

# In-vitro Permeability of the Human Nail and of a Keratin Membrane from Bovine Hooves: Influence of the Partition Coefficient Octanol/Water and the Water Solubility of Drugs on their Permeability and Maximum Flux

DIRK MERTIN AND BERNHARD C. LIPPOLD

*Department of Pharmaceutical Technology, Heinrich-Heine-University, Universitätsstr. 1, D-40225 Düsseldorf, Germany*

## Abstract

Penetration of homologous nicotinic acid esters through the human nail and a keratin membrane from bovine hooves was investigated by modified Franz diffusion cells in-vitro to study the transport mechanism.

The partition coefficient octanol/water  $PC_{Oct/W}$  of the esters was over the range 7 to >51 000. The permeability coefficient  $P$  of the nail plate as well as the hoof membrane did not increase with increasing partition coefficient or lipophilicity of the penetrating substance. This indicates that both barriers behave like hydrophilic gel membranes rather than lipophilic partition membranes as in the case of the stratum corneum. Penetration studies with the model compounds paracetamol and phenacetin showed that the maximum flux was first a function of the drug solubility in water or in the swollen keratin matrix. Dissociation hindered the diffusion of benzoic acid and pyridine through the hoof membrane. Since keratin, a protein with an isoelectric point of about 5, is also charged, this reduction can be attributed to an exclusion of the dissociating substance due to the Donnan equilibrium. Nevertheless, the simultaneous enhancement of the water solubility makes a distinct increase of the maximum flux possible.

In order to screen drugs for potential topical application to the nail plate, attention has to be paid mainly to the water solubility of the compound. The bovine hoof membrane may serve as an appropriate model for the nail.

Fungal diseases of the nail plate account for up to 30–45% of all topical mycoses and the prevalence among the population of the industrial nations is about 5% (Evans 1990). Serious infections are treated with systemic antimycotics over several months, which exposes the organism to a considerable amount of drug. Since the introduction of nail lacquers, topical treatment has become more and more successful for light and intermediate mycoses (Qadripur et al 1981; Polak & Zaug 1990; Meisel 1992; Nolting & Seebacher 1993; Haria & Bryson 1995).

Until now there has been no commonly accepted in-vitro model for testing new, topically applicable drugs. Because human nails are not available in a sufficient number, a model based on membranes from the bovine hoof was developed, the structure and permeability of which equates with those of the human nail. Up to now the mechanism of nail penetration is largely uncertain. From investigations of the penetration of homologous alcohols through the nail plate, Walters et al (1983, 1985b) concluded that it is a hydrophilic gel membrane. They postulated an additional lipophilic route for the diffusion of lipophilic substances. Therefore, the present work examines the dependency of the permeability of both the nail plate and the hoof membrane on the partition coefficient between 1-octanol and water ( $PC_{Oct/W}$ ). Homologous nicotinic acid esters were used as model compounds with  $PC_{Oct/W}$  values over the range 7 to >51 000 (Table 1).

However, the maximum amount of drug, reaching the side of action per time after application to an area  $A$ , is more interesting than the permeability. While in the case of a lipophilic partition membrane the maximum flux depends on the drug solubility in the vehicle and the partition coefficient (Hagedorn-Leweke & Lippold 1995), it increases with growing water solubility in the case of hydrophilic gel membranes. The dependency of the maximum flux through the nail plate and the hoof membrane was investigated with the model compounds paracetamol and phenacetin whose solubilities in water differ from each other by a factor of 17 ( $C_{sw} = 16\ 800$  and  $950\ \text{mg L}^{-1}$ ,  $PC_{Oct/W} = 5.7$  and  $70.8$ , respectively).

The solubility of acidic or basic substances in water can be increased greatly by dissociation. Enhancement of the maximum flux through the nail plate is possible only when the decrease of the permeability coefficient due to dissociation is not too strong. Ions cannot penetrate lipid membranes due to low partitioning into the membrane. However, they are able to overcome hydrophilic gel membranes (Zaikov et al 1988). In this respect the penetration of the model compounds benzoic acid ( $pK_a$  4.19) and pyridine ( $pK_a$  5.19) through the hoof membrane was investigated at pH 2.0 and 7.4. In these milieus they are nearly completely dissociated or undissociated. The neutral benzyl alcohol served as a standard in order to consider the possible influence of the pH value on the keratin swelling.

## Materials and Methods

### Chemicals

Phosphate buffer solution with sodium chloride pH 7.4 (DAB 1996), hydrochloric acid buffer pH 2.0 or ammonium chloride

Table 1. Physical and chemical parameters of the nicotinic acid esters (Le &amp; Lippold 1995).

Substance	MW	$C_{sW}$ (mg mL <sup>-1</sup> )	$C_{sOct}$ (mg mL <sup>-1</sup> )	$PC_{Oct/W}$
Methyl nicotinate	137.1	1106	7624	6.9
Ethyl nicotinate	151.1	47	1034	22
Butyl nicotinate	179.2	2.45	715	292
Hexyl nicotinate	207.3	0.17	548	3233
Octyl nicotinate	235.3	0.01	527	51182

MW = molecular weight,  $C_{sW}$  = solubility in water,  $C_{sOct}$  = solubility in 1-octanol,  $PC_{Oct/W}$  = partition coefficient between 1-octanol and water.

buffer pH 10.0 (DAB 1996) were used as vehicles. All solutions were standardized to an ionic strength of 0.158. Methyl, ethyl, butyl and hexyl nicotinate were obtained from Aldrich-Chemie (Steinheim), octyl nicotinate from the Department of Pharmaceutical Chemistry of the University of Düsseldorf, paracetamol from Boehringer Ingelheim KG (Ingelheim), phenacetin from Bayer AG (Leverkusen), benzoic acid from Caesar & Lorentz (Hilden), benzyl alcohol from Janssen Chimica (Geel, Belgium) and pyridine from Kraft GmbH (Duisburg). HPLC grade methanol and acetonitrile are products of Riedel-de Haën (Seelze, Germany). Water was used freshly distilled.

#### Diffusion cells

Two different diffusion cells were used for the investigations of the nail plates and hoof membranes (Mertin 1995). Both cells were modifications of the Franz diffusion chamber (Franz 1975). The vertically oriented donor and acceptor compartment were joined together by clamps and contained 100 or 50 mL, respectively. As curved nails must be fixed under pressure to seal the cell, the two-part donor compartment was built from stainless steel. The nail plate was fixed between these two parts screwing them together.

#### Preparation of the nail plates and hoof membranes

Healthy nails of the big toe were taken from dead men and women, aged 19–61 years, 24 h post mortem at the latest. Adhering tissue of the nail bed or nail fold was removed by the method of Kligman and Christophers (Kligman & Christophers 1963). With the ventral side downwards the nails were placed on a cotton pad which was soaked with 0.5% trypsin in phosphate buffer pH 7.4 and tempered at 37°C over a period of 10 h. Afterwards the tissue was removed with tweezers, the nails rinsed with distilled water and put into water for 24 h to remove remaining trypsin. Because keratin is hardly decomposed by proteinases (Falbe & Regitz 1989), this method enabled receipt of intact nail plates.

Hooves were taken from freshly slaughtered cattle, rid of adhering connective and cartilaginous tissue and put into water for 24 h. Afterwards about 100 µm thick membranes were taken from the distal part of the ball horn with a microtome (Reichert & Jung, Nußloch). The hoof pieces were strong enough not need embedding, even in the swollen state. The membranes were punched with a diameter of 25 mm and dried at room temperature. Careful inspection of the hoof membranes by electron microscopy did not show any pores through the membranes but only cavities. The thickness of the swollen nails and membranes were measured with a micrometer caliper (Tesa Micromaster, Renens, Switzerland), in the case of the nails with the help of a metal ball (5.5 mm diameter).

#### Analytical conditions

In the case of hoof membranes the determination of the acceptor concentration was carried out spectrophotometrically (Zeiss DMR 10, Oberkochen). The hourly-collected samples were measured at the long-wave maximum and thereafter returned. In the case of the nail plates the samples had to be analysed by HPLC due to the smaller fluxes. The concentration was assayed by a modular HPLC unit (LC-6A, Shimadzu, Duisburg) equipped with an automatic sample injection module (SIL-6B), system controller (SCL-6B), UV-VIS-spectrophotometer (SPD-6AV) and an integrator (CR 4AX Chromatopac). The analyses were carried out at ambient temperature with a 125 × 4 mm column packed with LiChrospher 100 RP-18, 5 µm particle diameter (E. Merck, Darmstadt). The mobile phase, consisting of methanol/water or acetonitrile/water mixtures was pumped at flow rates ranging from 1 to 2 mL min<sup>-1</sup>. Injection volumes varied between 5 and 150 µL. The concentration of the samples was calculated from peak areas by the external standard method.

#### Solubilities

After a rough estimate in a preliminary experiment, the amount of substance corresponding to twice the solubility was mixed with 20–50 mL solvent in a 100 mL glass bottle by rotating at 32°C. After 24 h the samples were filtered first by paper (Schleicher & Schuell, Dassel) and after that by cellulose acetate membrane filter (0.45 µm, 25 mm Ø) with a filter device of stainless steel (Sartorius, Göttingen). All materials were equilibrated at 32°C. Spectrophotometrical assay of the concentration followed immediately to prevent precipitation.

#### Penetration studies

After filling the acceptor compartment, the swollen nail plate or hoof membrane was inserted in the diffusion cell. The cells were equilibrated at 32°C in a water bath (thermostat Julabo Paratherm IM, Juchheim Labortechnik, Seelbach), then 100 mL donor liquid was added and samples were taken from the acceptor periodically. The measuring interval was determined by the penetration rate. If it was high enough (hoof membrane) the samples were taken hourly. In the case of the nail plate samples were collected daily or weekly because of the low permeability and therefore long test period. Since all investigations with nail plates were analysed by HPLC, the samples (1.7 mL) could not be returned but had to be replaced by buffer solution. The acceptor dilution was corrected arithmetically (computer-aided). The media were preserved with 0.02% sodium azide in the case of a long test period. The acceptor was mixed by a magnetic stirrer (Ikamag EOA 9 with

controller ES 5, Janke & Kunkel, Staufen i. Br., Germany) during the whole course.

The donor concentration of methyl, ethyl and butyl nicotine was about  $1000 \text{ mg L}^{-1}$ , while hexyl and octyl nicotine were used as saturated solutions at  $32^\circ\text{C}$ . Octyl nicotine was investigated only with the hoof membrane due to its low solubility and low flux. Paracetamol and phenacetin were used as saturated solutions at  $32^\circ\text{C}$ . In the case of the investigations of charge influence the donor concentrations were between  $1000$  (pyridine) and  $5000 \text{ mg L}^{-1}$  (benzoic acid).

## Results and Discussion

### Influence of the partition coefficient octanol/water on the permeability coefficient

The evaluation of the data is based on Fick's law:

$$\frac{dM}{dt} = \frac{D_B \cdot A}{h_B} \cdot (C_{BD} - C_{BA}) \quad (1)$$

in which  $dM/dt$  is the penetrating amount per time,  $D_B$  the effective diffusion coefficient in the barrier,  $h_B$  the thickness of the barrier, and  $C_{BD}$  and  $C_{BA}$  the concentration in the barrier on the donor and the acceptor side, respectively. As the concentration in the barrier is normally unknown,  $C_{BD}$  is replaced by  $PC_{B/V} \cdot C_V$ , in which  $PC_{B/V}$  is the partition coefficient barrier/vehicle and  $C_V$  is the vehicle concentration of the penetrating compound in the donor. The term  $D_B \cdot PC_{B/V}$  is called permeability coefficient  $P$ . On the condition that not more than 10% of the drug amount in the donor penetrates,  $P$  can be calculated from the slope of a plot  $M$  vs  $t$ :

$$M = \frac{D_B \cdot PC_{B/V} \cdot A \cdot C_V}{h_B} \cdot t = \frac{P \cdot A \cdot C_V}{h_B} \cdot t \quad (2)$$

If  $P$  is dependent on the lipophilicity, then it grows with increasing  $PC_{B/V}$ . This term is normally unknown and its determination is difficult. So  $PC_{B/V}$  is approached by the partition coefficient octanol/water ( $PC_{Oct/W}$ ) in the case of biological membranes. Considering the equation of Collander (1947), who quantified the relationship between  $PC_{Oct/W}$  and  $PC_{B/V}$ ,  $\log P$  can be calculated from:

$$\log P = \log D_B + b + a \cdot \log PC_{Oct/W} \quad (3)$$

If the solubility properties of octanol and the lipid barrier are not the same, the slope  $a$  differs more or less from the ideal value of unity but is distinctly greater than 0. But if the barrier behaves like a hydrophilic gel membrane, then the permeability is independent of the  $PC_{Oct/W}$  and the slope becomes 0.

The permeability coefficients of the hoof membrane ( $P_H$ ) exceeded that of the nail plate ( $P_N$ ) 10- to 30-fold (Fig. 1). Unlike the hoof membrane, where the lag-time was only a few minutes, steady-state penetration through the nail plate occurred after 10 (methyl nicotine) up to 80 (hexyl nicotine) h. The plot of  $P$  vs  $PC_{Oct/W}$  in a logarithmic scale according to equation 3 does not show a positive slope. Rather the permeability of the hoof membrane is independent of the lipophilicity of the penetrating substances ( $P=0.05$ ). However, the decrease of the permeability in case of the nail plate is highly significant ( $P=0.01$ ), but also contradicts the model of a partition membrane. It can be explained by the decreasing diffusion coefficient due to the increasing molecular volume. It can only be assumed why this factor has no influence in the

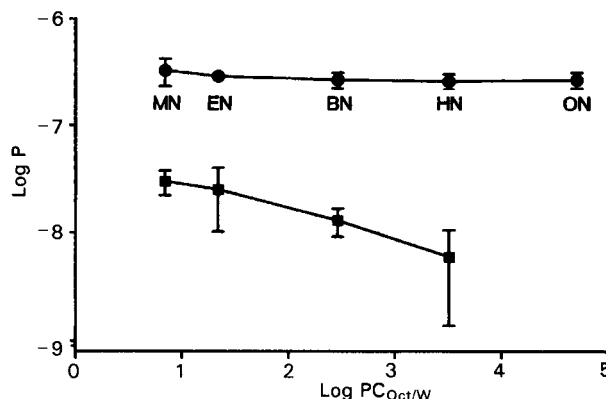


FIG. 1. Relationship between permeability coefficient  $P$  and partition coefficient octanol/water  $PC_{Oct/W}$  of the nicotinic acid esters across human nail plate (■) and bovine hoof membrane (●) at  $32^\circ\text{C}$  ( $n=3-8$ , means  $\pm$  s.d.). MN = methyl nicotine, EN = ethyl nicotine, BN = butyl nicotine, HN = hexyl nicotine, ON = octyl nicotine.  $P$  in  $\text{cm}^2 \text{s}^{-1}$

case of the hoof membrane: compared with the nail plate the keratin matrix in the hoof membrane is probably wider-meshed, so that the increasing molecular weight reduces the diffusion coefficient only insignificantly. The enlargement of a molecule only in one direction, as is the case with homologs, merely leads to a small decrease of  $D$ , because the cross-section remains constant assuming orientation in the direction of diffusion (Flynn et al 1974).

Although the permeabilities presented here are in the same order of magnitude as the results of Walters et al (1983, 1985b), there is an essential difference in that the decrease of the permeabilities from the  $C_2$ - to the  $C_8$ -alcohol (from  $1.6 \times 10^{-6}$  to  $7.5 \times 10^{-8} \text{ cm s}^{-1}$ ) is much stronger but the diffusion coefficients are nearly the same. The authors justified the assumption of a decreasing partition coefficient nail/water with an endergonic transfer of a methyl group from water into the nail compartment. This explanation is only conclusive when the affinity of a substance to the nail plate decreases faster than its affinity to water with increasing lipophilicity. Harrison & Speakman (1958) also reported a decreasing diffusion of  $n$ -alcohols in keratin with increasing chain length. As pure alcohols were used for these investigations, the permeabilities decreased probably because of the reduced swelling of keratin or increasing molecular volume. Our results with homologous nicotinic acid esters correspond with the investigations of Walters et al (1983, 1985b) as far as the nail plate is characterized as a hydrophilic gel membrane rather than a lipophilic partition membrane. In contrast to the stratum corneum, the lipid content of the nail plate is much lower (0.1–1% (Walters & Flynn 1983); stratum corneum: 15% of dry weight (Flynn 1985)). The high water content of the swollen nail plate (about 27% (Mertin 1995)) indicates the presence of a hydrophilic gel membrane, too. The hoof membrane behaves similarly: there is no dependency of the  $PC_{Oct/W}$  or the lipophilicity on the permeability of the penetrating drug.

### Maximum fluxes and water solubilities

The equation for calculating the maximum flux ( $J_{max}$ ) follows directly from the first Fick's law (eqn 1).  $J_{max}$  is the amount of substance that penetrates through a barrier of the area  $A$  and the thickness  $h_B$  per unit of time from a saturated solution

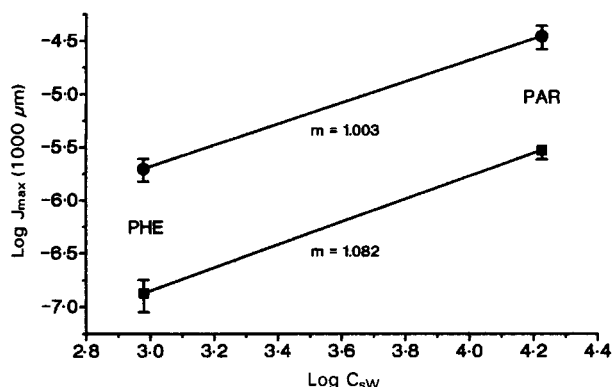


FIG. 2. Relationship between the logarithm of the maximum flux  $J_{\max}$  (1000  $\mu\text{m}$ ) and the logarithm of the water solubility of the model compounds paracetamol (PAR) and phenacetin (PHE) across human nail plate (■) and bovine hoof membrane (●) at 32°C ( $n=4$ , means  $\pm$  s.d.).  $J_{\max}$  (1000  $\mu\text{m}$ ) in  $\text{mg cm}^{-2} \text{s}^{-1}$ ;  $C_{\text{sw}}$  in  $\text{mg L}^{-1}$ .

(sink-conditions):

$$J_{\max} = \frac{dM_{\max}}{dt \cdot A} = \frac{P}{h_B} \cdot C_{\text{sv}} \quad (4)$$

Taking the logarithms results in:

$$\log J_{\max} = \log \frac{P}{h_B} + \log C_{\text{sv}} \quad (5)$$

Neglecting effects of the molecular volume and assuming a hydrophilic gel membrane, the term  $\log P/h_B$  in equation 5 is constant and the maximum flux is only dependent on the solubility of the drug in water. Then we expect, for a plot of logarithmic maximum fluxes vs logarithmic water solubilities, a linear correlation with the ideal slope of unity. This was confirmed by the results of the nail plate (slope 1.082) as well as the hoof membrane (slope 1.003, Fig. 2). For better comparability the maximum fluxes were standardized to a barrier thickness of 1000  $\mu\text{m}$ . The slight positive deviation of the slope in the case of the nail plate was explained by the stronger influence of the molecular size on the permeability. The denser network of filamentous keratin compared with that of the hoof membrane exhibited more resistance to the more voluminous phenacetin than to paracetamol. This was expressed not only by the lower maximum flux (Fig. 2), but also by the lower permeability coefficient calculated from these results:  $1.78 \pm 0.32 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$  for paracetamol and  $1.40 \pm 0.47 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$  for phenacetin respectively.

It has to be emphasized that the maximum flux of a substance firstly depends on its water solubility and, considering

that, this investigation with only two model compounds must be interpreted carefully. This agrees with the model of a hydrophilic gel membrane where the solubility in the barrier, which determines the maximum flux, corresponds to the solubility in the swelling medium that is water. In contrast, the maximum flux through a lipophilic partition membrane is a function of the partition coefficient and the solubility in the vehicle. Approaching the solubility in the barrier by the solubility in octanol, complex equations result. With their help maximum fluxes can be predicted by knowledge of the drug solubility in the vehicle and in octanol (Hagedorn-Leweke & Lippold 1995). The consequence is that one has to pay attention primarily to the highly different water solubilities and only secondly to the more similar permeability coefficients in order to screen drugs for potential topical application to the nail plate.

#### Penetration of electrolytes

Dissociation of benzoic acid and pyridine led to a reduction of their penetration rate through the hoof membrane. Table 2 shows that the permeability coefficients of the ionic forms were significantly lower than those of the neutral form. Because the penetration of benzyl alcohol decreases significantly by a half ( $t$ -test,  $P=0.05$ ) after the transition from an acidic to a neutral or basic milieu, the neutral compound serves as a standard. To this end the quotients of the permeability coefficients  $P_{\text{benzoic acid}}$  or  $P_{\text{pyridine}}$  and  $P_{\text{benzyl alcohol}}$  are built and compared with each other (Table 3).

Dissociation reduces the diffusion rate of benzoic acid to a fifth and that of pyridine to a quarter. Assuming the isoelectric point of keratin is about 5 (Marshall 1983), it is positively charged at pH 2.0 and negatively charged at pH 7.4. However, nothing is known about the charge density. The decrease of penetration caused by dissociation is due to the Donnan effect (Meares 1968; Higa et al 1990, 1991) or the electrostatic repulsion between the membrane and the diffusing molecule (Kobayashi et al 1994). At pH 7.4 benzoate is a co-ion to the negatively charged keratin and hence it is displaced from the membrane; the lower concentration gradient then leads to a reduction of the permeability. In the case of an acidic milieu, the pyridinium-cation is displaced from the now positively charged keratin leading to a decreased penetration compared with the neutral form. Nevertheless, diffusion of ionic substances through the hoof membrane confirm the assumption of a hydrophilic gel membrane (Zaikov et al 1988).

Some authors attribute the lowering of the electrolyte diffusion through charged polymers to sorption effects (Medley 1957; Meares 1968; Zaikov et al 1988). Since only the free

Table 2. Permeability coefficients of benzyl alcohol, benzoic acid and pyridine through the bovine hoof membrane.

	pH	Permeability coefficient ( $10^{-8} \text{ cm}^2 \text{ s}^{-1}$ )
Benzyl alcohol	2.0	$78.24 \pm 16.45$
	7.4	$41.72 \pm 7.07$
	10.0	$48.78 \pm 8.40$
Benzoic acid	2.0	$78.62 \pm 16.43$
	7.4	$8.29 \pm 1.36$
Pyridine	2.0	$19.14 \pm 7.05$
	7.4	$44.80 \pm 10.35$

Temperature = 32°C ( $n=4$ , means  $\pm$  s.d.).

Table 3. Permeability coefficients of benzoic acid ( $P_{BAC}$ ) and pyridine ( $P_{PYR}$ ) through the bovine hoof membrane at pH 2.0 and 7.4, standardized to benzyl alcohol ( $P_{BAL}$ ).

	pH	$P_{BAC}/P_{BAL}$	$P_{PYR}/P_{BAL}$
Benzoic acid	2.0	1.00	
	7.4	0.20	
Pyridine	2.0		0.24
	7.4		1.07

Temperature = 32°C (n = 4).

fraction is able to penetrate, sorption to functional groups could lead to a decrease of the concentration gradient. However, investigations with polydimethylsiloxane films containing a silica filler show that sorption only causes a prolongation of the lag-time but not a reduced steady-state flux (Flynn & Roseman 1971). Due to the dynamic exchange of the free substance between membrane and donor solution, the resulting concentration of the free drug in the membrane will not alter after the adjustment of the sorption equilibrium at the steady state and is independent of the degree of saturation of the functional groups, on the condition that there is no decrease of the donor concentration by the sorption. Therefore, an interpretation of the hindered penetration of charged molecules is preferred on the basis of the Donnan potential.

The reduced permeability of the neutral compound benzyl alcohol at pH 7.4 as compared with pH 2.0 is probably caused by a decrease of the keratin swelling due to the charge inversion of the keratin at the transition from an acidic to a neutral or basic environment. The weak, not significant increase of the penetration of the benzyl alcohol changing the pH from 7.4 to 10.0 lends further support to this hypothesis, because the number of negatively charged groups in keratin (IEP ~ 5) at pH 10.0 is nearly the same as in a milieu of pH 7.4.

Applying these results to the human nail, they contradict the investigations of Walters et al (1985a), who could not find a dependency of the nail permeability of miconazole on the degree of dissociation. Maybe this contradiction is explained by the higher ionic strength of the solutions; in this case the influence of the Donnan equilibrium is lowered. Since the amino acid composition of keratin is dependent on the species (Fraser & MacRae 1973), the different results could also be put down to varying charge densities of hoof and nail keratin. The reduction of the hoof permeability to a quarter caused by complete dissociation of the substance is much lower than the possible enhancement of the solubility of hardly soluble acids and bases. So the solubility is increased a hundred fold at 99% degree of ionization (assuming sufficient solubility of the salt). Since the maximum flux is primarily a function of the solubility in water (see above), it is possible to heighten the permeability of both the hoof membrane and the nail plate by dissociation of the penetrating drug if the milieu in the barrier causes no precipitation of the substance.

### References

Collander, R. (1947) On "lipoid solubility". *Acta Physiol. Scand.* 13: 363-381  
 Evans, E. G. V. (1990) Nail dermatophytosis: the nature and scale of the problem. *J. Dermatol. Treat.* 1 (Suppl. 2): 47-48  
 Falbe, J., Regitz, M. (1989) *Römpf Chemie-Lexikon*. G. Thieme Verlag, Stuttgart, pp 2195-2196

Flynn, G. L. (1985) Mechanism of percutaneous absorption from physicochemical evidence. In: Bronaugh, R. L., Maibach, H. I. (eds) *Percutaneous Absorption: Mechanisms - Methodology - Drug Delivery* (Dermatology, Vol. 6). Marcel Dekker Inc., New York, pp 17-41  
 Flynn, G. L., Roseman, T. J. (1971) Membrane diffusion II: influence of physical adsorption on the molecular flux through heterogeneous dimethylpolysiloxane barriers. *J. Pharm. Sci.* 60: 1788-1796  
 Flynn, G. L., Yalkowsky, S. H., Roseman, T. J. (1974) Mass transport phenomena and models: theoretical concepts. *J. Pharm. Sci.* 63: 479-510  
 Franz, T. J. (1975) Percutaneous absorption. On the relevance of in vitro data. *J. Invest. Dermatol.* 64: 190-195  
 Fraser, R. D. B., MacRae, T. P. (1973) Conformation in fibrous proteins: chapter 16: keratins. Academic Press, New York, pp 469-481  
 Hagedorn-Leweke, U., Lippold, B. C. (1995) Absorption of sunscreens and other compounds through human skin in vivo: derivation of a method to predict maximum fluxes. *Pharm. Res.* 12: 1354-1360  
 Haria, M., Bryson, H. M. (1995) Amorolfine - a review of its pharmacological properties and therapeutic potential in the treatment of onychomycosis and other superficial fungal infections. *Drugs* 49: 103-120  
 Harrison, D., Speakman, J. B. (1958) The pore size of keratin. *Text. Res. J.* 28: 1005-1008  
 Higa, M., Tanioka, A., Miyasaka, K. (1990) A study of ion permeation across a charged membrane in multicomponent ion systems as a function of membrane charge density. *J. Membr. Sci.* 49: 145-169  
 Higa, M., Tanioka, A., Miyasaka, K. (1991) An experimental study of ion permeation in multicomponent ion systems as a function of membrane charge density. *J. Membr. Sci.* 64: 255-262  
 Kligman, A. M., Christophers, E. (1963) Preparation of isolated sheets of human stratum corneum. *Arch. Dermatol.* 88: 702-705  
 Kobayashi, T., Nagai, T., Suzuki, T., Nosaka, Y., Fujii, N. (1994) Restricted permeation of dextranulfates by electrostatic barrier of negatively charged ultrafiltration membranes: salt effect on the permeation. *J. Membr. Sci.* 86: 47-56  
 Le, V. H., Lippold, B. C. (1995) Influence of physicochemical properties of homologous esters of nicotinic acid on the skin permeability and maximum flux. *Int. J. Pharm.* 124: 285-292  
 Marshall, R. C. (1983) Characterization of the proteins of human hair and nail by electrophoresis. *J. Invest. Dermatol.* 80: 519-524  
 Meares, P. (1968) Transport in ion-exchange polymers. In: Crank, J., Park, G. S. (eds) *Diffusion in Polymers*. Academic Press, New York, pp 373-428  
 Medley, J. A. (1957) The diffusion of acid ions in keratin. *Trans. Faraday Soc.* 53: 1380-1388  
 Meisel, C. W. (1992) International symposium on amorolfine. *Haut* 3: 64-70  
 Martin, D. (1995) Permeabilität der menschlichen Nagelplatte und ihre Vorhersagemöglichkeit mittels einer Keratinmembran tierischer Herkunft. Verlag Mainz, Aachen  
 Nolting, S., Seebacher, C. (1993) *Ciclopiroxolamin - Wegweiser topischer Mykose-Therapie*. Universitätsverlag Jena, Jena, pp 46-68  
 Polak, A., Zaug, M. (1990) Amorolfine. In: Ryley, J. F. (ed.) *Chemotherapy of Fungal Disease (Handbook of Experimental Pharmacology, Vol. 96)*. Springer-Verlag, Berlin, pp 505-521  
 Qadripur, S. A., Horn, G., Höhler, T. (1981) Zur Lokalwirksamkeit von Ciclopiroxolamin bei Nagelmykosen. *Arzneim. Forsch.* 31: 1369-1372  
 Walters, K. A., Flynn, G. L. (1983) Permeability characteristics of the human nail plate. *Int. J. Cosmet. Sci.* 5: 231-246  
 Walters, K. A., Flynn, G. L., Marvel, J. R. (1983) Physicochemical characterization of the human nail: permeation pattern for water and the homologous alcohols and differences with respect to the stratum corneum. *J. Pharm. Pharmacol.* 35: 28-33  
 Walters, K. A., Flynn, G. L., Marvel, J. R. (1985a) Penetration of the human nail plate: the effects of vehicle pH on the permeation of miconazole. *J. Pharm. Pharmacol.* 37: 498-499  
 Walters, K. A., Flynn, G. L., Marvel, J. R. (1985b) Physicochemical characterization of the human nail: solvent effects on the permeation of homologous alcohols. *J. Pharm. Pharmacol.* 37: 771-775  
 Zaikov, G. E., Iordanskii, A. L., Markin, V. S. (1988) Diffusion of Electrolytes in Polymers. VSP, Utrecht, pp 73-126