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The influence of physico-chemical properties of homologous nicotinic acid esters on the permeability and maximum flux through an octanol membrane

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Abstract

In a Schulman-type three compartment model with water as donor phase A and acceptor phase B and octanol as the lipophilic phase between them, rate constants of transfer from A to B, k_{AB}, have been experimentally determined for homologous nicotinic acid esters (methyl nicotinate, MN, ethyl nicotinate, EN, butyl nicotinate, BN, hexyl nicotinate, HN, and octyl nicotinate, ON). The k_{AB}-values are rather independent of the partition coefficient octanol/water $PC_{oct/W}$ of the respective esters, demonstrating diffusion control in aqueous boundary layers. Thus, the calculated permeabilities of the homologous esters for a three layer membrane water/octanol/water also show values of similar magnitude. The logarithms of the maximum fluxes J_{max} of the esters through this three layer membrane exhibit an inverse proportionality to the number of C-atoms in the alkyl chain. The slope of the respective straight line corresponds well with the incremental constant δ for the relationship between the logarithms of the water solubilities and the alkyl chain length. This confirms the distinctive influence of aqueous boundary layers on the drug transfer through octanol membranes in vitro.

Keywords: Homologous esters; Maximum flux; Nicotinic acid esters; Partition coefficient; Permeability; Rate constant of transfer; Solubility; Three compartment model

1. Introduction

To understand the influence of physico-chemical properties of drugs on their permeability coefficients for biological membranes and on the maximum flux of drugs into the body, the study of liquid model membrane with aqueous and lipid barriers in series can be of advantage (Stehle and Higuchi, 1972). The significance of aqueous boundary layers adherent to a lipid membrane area is detectable with the help of hydrodynamic investigations (Stehle and Higuchi, 1972; Lippold

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and Schneider, 1975; Fürst et al., 1980). These aqueous boundary layers in vitro may simulate stagnant aqueous layers on the gut mucosa or water filled spaces between the intercellular lipid double layers of the stratum corneum.

A modified three compartment model (Rosano et al., 1961; Lippold and Schneider, 1975) is used to investigate the membrane transport of homologous nicotinic acid esters as model compounds. The permeability and the maximum flux of the esters are calculated with the help of the experimentally obtained rate constants of transfer. These in vitro results are the basis for the correlation regarding in-vivo experiments (Le and Lippold, 1995).

2. Materials and methods

2.1. Homologous esters of nicotinic acid

Methyl (MN), ethyl (EN), butyl (BN), and hexyl nicotinate (HN) were used as obtained (Aldrich-Chemie, Germany). Octyl nicotinate (ON) was synthesized from nicotinic acid and *n*-octanol (Beilstein).

2.2. Experimental set-up of the three compartment model

The set-up for the experiments is directly comparable to the Schulman-type cell as presented in Fig. 1. The three compartment model consists of a glass cylinder which measures 10 cm in diameter and a height of 15 cm. A glass plate measuring about 10×10 cm, which is fixed vertically by sintering, separates the cylinder into two compartments of the same size. These are filled with aqueous solutions of the ester (donor phase) or 0.1 mol l^{-1} HCl (acceptor phase). The filling volume is 265 ml for each phase. Sink conditions are maintained in the acceptor because the basic molecules are protonated here and therefore not redistributed to the donor phase. The aqueous phases are covered with 45 ml of octanol. In the aqueous phases glass stirrers are fixed to the boundary layers leading to the octanol. The stirring speed is 80 upm, providing effective mixing without disturbing the boundary area (Le, 1993). The apparatus is brought to a temperature of 32 ± 1°C in a water bath, allowing for saturation of the different phases. The change of concentration in the donor and acceptor phase is continuously measured spectrophotometrically with flow-through cells at the wave length of the isosbestic point ($\lambda = 270.5$ nm) for about 3.5 h; pumping speed is 600 ml h^{-1}. To prevent sorption of the esters by the tubes leading to and from the flow-through cell, they consist of PTFE, those within the roller pump of fluor rubber (Viton®). In the case of hexyl and octyl nicotinate, a piston pump (Sotax, Basel, Switzerland) is used. The esters do not hydrolyse under the described experimental conditions (Le, 1993).

2.3. Principles of the kinetics in the three compartment model

The transfer of the substance from the donor to the octanol can be described by the rate constant of transfer of the first order k_{AB}, from the octanol to the acceptor by k_{BA} (Lippold and Schneider, 1975). In principle, two unstirred boundary layers or membranes exist, which develop on the boundary between water and octanol (Fig. 2).

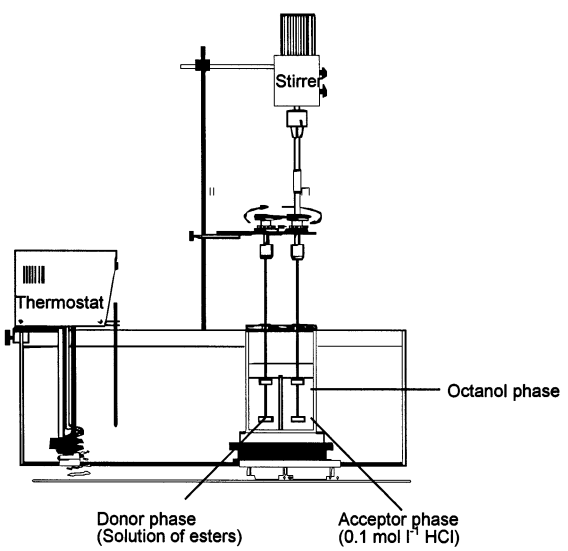

Fig. 1. Three compartment model to investigate the rate constants of transfer.

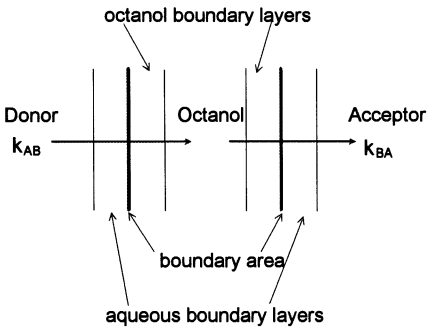

Fig. 2. Rate constants in the three compartment model with sink condition.

Their thickness depends mainly on the intensity of the stirring but also on the temperature (compare Section 3.1.1). The thickness is about 600 μm under unstirred conditions (Neubert and Fürst, 1989), while it is in the order of about 50 μm at intensive agitation (Hadgraft and Ridout, 1988). The thickness of these stagnant layers determines the rate constants of transfer k_{AB} and k_{BA}. One of the two layers, the aqueous or the lipophilic, can be rate determining.

2.4. Determination of the rates constant of transfer k_{AB} and k_{BA}

Typical concentration courses in the donor, octanol and acceptor phase are presented in Figs. 3

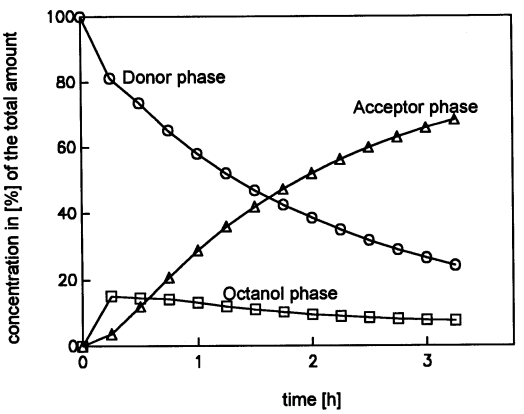

Fig. 3. Concentration course of methyl nicotinate in the donor and acceptor phase as well as in the octanol phase (calculated, single experiment, $C_{A_O} = 10^{-3}$ mol l^{-1}), Temperature = 32 ± 1°C.

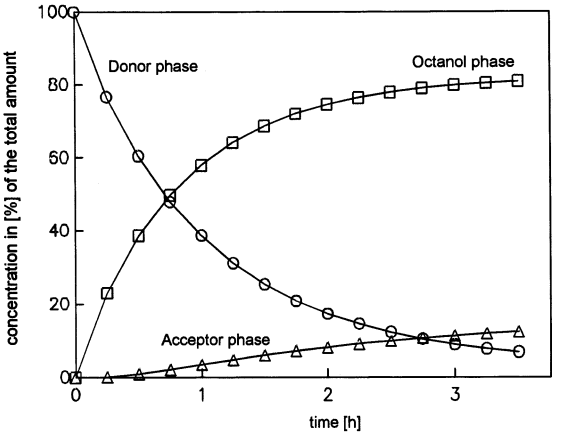

Fig. 4. Concentration course of hexyl nicotinate in the donor and acceptor phase as well as in the octanol phase (calculated, single experiment, $C_{A_O} = 0.4$ mol l^{-1}), Temperature = 32 ± 1°C.

and 4. The decrease of the concentration in the donor phase runs exponentially according to a first order kinetic:

$$-\frac{dc_A}{dt} = k_{AB} \cdot c_A \quad (1)$$

This leads to

$$\log c_A = \log c_{A_0} - \frac{k_{AB}}{2303} \cdot t \quad (2)$$

In Eqs. (1) and (2), c_{A_0} and c_A describe the initial concentration and the concentration at the time t in the donor phase. The plot of the logarithms of these concentrations c_A versus time results in a straight line with $k_{AB} \cdot 2.303^{-1}$ as its slope (Fig. 5).

Figs. 3 and 4 demonstrate that the appearance in the acceptor phase occurs very slowly when taking lipophilic esters (butyl, hexyl and octyl ester). The octanol phase here absorbs a lot of the substance. Quasi stationary conditions do not exist any more (Suzuki et al., 1970; Lippold et al., 1975) as in the case of the hydrophilic esters (methyl and ethyl nicotinate). To determine the rate constant k_{AB} of the lipophilic esters, only the decrease of the concentration in the donor phase during the first 2–3 h is considered. Here the octanol phase functions as a sink (Suzuki et al., 1970; Lippold et al., 1975):

The rate constant of transfer k_{BA} for the transport of nicotinic acid ester molecules from the octanol phase to the aqueous acceptor phase (0.1 mol l^{-1} HCl) can be calculated according to Lippold et al. (Lippold and Schneider, 1975):

$$k_{BA} = k_{AB} \cdot \frac{1}{PC_{Oct/W}} \cdot \frac{V_W}{V_O} \quad (3)$$

$PC_{Oct/W}$ is the partition coefficient of the respective ester between octanol and water. The correction factor V_W/V_O results from the different volumes of the aqueous and octanol phase.

2.5. Partition coefficients octanol/water $PC_{Oct/W}$

Appropriate volumes of the aqueous and organic phase are filled into 100 ml cylindrical glass bottles with a PTFE-screw-top and the drugs are added. The partition occurs during rotation of the mixtures in a rotating-bottle-apparatus overnight at a temperature of 32 ± 1°C. The aqueous phase is used for a direct spectrophotometric measurement. The concentration of HN and ON in the aqueous phase is determined with the help of the HPLC-technique (Le and Lippold, 1995).

2.6. Water solubility c_{sW}

To determine the water solubility, excessive amounts of the liquid esters are mixed with distilled water. A magnetic stirrer at a low stirring rate guarantees the mixing and avoids the formation of emulsions. The experiments are carried out in a water bath at 32 ± 1°C for 24 h. The concentration of the octyl nicotinate is determined with the HPLC-technique (Le and Lippold, 1995), that of other esters spectrophotometrically after centrifugation.

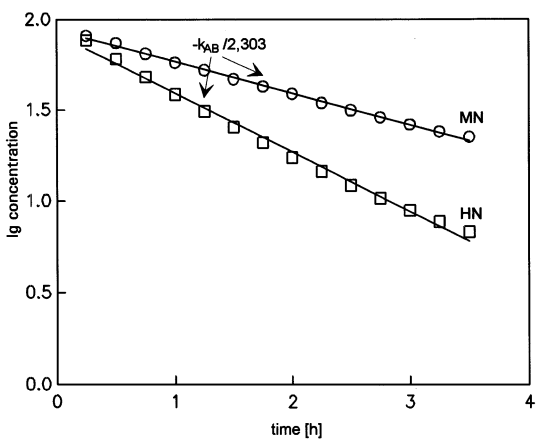

Fig. 5. Determination of the rate constants of transfer by plotting the logarithms of the concentrations in the donor phase (concentration in [%] of C_{A_O}) versus time, Temperature = 32 ± 1°C.

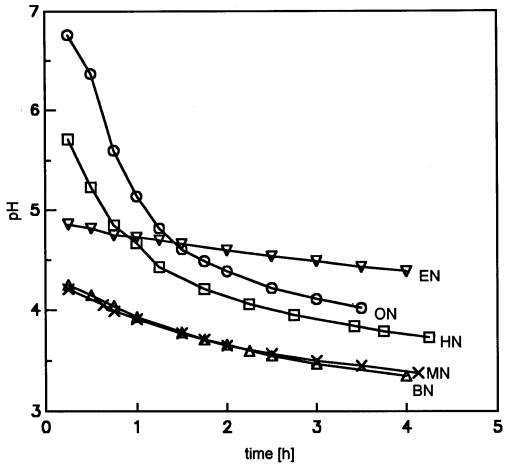

Fig. 6. pH-change in the donor phase of MN, EN, BN, HN and ON during the experiment, single runs. Temperature = 32 ± 1°C. ×, MN; ▽, EN; △, BN; □, HN; ○, ON.

2.7. Octanol solubility c_{sO}

The octanol solubility of the esters is calculated through the partition coefficient octanol/water $PC_{Oct/W}$ and the water solubility c_{sW}($c_{sO} = c_{sW} \cdot PC_{Oct/W}$).

3. Results and discussion

3.1. Validation of the investigations with the three compartment model

3.1.1. pH-change in the donor phase during the experiments

A decrease of the pH-value in the donor phase can be observed at an advanced stage of the experiment (Fig. 6). The reason could be that the

Table 1
Rate constants of transfer k_{AB} from the water phase in the octanol phase and the partition coefficients octanol/water $PC_{Oct/W}$ of homologous nicotinic acid esters as a function of the concentration, temperature = 32 ± 1°C

Ester/concentration	k_{AB}-values [h^{-1}]		$PC_{Oct/W}$	
	10^{-4} [mol l^{-1}]	10^{-3} [mol l^{-1}]	10^{-4} [mol l^{-1}]	10^{-3} [mol l^{-1}]
MN	0.43; 0.46	0.47	5.8	6.9
EN	0.54; 0.54	0.78	20.8	22.0
BN	0.69; 0.77	1.02	264.5	292.0
HN	0.62; 0.63	0.91[b]	n.d.[c]	3233
ON	0.40[a]	n.d.	n.d.	51182

Column 2: single values, columne 3 to 5: means.
Relative standard deviations for k_{AB} at $C_{A_O} = 10^{-3}$ mol l^{-1}: 5–17% ($n = 5$), for $PC_{Oct/W}$: 2–10% ($n = 4$–12).
[a] Initial concentration = $0.4 \cdot 10^{-4}$ [mol l^{-1}], $n = 4$.
[b] Initial concentration = $0.72 \cdot 10^{-3}$ [mol l^{-1}].
[c] n.d., Cannot be determined due to low solubility.

ion pairs $H_3O^+Cl^-$ leave the acceptor to enter the donor phase, possibly as water-centred aggregates (Lippold and Adel, 1972). Water is soluble in octanol to 0.05% (Scheuplein and Blank, 1971). The same may be assumed for ester ion pairs, e.g. protonated ester hydrochlorides. The transports of ion pairs through lipophilic membranes were also detectable for indomethacin (Inagi et al., 1981), phenylbutezone (Lovering and Black, 1974) and homologous quaternary esters of benzilic acid (Lippold and Schneider, 1974, 1975). Furthermore, the transport of hydrophilic cationic drugs from the acceptor phase through a dodecanol-collodium membrane could be detected (Neubert and Fürst, 1989). The influence of this pH-change in the donor phase will be examined in Section 3.1.2.

3.1.2. Influence of the pH-value on the rate constant of transfer

To examine how far the pH-changes in the donor phase influences the rate constants of transfer, phosphate puffer pH 7.0 as a donor medium is used in the case of ethyl nicotinate and water as acceptor medium with the lipophilic butyl nicotinate. The respective k_{AB}-values for the varying pH-conditions of the phases are in the confidence interval of the mean values of the main experiments. This phenomenon can on the one hand be explained by the pK_a-values of the ester (pK_a-values in the area of 3.1–3.4): As long as the pH-value in the donor phase does not clearly fall below 4.1–4.4, pH-decreases should hardly have an effect. Apart from that, dissociated compounds also contribute to a certain extent to the transport (Lovering and Black, 1974; Lippold and Schneider, 1974, 1975; Inagi et al., 1981), even if their $PC_{Oct/W}$-values are clearly lower than those of the neutral ones (Le, 1993). The missing influence of the exchange of 0.1 mol l^{-1} HCl by water in the acceptor clearly demonstrates that the octanol phase already functions as a sink for both esters (compare Section 2.4).

3.1.3. Concentration dependence of the k_{AB}-values

Experiments with MN, EN, BN and HN with two different initial concentrations in the donor phase, namely 10^{-3} and 10^{-4} mol l^{-1}, show that k_{AB}-values increase with increasing concentration. The k_{AB}-values are listed in Table 1. While the concentration increase only has little influence on the transport of methyl nicotinate, it has a stronger influence on the rate constant of transfer of lipophilic homologous. The increase can amount to 45%. Since the k_{AB}-values are often dependent on the partition coefficient octanol/water, the increase of the k_{AB}-values with increasing concentration in the donor phase could be ex-

plained by the concentration dependence of the $PC_{Oct/W}$-values (Lippold and Schneider, 1975). The $PC_{Oct/W}$-values also increase with increasing concentration (Table 1). The experimentally determined $PC_{Oct/W}$-values of MN, EM and HN correspond very well with data from the literature, whereas BN is found lower (Houk and Guy, 1988).

However, as further analysis will show, the transport is not directly dependent on membrane control (control through the boundary octanol layer), but mainly on diffusion control (control through the aqueous boundary layer) and the detected dependence of the k_{AB}-values on the concentration cannot be explained with the help of the concentration dependence of the partition coefficients. For further analysis, the data for higher concentrations with higher reliability (Table 1) are used.

3.2. Rate constants of transfer k_{AB} and k_{BA}

Fig. 7 shows the rate constants for the transport of the esters from the aqueous into the octanol phase k_{AB} and the calculated k_{BA}-values (Eq. (3)) for the transport from the octanol into the acceptor phase, plotted logarithmically against the number of C-atoms in the alkyl chain. The logarithms of the k_{AB}-values slightly increase between MN and EN by 0.22 (Fig. 7). From EN onwards, the curve reaches a plateau despite the increasing number of C-atoms in the alkyl chain. ON demonstrates a slight decrease of the k_{AB}-values.

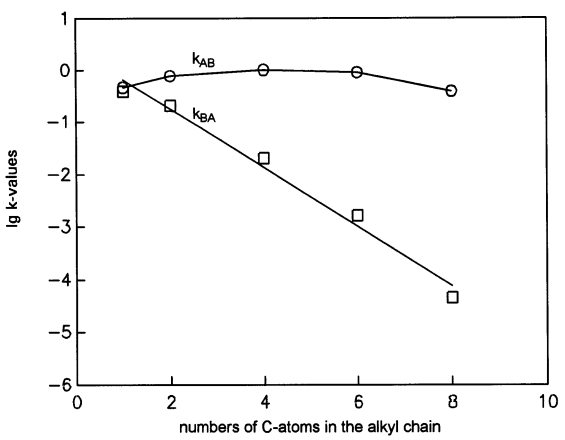

Fig. 7. Dependence of the logarithms of the k-values on the number of C-atoms in the alkyl chain, k-values in $[h^{-1}]$.

If the transfer of the esters from the donor to the octanol phase were dependent on the $PC_{Oct/W}$ which would mean dependent on membrane control, then the increase in Fig. 7 should approximately correspond to the π-constant according to Hansch (Fujita et al., 1964; Leo et al., 1971) ($\pi = 0.55$ for the nicotinic acid esters) (Le, 1993). The experimental values show, that the transport from MN onwards changes from membrane to diffusion control, and while $PC_{Oct/W}$ loses its influence on the transport, aqueous boundary layers gain in importance.

In contrast to this, the curve of the logarithms of the k_{BA}-values drops steadily, the slope of the straight line is 0.56 (Fig. 7). The transfer of the ester from the octanol into the acceptor phase is determined by the reciprocal $PC_{Oct/W}$ in contrast to the transfer donor/octanol (Eq. (3)). The slope of 0.56 corresponds very well to the π-constant of 0.55. This reciprocal dependence of the k_{BA}-values on $PC_{Oct/W}$ can be explained again only by diffusion control, meaning that the k_{AB}-values do not contain $PC_{Oct/W}$.

For the interpretation of rate constants of transfer of different homologous compounds in two or three compartment models, the bi-linear model is very useful (Lippold and Schneider, 1975; Kubinyi, 1976, 1979a,b; van de Waterbeemd, 1980). Depending on the chain length of the homologous, the plateau of the k_{AB}- and k_{BA}-values is more or less visible. The k_{AB}-values of HN and ON for the here examined nicotinic acid ester slightly deviate from the plateau. The fact that the diffusion of these larger molecules is hindered to some extent, caused by the chain lengthening, seems to be a reason for this (Section 3.3.2).

The situation of lipophilic membranes, which do not demonstrate a strong substance enrichment as in the case of the huge octanol phase in the three compartment model, is of special interest with respect to an in vitro/in vivo-correlation. So in the following, the permeabilities of the esters, which are independent of the experimental

quantities area and volume, will be discussed for a thin octanol membrane with aqueous boundary layers.

3.3. Permeability of a three layer membrane water/octanol/water for the esters

The overall permeability of a three-layer membrane water/octanol/water can, depending on the substance properties, be influenced by the $PC_{Oct/W}$ or by aqueous boundary layers and thus by the diffusion coefficient in the organic or aqueous layer. Furthermore, the permeability is dependent on the thickness of the layers (Roberts, 1985, 1991).

3.3.1. Investigation of the permeability

The calculation of the overall permeability is based on a thin octanol membrane with aqueous boundary layers on the donor and acceptor side (compare Section 2.3), therefore no real substance enrichment is assumed in this three layer membrane. The overall permeability $P_{overall}$ describes the diffusion ability of the ester molecules from the donor compartment into the acceptor compartment, that is the moving through the in line arranged layers (aqueous boundary layer, octanol layer, aqueous boundary layer). It can be calculated according to Eq. (4) (Flynn et al., 1974):

$$P_{overall} = \frac{D_W \cdot D_O \cdot PC_{Oct/W}}{d_O \cdot D_W + 2d_W \cdot D_O \cdot PC_{Oct/W}} \quad (4)$$

D stands for the diffusion coefficient and d for the thickness of the layers. The indices O and W mean octanol and water respectively.

Apart from d_O and d_W the other parameters (D_W as well as $PC_{Oct/W}$) are quantities, to be obtained through calculation or experiment (compare Tables 1 and 2). The calculation of the quantities d_O and d_W is possible with the help of the k_{AB}-values. Knowing V_W, the volume of the aqueous phase, as well as the size of the contact area A between the donor and octanol phase, the permeabilities P_{AB} for the transfer from water to octanol can be calculated from the k_{AB}-values according to the 1. Fickian law:

Table 2
Calculated permeabilities $P_{overall}$ of the three layer membrane water/octanol/water as well as the diffusion coefficients in octanol D_O and water D_W of homologous nicotinic acid esters, temperature = 32 ± 1°C

Ester	$P_{overall}$ [cm h^{-1}]	D_w^a [10^{-6} cm^2 s^{-1}]	D_O^a
MN	2.68	9.7	3.3
EN	3.64	9.0	3.0
BN	4.02	8.0	2.5
HN	3.59	7.2	2.2
ON	1.58	6.7	1.9

[a] Calculated according to Scheibel and Wilke and Chang (Scheibel, 1954; Wilke and Chang, 1955); the diffusion coefficient of MN in water was experimentally determined to $8.1 \cdot 10^{-6}$ cm^2 s^{-1} (Cadman et al., 1981).

$$P_{AB} = k_{AB} \cdot \frac{V_W}{A} \quad (5)$$

If diffusion control predominates, which is supposed according to the plot of the logarithms of the k_{AB}-values versus the number of C-atoms from the BN onwards (Fig. 7), then $PC_{Oct/W}$ has no influence on the transfer. Thus, the permeability P_{AB} is merely dependent on the diffusion through the aqueous boundary layer on the donor side:

$$P_{AB} = k_{AB} \cdot \frac{V_W}{A} = \frac{D_W}{d_W} \quad (6)$$

Solving for d_W yields:

$$d_W = \frac{D_W \cdot A}{V_W \cdot k_{AB}} \quad (7)$$

If membrane control is significant at the same time, then the permeability P_{AB} is determined not only by D_W and d_W but also by d_O, D_W and $PC_{Oct/W}$:

$$P_{AB} = k_{AB} \cdot \frac{V_W}{A} = \frac{D_W \cdot D_O \cdot PC_{Oct/W}}{d_O \cdot D_W + d_W \cdot D_O \cdot PC_{Oct/W}} \quad (8)$$

The thickness of the octanol layer d_O may be calculated accordingly:

$$d_O = \frac{D_O \cdot PC_{Oct/W}(D_W \cdot A - d_W \cdot k_{AB} \cdot V_W}{D_W \cdot k_{AB} \cdot V_W} \quad (9)$$

Taking HN (pure diffusion control) as an example, first the thickness of the aqueous boundary

layer d_W is calculated according to Eq. (7). It has a thickness of 36 μm, this should also apply for the other esters under the same experimental conditions. The thickness of the octanol layer is investigated by using the now known d_W as well as the k_{AB}-values for MN and EN according to Eq. (9). The thickness of the investigated octanol layer is about 130 μm. Since the octanol phase is not really stirred and octanol has a 6.6 times higher viscosity than water, the thickness d_O is much higher in comparison to d_W of the aqueous boundary layers.

Eq. (4) is used to calculate the overall permeability under the influence of the aqueous and octanol layers (MN and EN). If diffusion control alone predominates (BN, HN and ON) the product $2 \cdot d_W \cdot D_O \cdot PC_{Oct/W} \gg d_O \cdot D_W$ in Eq. (4); therefore Eq. (4) can be simplified to:

$$P_{overall} = \frac{D_W}{2 \cdot d_W} \quad (10)$$

3.3.2. Permeability values

Table 2 presents the permeabilities $P_{overall}$ calculated according to Eq. (4) (MN, EN) and Eq. (10) (BN, HN, ON). Since for these estimations various quantities are considered to be constant and without variation, no standard deviations are given. The permeabilities increase from MN to EN, they stay more or less constant from here on and even decrease slightly at ON.

To demonstrate the dependence of the permeability on the chain length more clearly while considering the molar volume V_M, the logarithms of the product from the permeability and the cube root of the molar volume (Wilke and Chang, 1955) are plotted versus the number of C-atoms in Fig. 8. This correction enables the elimination of influences of the variable diffusion coefficient on the permeability. Therefore, the potential influence of $PC_{Oct/W}$ should be more evident in this plot. Here again the logarithms of $P_{overall} \cdot V_M^{1/3}$ slightly increase with the lengthening of the alkyl chain between MN and EN. The slope of this section is merely 0.15, clearly lower than the π-constant of 0.55. From the MN onwards the aqueous boundary layers become more and more significant for the permeability in accordance with the k_{AB}-values. The curve in Fig. 8 heads for a plateau from the EN onwards. $PC_{Oct/W}$ loses its significance, the permeability is only dominated by aqueous boundary layers.

If the permeabilities were directly proportional to $PC_{Oct/W}$, that is if there were membrane control with $P_{overall} = D_O \cdot PC_{Oct/W}/d_O$, then a straight line with a slope of unity should result for a logarithmic presentation of $P \cdot V_M^{1/3}$ against $PC_{Oct/W}$. The slope of the straight line between MN and EN is only 0.31, then, as expected, a plateau is reached again (Le, 1993).

The decrease of $P_{overall}$—or the corrected $P_{overall}$-value of ON (Table 2 or Fig. 8) cannot be theoretically explained. It is possibly a consequence of experimental errors when determining the k_{AB}-value, due to extremely low concentrations.

Other authors (Houk and Guy, 1988) studied experimentally the diffusion of nicotinic acid esters in a rotating diffusion cell with isopropyl myristate as lipid layer. They found P-values in the same order of magnitude. Although the observed plateau, that means in principle independence of the P-values of PC, was interpreted as a parabolic relationship, the size of the constant π being left out of consideration.

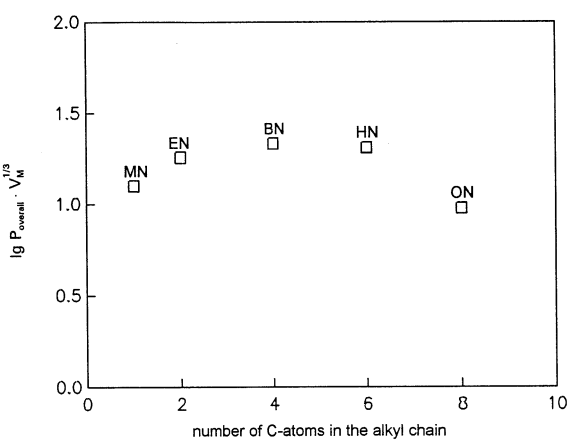

Fig. 8. Dependence of the logarithms of the permeabilities $P_{overall}$, corrected with the molar volume $V_M^{1/3}$, on the number of C-atoms in the alkyl chain, $P_{overall} \cdot V_M^{1/3}$ in [cm^2 h^{-1} mol$^{-1/3}$].

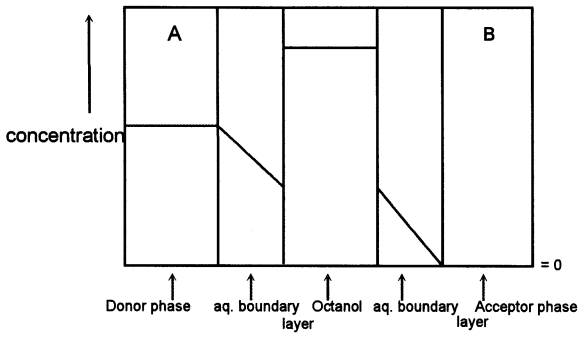

Fig. 9. Concentration course with rate determining aqueous boundary layers in the three layer model water/octanol/water for lipophilic nicotinic acid esters.

3.3.3. Discussion concerning the permeability

Fig. 8 shows that the chain length is of minor importance for the mechanism of transport through the three layer membrane and therefore for the permeability of homologous nicotinic acid esters. According to the 1. Fickian law, the permeability independence of the chain length can be explained by the existence of aqueous boundary layers on both sides of the organic layer (Stehle and Higuchi, 1972). Since diffusion control mainly exists for the investigated nicotinic acid esters, the substance transport is described in the following as transport through the main barrier, the aqueous boundary layers (Fig. 9). Due to the geometrical symmetry of the model, one can assume that the thicknesses d_W of the aqueous boundary layer in the donor and acceptor phase are identical. As a pre-condition it is assumed that the concentration equilibration in the stirred phases takes place immediately. The concentration gradient in the steady state is represented by decreasing straight lines in Fig. 9. Horizontal lines describe homogeneous concentrations or immediate concentration equilibration, these regions cannot present a barrier. Furthermore, a concentration dependence of the diffusion coefficient D should not exist. The transfer through the liquid octanol membrane is caused by the diffusion of the drug through the aqueous boundary layer of phase A, by the partition in the octanol membrane, and diffusion therein and again a partition in the next aqueous boundary layer with a subsequent diffusion here into the next aqueous phase B (Fig. 9). Concentration gradients develop in the aqueous layers. The transport is controlled by both aqueous layers before and after the octanol membrane (diffusion control). Due to the high $PC_{Oct/W}$-values of the ester, the transport through the octanol layer is so fast, that concentration equilibration regularly take place there. It can be assumed, that the parameters for the control by the lipophilic layer, that is $PC_{Oct/W}$, D_O and d_O only have a slight influence on the rate of transfer of the hydrophilic ester MN and EN. So a concentration gradient within the octanol layer develops (the horizontal line in the octanol layer in Fig. 9 receives an inclination). The transition from membrane to diffusion control probably takes place from the methyl ester onwards.

3.4. Maximum flux through a three layer membrane water/octanol/water for the esters

3.4.1. Calculation of the maximum flux

The maximum flux J_{max} through a three layer membrane can be calculated with the help of the determined permeability. The maximum flux J_{max} results from the product of the overall permeability and the solubility in the donor phase (in this case water):

$$J_{max} = P_{overall} \cdot c_{sW} \quad (11)$$

The agreement between the calculated maximum flux and the experimentally determined maximum flux is examined by taking butyl nicotinate as an example.

Table 3 shows the according to Eq. (11) calculated J_{max}-values for the nicotinic acid esters in mg cm^{-1} h^{-1} as well as in mmol cm^{-1} h^{-1}. The standard deviations are not given for the same reason as for the permeability. J_{max} decreases exponentially in the order MN, EN, BN, HN, ON. The maximum flux of the highly water soluble methyl ester is 20 000 or 50 000 higher (mass- or molar flux) than that of the octyl ester, which is not readily soluble in water.

For the experimental determination of J_{max}, an ester would be favoured, which does not show a distinctive substance enrichment in the octanol phase of the three compartment model. Here it would be best to take MN. Since MN is readily

Table 3
Calculated maximum fluxes J_{max} of homologous nicotinic acid esters through the three layer membrane and their solubilities in water c_{sW} and octanol c_{sO}, temperature = 32 ± 1°C

	J_{max} [mg cm^{-2} h^{-1}]	J_{max} [mmol cm^{-2} h^{-1}]	c_{sW} [mg ml^{-1}]	c_{sO} [mg ml^{-1}]
MN	2964.1	21.64	1106	7624
EN	171.1	1.13	47	1034
BN	9.8	0.055	2.45	715
HN	0.6	0.0029	0.17	548
ON	0.016	0.000068	0.01	527

soluble in water, its usage is not possible with a saturated solution in the donor. Therefore, to check the calculation of the maximum flux according to Eq. (11), an experiment with BN is carried out, whose solubility takes up a middle position within the investigated homologous compounds. Apart from the usage of a saturated solution in the donor, the conventional donor volume of 265 ml is increased to 1000 ml to ensure the nearly constant concentration.

J_{max} is here 16.5 mg h^{-1} cm^{-1}. This experimental value is 70% higher than the calculated value ($J_{max} = 9.8$ mg h^{-1} cm^{-2}), but in fairly good agreement, especially if the J_{max}-values of EN and HN are also considered.

3.4.2. Discussion concerning the maximum flux

To interpret the results of the maximum flux with the help of Flynn and Yalkowsky's model (Flynn and Yalkowsky, 1972), the relationship between the logarithm of J_{max}, and the number of C-atoms is plotted in Fig. 10. The values decrease linearly with the lengthening of the alkyl chain.

According to Flynn and Yalkowsky, the J_{max}-values of homologous compounds are dependent on two physico-chemical quantities, namely π and δ (Flynn and Yalkowsky, 1972). π is again the incremental factor, through which the logarithms of the partition coefficients increase linearly when adding a CH_2-group to the alkyl chain; δ is the incremental factor, with which the logarithms of the water solubilities decrease linearly when adding a CH_2-group. (Flynn et al., 1974). The increase of the partition coefficient of the homologous when the alkyl chain is lengthened is clearly lower in comparison to the decrease of the water solubility: $\pi = 0.55$ and $\delta = 0.64$ (Tables 1 and 3) (Le, 1993). Since a negative value results as the difference between both constants, a decrease of the maximum flux with chain lengthening of the homolog is expected according to the model for membrane control. The plot of the logarithms of the maximum fluxes versus the number of C-atoms should result in a decreasing straight line with a slope which corresponds to this difference ($= -0.09$). In the case of diffusion control (control through aqueous boundary layers), the decreasing straight line should have the slope of the δ-constant ($= 0.64$). The slope in Fig. 10 is 0.74, which corresponds quite well with the value of δ. The reason for the deviation of 18% upwards should be the extremely high and somewhat out of place water solubility of MN (Le, 1993), which causes a very high maximum flux value for the calculation and also causes the regression line to

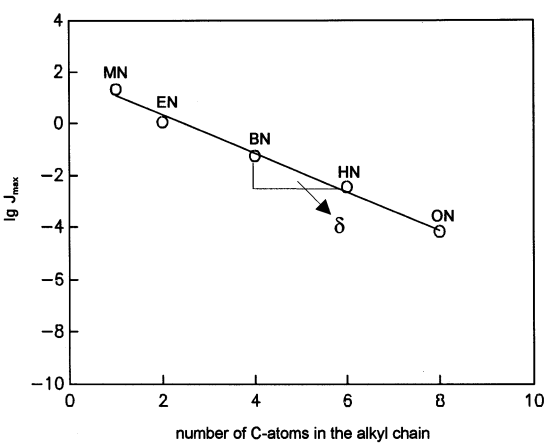

Fig. 10. Relationship between the logarithms of the maximum fluxes J_{max} and the number of C-atoms in the alkyl chain, maximum flux in [mmol cm^{-2} h^{-1}].

have a rather steep slope. The exclusion of MN results in a straight line with a slope of 0.69. Thus, one can assume that diffusion control of J_{max} takes place. The maximum flux is not influenced by the partition coefficient octanol/water as in the case of membrane control (Hagedorn-Leweke and Lippold, 1995; Le and Lippold, 1995). With a high enough $PC_{Oct/W}$ (control to large extent through aqueous boundary layers), the methyl ester has about the same permeability as the octyl ester, but a much higher solubility in water, so that MN has the greatest maximum flux in comparison with the other ester.

A lipophilic membrane in combination with aqueous boundary layers could be arranged in series, so that the permeability not only heads for a plateau, but can also run parabolically (Lien et al., 1970; Hansch and Dunn, 1972; Hansch and Clayton, 1973; Lien and Tong, 1973; Lien, 1975) or bi-linearly (Kubinyi, 1976, 1979a,b) in the log-permeability/PC-profile. Thus, complex relations to the maximum flux result.

The penetration of the nicotinic acid esters through the stratum corneum shows that diffusion control cannot be proven in vivo. The results rather show that permeability and maximum flux correspond to membrane control (Le and Lippold, 1995).

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