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Effect of drug lipophilicity on in vitro release rate from oil vehicles using nicotinic acid esters as model prodrug derivatives

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Abstract

The rate constants for transfer of a homologous series of nicotinic acid esters from oil vehicles to aqueous buffer phases were determined using a rotating dialysis cell. The chemical stability of butyl nicotinate has been investigated at 60°C over pH range 0.5–10. Maximum stability occurs at pH 4–5 and an inflection point was seen around the pK_a. For the nicotinic acid esters, a linear correlation was established between the first-order rate constant related to attainment of equilibrium, k_{obs} and the apparent partition coefficient, P_{app}: $\log k_{obs} = -0.83 \log P_{app} + 0.26$ (k_{obs} in h^{-1}, $n = 9$). For hexyl nicotinate with a true partition coefficient of 4 it was possible to determine k_{obs} by decreasing pH in the aqueous release medium to 2.05. Thus, under the latter experimental conditions estimation of the relative release rates for the esters were performed. The ratio between the specific rate constant k_{ow}, related to the transport from oil vehicle to aqueous phase, for ethyl and hexyl nicotinate was 139. The hydrophobic substituent constant for a methylene group, $\pi(CH_2)$, was determined for nicotinic acid esters in different oil/buffer partitioning systems to 0.54–0.58. Addition of hydroxypropyl-β-cyclodextrin to the aqueous release medium did not enhance the transport rate of the esters from the oil phase. © 2001 Published by Elsevier Science B.V.

Keywords: Parenteral depots; Prodrugs; Oily solutions; Lipophilicity; Relative release rate; Nicotinic acid ester

1. Introduction

Many long-acting injectables are formulated in the form of lipophilic prodrugs dissolved in oil vehicles. For a number of drugs pharmacokinetic data after intramuscular injection of such formulations have been reported (for review see Davis et al., 1994). In contrast only few studies have dealt with the relative influence of the lipophilicity of the transport group on the absorption rate or duration of action of the individual therapeutic agent: testosterone (James et al., 1969), flup-

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henazine (Dreyfuss et al., 1976), zuclopenthixol (Aaes-Jørgensen, 1989) and morphine (Jørgensen et al., 1994). Since the rate of drug appearance in the systemic circulation is governed by various presystemic absorption processes (Luo et al., 1997, 1998) the design of depot injections with optimal delivery characteristics requires understanding of these underlying mechanisms, in particular the rate of release of the prodrug from the oil vehicle (Huang et al., 1995). Earlier, we have developed an in vitro release model based on a rotating dialysis cell, which was used for investigation of the influence of composition of the aqueous release medium on the release rates of chemical entities from oil solutions (Larsen et al., 2000a). Furthermore, the effect of different oil vehicle compositions on the release rates was investigated (Fredholt et al., 2000). This in vitro release model is limited to release studies of moderately lipophilic compounds having a log *P* value less than 2. The aim of the present study was to optimise the existing model by altering the composition of the release medium in order to enable determination of relative release rates of a homologous series of prodrug derivatives from various oil vehicles. Nicotinic acid esters are used as model drug substances (Fig. 1).

2. Materials and methods

2.1. Materials

Fractionated coconut oil (Viscoleo®) was obtained from P. Broeste A/S, Denmark. Sesame oil, castor oil, nicotinic acid, methyl, ethyl and hexyl nicotinate were purchased from Sigma Chemical Company (USA). Hydroxypropyl-β-cyclodextrin (HPβCD), isopropyl and butyl nicotinate were purchased from Aldrich-chemical Co. Chemicals for preparation of buffers and HPLC mobile phases were of analytical or chromatographic grade. Demineralised water was used throughout. Visking dialysis tubing size 27/32, 21.5 mm with a cut off at 12 000–14 000 Da was employed for the dialysis cell.

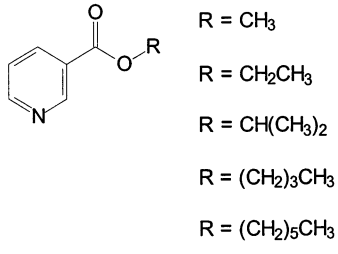


Fig. 1. Chemical structures of nicotinic acid esters (methyl, ethyl, isopropyl, butyl and hexyl ester).

2.2. Stability studies

The stability of butyl nicotinate was investigated in different buffer solutions in the pH-range 0.45–9.60 at 60 ± 0.5°C. The influence of the temperature on the degradation rate of the nicotinic acid ester was studied at pH 1.15 at 37 ± 0.5, 60 ± 0.5, 70 ± 0.5 and 80 ± 0.5°C. The stability of methyl, ethyl, isopropyl and hexyl nicotinate was studied at pH 1.15 at 60 ± 0.5°C. The buffers used were acetate (pH 4.00–5.00), phosphate (pH 3.00 and 6.80) and borate (pH 8.40 and 9.60) solutions at a total buffer concentration of 0.1 M. In the pH range 0.45–2.15, hydrochloric acid was used. A constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride.

The reactions were initiated by adding 100 μl of a 10^{-2} M stock solution of the compounds in acetonitrile to 9.9 ml of preheated buffer solution in screw-capped test tubes resulting in a final concentration of 10^{-4} M. The solutions were kept at constant temperature in a water bath and at appropriate times samples were withdrawn and chromatographed immediately. In case of fast reactions, samples were taken and hydrochloric acid or sodium hydroxide was added to pH 4–5 prior to analysis in order to stop the degradation process. For slowly proceeding reactions (pH range 0.45–6.8) the pseudo-first-order rate constants were derived by the initial rate method (Connors, 1973). The reactions were followed for a time period corresponding to 1–2% formation of nicotinic acid. Pseudo-first-order rate constants for the degradation of butyl nicotinate at pH 1.15, 8.40 and 9.6 were determined from the slopes of linear plots of the logarithm of intact nicotinic acid ester

against time. To evaluate the initial rate method the pseudo-first-order rate constants at pH 1.15 and 8.40 were also obtained by the initial rate method.

2.3. In vitro release experiments

The release studies were carried out at 37 ± 0.5°C using the rotating dialysis cell model described earlier (Larsen et al., 2000a). In brief, volumes of 1000 and 5 ml were used for the aqueous release medium and the oil phase, respectively. The release media used were 0.05 M aqueous buffer solutions pH 5.00 (acetate), pH 3.00 and 6.00 (phosphate) and pH 2.05 (hydrochloride acid). In the experiments involving cyclodextrin, 3% HPβCD was added to the aqueous release medium. The model drug compounds were initially added to the various oil vehicles at a concentration of 10 mg ml^{-1} and placed inside the dialysis cell. The revolution speed of the cell was maintained at 50 rpm. At appropriate intervals samples were withdrawn from the aqueous phase and analysed by HPLC. Sampling was continued until equilibrium between the two phases was established. The amount of drug released (M_t) in percent (corrected for sampling) into the aqueous phase was calculated according to Eq. (1):

$$M_t = \frac{V_s \sum_{n=1}^{n} C_{n-1} + V_m \times C_n}{M} \times 100 \quad (1)$$

where V_s and V_m are the volumes of sample taken and release medium, respectively. C_n is the drug concentration in sample n and M represents the total amount of drug initially applied to the dialysis cell.

The overall first-order rate constant (k_{obs}) related to attainment of equilibrium was calculated from the relationship:

$$\ln(M_e - M_t) = \ln M_e - k_{obs} t \quad (2)$$

where M_e is the total amount released at equilibrium.

For very slow reactions the rate constant k_{obs} was determined by using the Guggenheim method (Guggenheim, 1926) according to the equation:

$$\ln(M_{t+\Delta} - M_t) = -k_{obs} t + \ln(M(1 - e^{-k\Delta})) \quad (3)$$

where M_t and $M_{t+\Delta}$ represent the amount of solute released at time t and time $t + \Delta$, respectively and M represents the total amount of drug in the oil phase at time zero.

Release experiments were done in triplicate unless otherwise stated.

2.4. Determination of partition coefficients

Apparent partition coefficients (P_{app}) of the derivatives between oil vehicle and buffer solution were determined at 37 ± 0.5°C. Equilibrium was attained after rotating in a water bath for 24 h. Drug concentration in the aqueous phase before and after partition was measured by HPLC allowing the apparent partition coefficients to be calculated according to Eq. (4):

$$P_{app} = \left(\frac{C_i - C_w}{C_w}\right) \times \left(\frac{V_w}{V_o}\right) \quad (4)$$

where C_i and C_w represent the drug concentration in the aqueous buffer before and after partition, respectively. V_w and V_o are the volumes of the aqueous and the oil phase. The initial drug concentration in the aqueous buffer was approximately 0.1 mM. Apparent partition coefficients were calculated from partition experiments done in triplicate. The volumes of each phase were chosen so that the solute concentration in the aqueous phase could easily be measured. In a few partitioning experiments, 10^{-6}M nicotinic acid (corresponding to formation of 1% nicotinic acid from hydrolysis of the ester derivatives in the aqueous release media) was added to the aqueous buffer solution.

2.5. pK_a determination

The pK_a-value of butyl nicotinate has been estimated using two methods.

2.5.1. pK_a determination by UV spectrophotometry

The absorbances of butyl nicotinate in solutions with pH of 1.11, 2.99, 3.22, 3.67 and 11.89 at room temperature were recorded at 260.74 nm by

UV spectroscopy, using a Spekol 1200 spectrophotometer (Carl Zeiss Technology). The buffer solutions used were 0.01 M phosphate (pH 2.99 and 3.22), 0.01 M acetate (pH 3.67), hydrochloric acid (pH 1.11) and sodium hydroxide (pH 11.89). A constant ionic strength of 0.1 was maintained for all the solutions. The pK_a-value of butyl nicotinate was calculated according to Eq. (5):

$$pK_a = pH - \log \frac{A_A - A}{A - A_B} - \log \gamma_B + \log \gamma_A \quad (5)$$

where A_A and A_B are the absorbance of the acid and base form of butyl nicotinate, respectively and A is the absorbance in the buffer solution. γ_A and γ_B are the activity coefficient of the acid and base form, respectively.

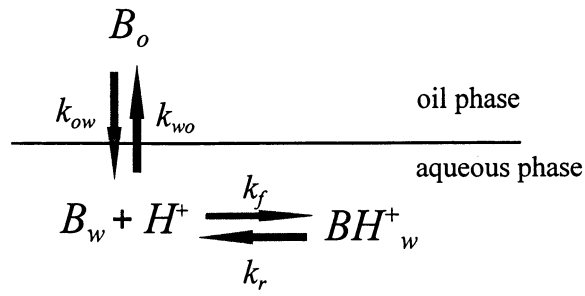
2.5.2. pK_a determination by the partition coefficients

The apparent partition coefficients for butyl nicotinate between Viscoleo and aqueous buffer phase at pH 1.19, 2.05, 3.00 and 4.00 at 37 ± 0.5°C were determined. The true partition coefficient of butyl nicotinate was determined at pH 7.00. The pK_a-value were calculated according to the relationship between the true partition coefficient (P) and the pH dependent partition coefficient (P_{app}) for a weak base expressed as:

$$P_{app} = \frac{P}{1 + 10^{pK_a - pH}} \quad (6)$$

2.5.3. HPLC analysis

Samples from the stability studies, the release studies and the partition experiments were analysed by HPLC. The system consisted of a Merck Hitachi L-6200 or a Shimadzu LC-6A pump and a Shimadzu SPD-6A or a Merck Hitachi L-4000 UV detector operating at 263 nm. For the release studies and the partition experiments a Merck Hitachi 655 A–40 auto sampler was used. Reversed phase chromatography was carried out using an Inertsil ODS-2 column (250 × 4.6 mm; 5 μm particles) equipped with an Inertsil ODS-2 precolumn (Chrompack, The Netherlands). The flow rate was set at 1 ml min^{-1}. The mobile phases were composed of 0.01 M phosphate buffer (pH 7.5) and 10% methanol for nicotinic acid and 50–65% acetonitrile or methanol for the nicotinic acid esters.



Scheme 1.

3. Results and discussion

The in vitro release experiments have been carried out using the rotating dialysis cell model characterised earlier (Larsen et al., 2000a). The partition process for the basic model prodrug derivatives can be described by reversible kinetics (Scheme 1), assuming that mass transfer between the two phases only involves non-ionised species: B_o and B_w represent the free base form in the oil phase and water phase, respectively. Due to dissociation both non-ionised base and the corresponding acid (BH^+_w) might be present in the aqueous phase. k_{ow} is the rate constant for transfer from the oil phase to the aqueous phase, whereas k_{wo} is the rate constant for the reverse transport process. k_f and k_r are the rate constants related to the acid-base equilibrium. The proton transfer reactions are expected to proceed extremely fast.

Earlier, enhanced release rates were observed by diminishing the apparent partition coefficient of the partitioning agent (Larsen et al., 2000a). Due to ionisation P_{app} of a weak base decreases with decreasing pH of the aqueous phase (the oil phase kept constant). In the present study the effect of decreasing pH of the release medium on the relative release rates for a homologous series of nicotinic acid esters has been determined. Since degradation data for nicotinic acid esters only are reported for hydrolysis at neutral and alkaline pH (Wernly-Chung et al., 1990) the pH-rate profile of

butyl nicotinate including the acidic range has been constructed. It was assumed that the homologous series of aliphatic esters used in the present study exhibit similar susceptibility to undergo pH-dependent hydrolysis.

3.1. Stability studies

For butyl nicotinate a pH-rate profile was constructed covering the pH range 0.45–9.60 at 60°C (Fig. 2). The degradation of butyl nicotinate at pH 1.15, 8.40 and 9.60 at 60°C followed pseudo-first-order kinetics for more than two half-lives, when monitoring the disappearance of intact ester as a function of time. In addition, the rate constants of butyl nicotinate at pH 1.15 and 8.40 were derived by using the initial rate method. Identical pseudo-first-order rate constants were obtained by employing the two different methods. For the slowly proceeding degradation reactions the initial rate method was used. In hydrochloric acid solutions, pH was calculated according to Eq. (7):

$$\mathrm{pH} = 0.15 - \log[\mathrm{H}^+] \quad (7)$$

where $[\mathrm{H}^+]$ is the hydrogen ion concentration. The logarithm to the activity coefficient at 60°C and $\mu = 0.5$ corresponds to -0.15 (Harned and Hamer, 1933).

From the pH-rate profile (Fig. 2) is seen that maximum stability for butyl nicotinate occurs at pH 4–5 and at about pH 3, an inflection point is observed. As apparent butyl nicotinate is relatively more stable at low pH values than in the alkaline region. The shape of the pH-rate profile can be accounted for in terms of a specific acid- (k_{H}^+) and a spontaneous or water- (k_0) catalysed reaction of the protonated form of butyl nicotinate and a spontaneous or water- (k'_0) and specific base- (k_{OH}^-) catalysed reaction of the free base. Accordingly, the overall pseudo- first-order rate constant (k_{obs}) can be expressed:

$$k_{\mathrm{obs}} = k_{\mathrm{H}^+} a_{\mathrm{H}^+} \frac{a_{\mathrm{H}^+}}{a_{\mathrm{H}^+} + K_{\mathrm{a}}} + k_0 \frac{a_{\mathrm{H}^+}}{a_{\mathrm{H}^+} + K_{\mathrm{a}}} + k'_0 \frac{K_{\mathrm{a}}}{a_{\mathrm{H}^+} + K_{\mathrm{a}}} + k_{\mathrm{OH}^-} a_{\mathrm{OH}^-} \frac{K_{\mathrm{a}}}{a_{\mathrm{H}^+} + K_{\mathrm{a}}} \quad (8)$$

where a_{H^+} and a_{OH^-} refer to the hydrogen ion and hydroxide ion activity, respectively. $k_{\mathrm{H}}+$ and k_{OH^-} are the second-order rate constants for specific acid- and base-catalysed hydrolysis, respectively. k_0 and k'_0 are the first-order rate constants related to spontaneous or water- catalysed hydrolysis and K_{a} is the ionisation constant (the pK_{a}-value for butyl nicotinate was determined to be 3.23 ± 0.02 and 3.15 ± 0.11 by spectrophotometry at room temperature and from the partitioning experiments at 37°C, respectively). A pK_{a}-value of 3.2 was used for the calculations. Good agreement between experimental values and the fitted curve is observed demonstrating the adequacy of Eq. (6). The following values were calculated for the rate constants at 60°C:

$$k_{\mathrm{H}^+} = 5 \times 10^{-4}\ \mathrm{M}^{-1}\ \mathrm{min}^{-1}$$

$$k_0 = 1.53 \times 10^{-6}\ \mathrm{min}^{-1}$$

$$k'_0 = 6.7 \times 10^{-7}\ \mathrm{min}^{-1}$$

$$k_{\mathrm{OH}^-} = 162\ \ \mathrm{M}^{-1}\ \mathrm{min}^{-1}$$

Assessment of the stability of the esters in acidic solution was done by determination of the degradation rate of methyl, ethyl, isopropyl and hexyl nicotinate at pH 1.15 (60°C). The pseudo-first-order rate constants for the esters are ranged in order of decreasing stability isopropyl, hexyl, butyl, ethyl and methyl nicotinate. A factor of 2.4 was found between the degradation rate for methyl and isopropyl nicotinate. A similar relative sensitivity towards hydrolysis of methyl, ethyl,

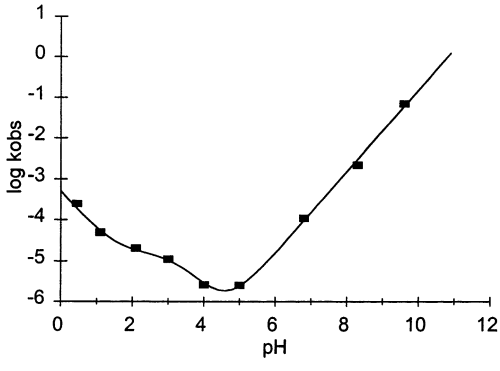


Fig. 2. pH-rate profile for the hydrolysis of butyl nicotinate at 60°C and $\mu = 0.5$. (k_{obs}: observed pseudo-first-order rate constant (min^{-1})). The full line has been calculated from Eq. (8).

isopropyl and butyl nicotinate at pH 7.4 have been reported (Wernly-Chung et al., 1990).

Since the release experiments were performed at 37°C estimation of the stability of the esters at 37°C was necessary. The influence of the temperature (37–80°C) on the degradation rate of butyl nicotinate was studied at pH 1.15. From the Arrhenius plot, an activation energy (E_a) of 70 kJ mol^{-1} was calculated.

Initial partitioning experiments were performed using aqueous buffer solutions with and without addition of 10^{-6} M nicotinic acid (corresponding to 1% formation of nicotinic acid from hydrolysis of the ester derivatives) revealing that in this concentration range nicotinic acid did not influence the magnitude of the apparent partition coefficients of the ester derivatives. Hence, in the release experiments and for the determination of apparent partition coefficients a maximum of 1% free nicotinic acid related to the nicotinic acid ester was allowed. The time period for 1% degradation ($t_{99\%}$) of the nicotinic acid esters at 37°C (acidic pH) was estimated by assuming an equal effect of temperature on the degradation rate of all ester. For methyl nicotinate, the least stable ester, $t_{99\%}$ at pH 2.15 was estimated to 1.8 days. By HPLC analysis it was ensured that the concentration of nicotinic acid was below 1% in the aqueous phase after end of the individual release experiments.

3.2. Release experiments

The in vitro release experiments were carried out by using a rotating dialysis cell model (Larsen et al., 2000a). The experimental conditions were kept constant except for the composition of the release medium. Release experiments were done in triplicate with relative standard deviations (R.S.D.) in the range 1–7%. In the two-phase partitioning systems, the rate constants related to attainment of equilibrium (k_{obs}) for the nicotinic acid esters were calculated according to Eq. (2) except from very slow release studies where k_{obs} was calculated by using the Guggenheim method Eq. (3).

In vitro release experiments with Viscoleo as the oil phase and 0.05 M acetate buffer pH 5.00 in the release medium were performed for all nicotinic acid esters and the accumulated amount of ester in the aqueous phase was calculated. From the release profiles for the methyl, ethyl, isopropyl and butyl nicotinate (Fig. 3) it appears that the order of release rate is methyl > ethyl > isopropyl > butyl nicotinate as expressed by the rate constant for attainment of equilibrium (k_{obs}) (Table 1). Hexyl nicotinate was too lipophilic to be released at the above described conditions. Thus, in order to enable estimation of the relative release rates of the nicotinic acid esters under identical experimental conditions the aqueous buffer phase was modified. The composition of the release medium was adjusted to enhance the aqueous solubility of the partitioning agents by lowering the pH or addition of cyclodextrins.

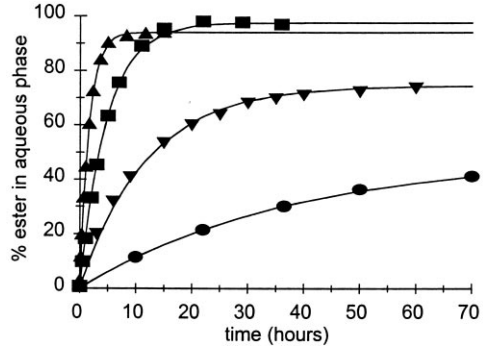


Fig. 3. Time dependence of the amount of ▲ methyl nicotinate ■ ethyl nicotinate ▼ isopropyl nicotinate and ● butyl nicotinate in the aqueous phase presented as percentage of the initial amount of ester applied to Viscoleo in a Viscoleo/acetate buffer pH 5.00 partitioning system at 37°C.

As expected, the k_{obs}-values for nicotinic acid esters increased due to the more acidic pH of the aqueous release medium (Table 1). At pH 2.05, where the ester derivatives are almost fully protonated the most lipophilic ester, hexyl nicotinate, was released over an acceptable period of time. Thus, optimisation of pH in the aqueous release medium enables a lipophilic compound with a log P of approximately 4 to be tested in the present model. Thus, at the above described experimental conditions a comparison of the relative release rate of nicotinic acid esters from Viscoleo to the aqueous phase as expressed by the rate constant

(k_{ow}) was possible. k_{ow}-values independent of interfacial area and volumes were calculated according to Eq. (9) (Yunker and Borodkin, 1971; Larsen et al., 2000b):

$$k_{ow} = \frac{k_{obs}}{A\left(\frac{1}{V_o} + \frac{P_{app}}{V_w}\right)} \quad (9)$$

where V_o and V_w are volume of the oil and water phase, respectively, and A is the interfacial area between oil and aqueous phase. Although A is unknown, it is expected that the interfacial area remains constant under the experimental conditions employed due to the construction of the rotating dialysis cell. Thus, at pH 2.05 the ratios, k_{ow}(ethyl ester):k_{ow}(butyl ester) and k_{ow}(ethyl ester):k_{ow}(hexyl ester) amounts to 8.2 and 139, respectively. At physiological pH, the difference in release rates (k_{ow}) of the ethyl and butyl ester has been estimated to 12 using an ionisation constant of $10^{-3.2}$ for both derivatives. In case of parenteral oil depot injections, drug release rate has been suggested to be controlled by the partition coefficient of the drug between the oil vehicle and the tissue fluid (Chien, 1981; Hirano et al., 1981). To this end, duration of actions of intramuscularly administered zuclopenthixol acetate and decanoate of 2–3 days and 2–4 weeks, respectively, has been reported (Aaes-Jørgensen, 1989). In a study of the slow-release characteristics of ^{14}C-fluphenazine enanthate and decanoate esters administered in sesame oil to dogs (Dreyfuss et al., 1976), it was concluded that the decanoate ester was released from the depot at less than one-half the rate of the enanthate ester. The lipophilicity of the zuclopenthixol prodrugs differs by a factor of 10^4, whereas the $\Delta\log P$ for the fluphenazine derivatives is about 1.5. Thus, for partition controlled drug release a much greater difference in relative duration of action of the prodrug derivatives, which at least partly reflects drug release rate from the oil solution, was to be expected. The data suggest that for highly lipophilic compounds the duration of action might not solely be explained by drug partitioning into the tissue fluid.

3.3. The relationship between log k_{obs} and log P_{app}

Earlier, a linear correlation was found between log k_{obs} and log P_{app} for the partition of the model drugs naproxen and lidocaine between different oils and aqueous buffers pH 5.00–7.40 (Fredholt et al. 2000; Larsen et al., 2000a). Likewise, for the nicotinic acid esters, a linear relationship between log k_{obs} and log P_{app} was established ($r = 0.997$, $n = 9$):

$$\log k_{obs} = -0.831 \ \log P_{app} + 0.26 \quad (k_{obs} \text{ in } h^{-1}) \quad (10)$$

As seen from Fig. 4, the linear correlations obtained in the present study differs to some

Table 1
Apparent partition coefficients (P_{app}) and apparent overall rate constants (k_{obs}) for partitioning of nicotinic acid esters between Viscoleo and buffer solutions with or without addition of 3% hydroxypropyl-β-cyclodextrin at 37°C

Ester	pH	±Cyclodextrin	k_{obs} (h^{-1})	P_{app}
Methyl nicotinate	5.00	−	0.62	3.71
Methyl nicotinate	6.00	−	0.69	3.80
Ethyl nicotinate	2.05	−	1.44	1.31
Ethyl nicotinate	5.00	−	0.21	13.9
Isopropyl nicotinate	5.00	−	0.085[a]	38.4
Butyl nicotinate	2.05	−	0.19	13.2
Butyl nicotinate	3.00	−	0.042	82.8
Butyl nicotinate	5.00	−	0.027	174
Butyl nicotinate	5.00	+	0.028[a]	23.0
Hexyl nicotinate	2.05	−	0.020[a]	197
Hexyl nicotinate	5.00	+	0.007[a]	123

[a] $n = 2$; mean values have been calculated from experiments done in triplicate.

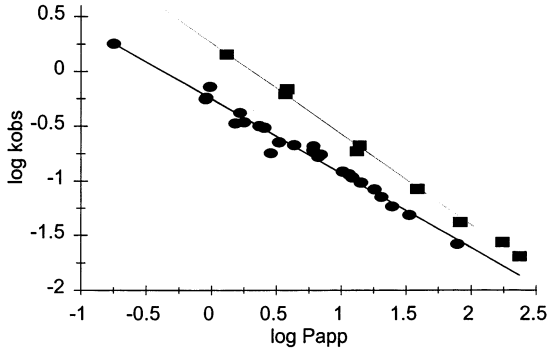


Fig. 4. Relationship between log k_{obs} and log P_{app} determined for partition between different oil/oil mixture and different aqueous buffers at 37°C. ● naproxen and lidocaine (Fredholt et al., 2000; Larsen et al., 2000a). ■ nicotinic acid ester. (k_{obs}: the overall rate constant for attainment of equilibrium (h^{-1}), P_{app}: the apparent partition coefficient). The line ---- has been drawn according to Eq. (10). The line — has been drawn according to: log $k_{obs} = -0.68$ log $P_{app} - 0.25$ (Fredholt et al., 2000).

extent from the relationship reported earlier (Fredholt et al., 2000; Larsen et al., 2000a).

In contrast to the latter reported experiments, aqueous release media of relatively low pH have been used in the present study. Potentially, this acidic environment could alter the structure of the dialysis membrane and thereby, the release characteristics of the derivatives. This possibility, however, appears less likely since the rate constants and apparent partition coefficients obtained at low pH fitted nicely into the linear relationship between log k_{obs} and log P_{app} Eq. (10). In addition, in the pH range 5–6, release experiments have been performed for both naproxen/lidocaine and the nicotinic acid esters. Significant uncertainty in the determination of k_{obs} can be ruled out. It has been demonstrated that the drug transfer reactions apply to reversible kinetics (Fig. 5). First-order kinetics of the present release studies have been ensured from the obtained linear plots according to Eq. (2). Further, identical k_{obs}-values were found for methyl nicotinate in a Viscoleo/pH 5 buffer system using initial concentrations in the oil phase of 5 and 10 mg ml^{-1}, respectively. Slight concentration dependence of the magnitude of the partition coefficient (octanol/water) of nicotinic acid esters has been observed (Le and Lippold, 1998). In our oil–aqueous buffer partition systems such a tendency has not been found varying the solute concentration in the range 10^{-4}–10^{-5} M. Thus, the reason for the different correlations between log k_{obs} and log P_{app} illustrated in Fig. 4 remains obscure.

Earlier, Le and Lippold (1998) have investigated the transfer rates of nicotinic acid esters in a Schulman-type three compartment model. The rate constants were found to be rather independent of the partition coefficient of the respective esters and a diffusion control in the aqueous boundary layers was suggested as the rate limiting step. These observations are different from those found in this study, which most likely is a reflection of differences in apparatus and experimental setup (Byron et al., 1981). Thus, a comparison of the rate constants have not been performed.

3.4. Prediction of P_{app} values

Due to the linear relationship between log k_{obs} and log P_{app}, the release rate of an ester derivative might be estimated with knowledge of the apparent partition coefficient. Generally, for prediction of partition coefficients in a homologous series of aliphatic compounds the hydrophobic substituent

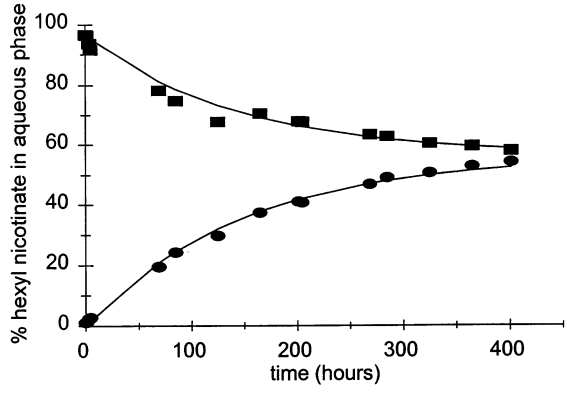


Fig. 5. Time dependence of the amount of hexyl nicotinate in the aqueous phase presented as percentage of the initial amount of ester applied to a partition system consisting of Viscoleo and acetate buffer pH 5.00 with addition of 3% hydroxypropyl-β-cyclodextrin at 37°C ■ 50 mg hexyl nicotinate initially applied to the aqueous buffer ● 50 mg hexyl nicotinate initially applied to Viscoleo. The full lines have been derived using Eq. (2).

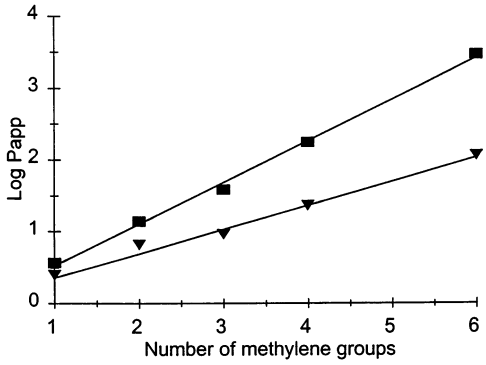


Fig. 6. Relationship between log P_{app} for nicotinic acid esters in a Viscoleo/acetate buffer pH 5.00 partitioning system at 37°C and the number of methylene groups in the alkyl chain. ■ without addition of cyclodextrins. ▼ with addition of 3% hydroxypropyl-β-cyclodextrin. (P_{app}: the apparent partition coefficient).

constant for a methylene group, $\pi(CH_2)$, is used (Hansch and Leo, 1979). Using methyl, ethyl, butyl, hexyl and octyl nicotinate a $\pi(CH_2)$-value of 0.55 has been determined in an octanol and water system (Le and Lippold, 1998). In the present experiments, almost identical $\pi(CH_2)$-values were calculated from different oil/buffer partitioning systems. A $\pi(CH_2)$-value of 0.55 was obtained for nicotinic acid esters in a Viscoleo/buffer pH 5.00 system (Fig. 6). For the partition of esters in systems consisting of buffer with pH 2.05 and Viscoleo, sesame oil or castor oil the $\pi(CH_2)$-values of 0.58, 0.57 and 0.54 were derived, respectively, indicating that at least for a homologous series of aliphatic compounds the hydrophobic substituent constant for an oil/buffer partitioning system is comparable with $\pi(CH_2)$-values generated from octanol/water partitioning systems.

3.5. Addition of cyclodextrins to the aqueous release medium

Addition of cyclodextrins to the release medium was expected to enhance the transport rate of derivatives from the oil phase to the aqueous phase due to an aqueous solubility enhancing effect of cyclodextrins. Preliminary data for the release of zuclopenthixol acetate from Viscoleo indicated that the amount of drug released increased significantly by addition of 2% HPβCD (hydroxypropyl-β-cyclodextrin) to the aqueous phase (Schultz, 1997). Furthermore, in a two-phase system Frijlink et al. (1989) observed a decrease in the transport of lipophilic drug substances from an aqueous phase to an organic phase consisting of cyclohexane by addition of βCD to the aqueous phase. Since preliminary partition experiments with different cyclodextrins have shown that HPβCD possesses the best characteristics for further studies, only addition of HPβCD to the aqueous phase was investigated in more detail in the present study. Addition of 3% HPβCD to the aqueous buffer phase in a Viscoleo/buffer pH 5.00 partitioning system resulted in a decrease in P_{app} of 96 and 88% for hexyl and butyl nicotinate, respectively. The decrease in P_{app} for butyl nicotinate did not result in an increase in k_{obs} (Table 1). For hexyl nicotinate it was not possible to determine k_{obs} without addition of HPβCD under these experimental conditions. Thus, for comparison of k_{obs} with and without HPβCD a k_{obs} for hexyl nicotinate was calculated. The k_{obs}-value of 0.002 h^{-1} was calculated using the P_{app}-value obtained in a Viscoleo/buffer pH 5.00 system and the relationship between log k_{obs} and log P_{app} Eq. (10). Thus, in contrast to butyl nicotinate an increase in k_{obs} with a factor of 3.5 was observed for hexyl nicotinate by addition of 3% HPβCD to the release medium. However, the increase in k_{obs} was not as high as expected from the relationship between log k_{obs} and log P_{app} Eq. (10). Furthermore, a $\pi(CH_2)$-value of 0.32 was determined for the nicotinic acid esters by addition of 3% HPβCD to the aqueous phase in a Viscoleo/buffer pH 5.00 partitioning system (Fig. 6). In conclusion, based on the experimental observations it appears less feasible to use cyclodextrins for evaluation of the relative release rate for a homologous series of drug derivatives. However, the use of cyclodextrin for assessment of the release rates of lipophilic non-electrolytes from different oil vehicles cannot be excluded.

4. Conclusion

Comparison of relative release rates for a homologous series of nicotinic acid esters from oil vehicles was accomplished by decreasing pH of the aqueous release medium. From the degradation studies, it was ensured that under the experimental conditions of the transfer experiments less than 1% of the esters was degraded. Using a release medium pH 2.05 the ratio between the rate constants, k_{ow}, related to transport from oil vehicle to the water phase, for ethyl and hexyl nicotinate was calculated to 139. In comparison, a much smaller difference in duration of action of commercially available oil depots containing various prodrugs differing significantly with respect to log P is observed. Thus, for highly lipophilic compounds in vivo release rates from oil vehicles might not solely governed by drug partitioning. A linear relationship was established between k_{obs} and P_{app} for the nicotinic acid esters. Results of the present study suggest that for a homologous series of prodrug derivatives estimation of partition coefficients can be based on tabulated hydrophobic substituent constants (n-octanol–water) since similar $\pi(CH_2)$ values were derived from partition of the nicotinic acid esters in different oil-buffer systems (0.54–0.58).

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