phospholipids and various concentrations of diclofenac diethylamine. (A) Microemulsion with 5% drug, (B) isotropic gel with 4% drug and (C,D) microemulsion gel with vesicles with (C) 3% drug and (D) 2% drug. (a) Typical permeation curves. (b) Stratum corneum permeability vs effective diffusion coefficient from the vehicles with 6% phospholipids.

Table 3
Permeability and lag-time of diclofenac diethylamine permeation through stratum corneum (source B) from systems with 6% phospholipids and various concentrations of diclofenac diethylamine

Type of system	Content of drug	Permeability coefficient ($\times 10^{-9}$) (cm/s)	Lag-time (min)
LMVs [a]	1%	no permeation	
LMVs [a]	1%	no permeation	
LMVs	4% PL + 1%	no permeation	
SUVs	2% [c]	no permeation	
SUVs [b]	2% [c]	no permeation	
SUVs [b]	2% [c]	no permeation	
Microemulsion gel with vesicles	2% [c]	4.72	580
Microemulsion gel with vesicles	3%	8.00	325
Isotropic gel	4%	11.70	290
Microemulsion	5%	11.90	64

PL, phospholipids.
[a] Source E; [b] source F; [c] the microstructure of systems with 6% phospholipids and 2% diclofenac diethylamine depends on the method of preparation.

drug transport across the stratum corneum is the release from the vehicle. For vehicle with a high release rate, the diffusional resistance inside the stratum corneum is greater than that in the vehicle and the permeability is stratum corneum controlled.

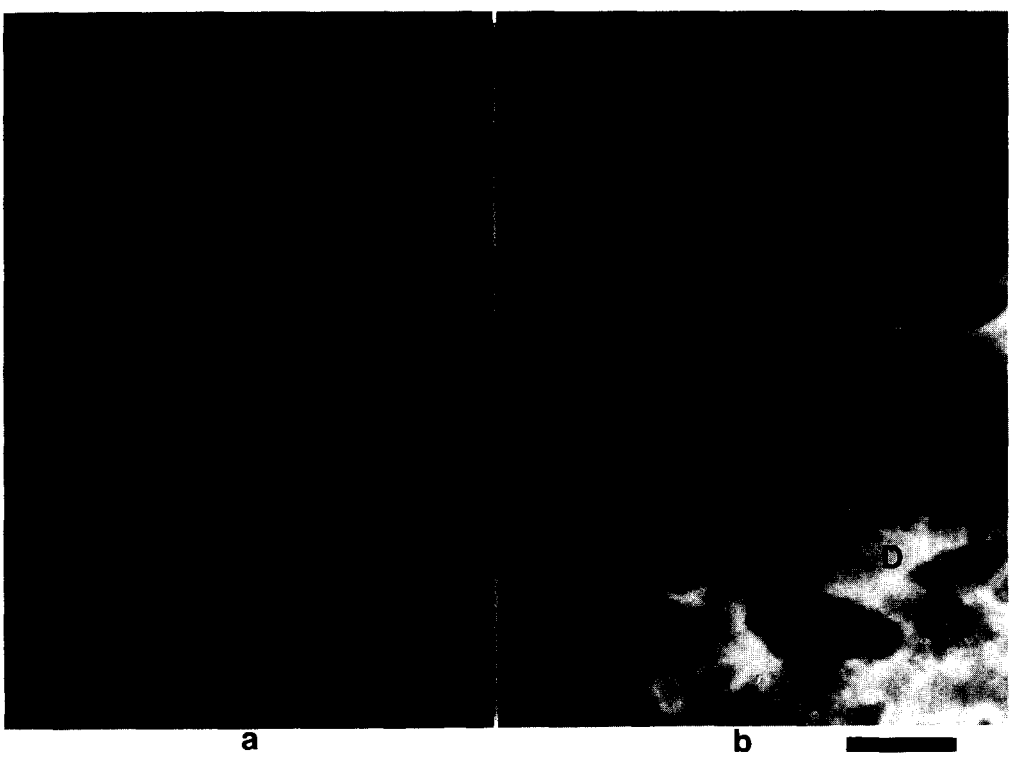

Fig. 7. Fluoromicrographs of human skin 24 h after application of (a) small unilamellar liposomes (6% phospholipids and 1% diclofenac diethylamine) and (b) a microemulsion (6% phospholipids and 5% diclofenac diethylamine) with the fluorescence probe triamteren (bar = 400 μm). S, stratum corneum; E, epidermis; D, dermis.

Permeation through the stratum corneum of diclofenac diethylamine from systems with 6% phospholipids and various concentrations of drug was also measured. There was no permeation through the horny layer from pure liposomal formulations, either from large multilamellar vesicles or from small unilamellar vesicles over a period of 24 h (Table 3). This can be explained by the high affinity of the drug to the liposomes. Diclofenac diethylamine participates in the lamellar layers (Kriwet and Müller-Goymann, 1994), therefore the release of the drug is too slow. The intact liposomes cannot permeate the stratum corneum and reach the aqueous solution.

Fig. 7a shows a fluoromicrograph of cryosection of human skin after application of fluorescence labeled small unilamellar liposomes within 24 h. Fluorescence is visible in the first stratum corneum cell layers. Intact fluorescence labeled liposomes cannot penetrate deeper into the epidermis. Their transition into the hydrophilic part of the skin is hindered. The fluorescence micrograph cannot answer the question whether the liposomes distribute into the whole stratum corneum or whether the fluorescence probe leaves the liposomes and dissolves in the intercellular lipid lamellae of the horny layer. The lipophilic probe cannot penetrate deeper into the skin.

In contrast to this observation, a flux of diclofenac diethylamine from microemulsion gel with vesicle can be detected (Fig. 6a, B–D). The permeability coefficients increase with an increasing content of diclofenac diethylamine (Table 3). Phase transformation occurs and pure microemulsions with low viscosity and high drug stratum corneum permeability arise (Fig. 6a, A). From the microemulsion the lipophilic fluorescence probe can penetrate deep into the skin. Fluorescence is visible in the whole skin (Fig. 7b).

A linear relationship between effective diffusion coefficients from the in vitro release experiment and the stratum corneum permeability of diclofenac diethylamine is observed again only at low effective diffusion coefficients (Fig. 6b). For systems with a higher drug release rate diffusion through the stratum corneum is the rate limiting step.

The permeation flux of diclofenac diethylamine from microemulsion is higher than the flux from aqueous solution (Table 1). The microemulsions enhance the permeation of diclofenac diethylamine through the stratum corneum. The stratum corneum controls the drug transport across the horny layer in both cases. Enhancing properties of the skin permeation of diclofenac have been reported for phospholipids (Nishihata et al., 1987). Our investigation shows that the enhancing efficiency is depending on the integration of the phospholipids and the drug in the microstructure of the vehicle. Only the microemulsion enhances the stratum corneum permeation of diclofenac diethylamine. The phospholipids are able to interact with the structures of the stratum corneum, if they are applied as microemulsion. The drug and the phospholipids are strongly bound in the liposomal formulations and the other investigated systems and an enhancer effect is not detectable.

4. Conclusion

The complexity of the process of percutaneous absorption results from a multitude of vehicle drug skin interactions. Our results confirm that the influence of the colloidal structure of the chosen vehicle on the stratum corneum permeability is distinct (Wilisch and Müller-Goymann, 1993). For vehicle with a low drug release rate the drug delivery is the rate limiting step in drug permeation. In contrast to this observation drug transport across the stratum corneum from aqueous solution and from vehicles with high effective diffusion coefficients is controlled by the stratum corneum, since the diffusion resistance in the stratum corneum becomes greater than that inside the vehicle.

Acknowledgements

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