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Partition and transport of verapamil and nicotine through artificial membranes

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Summary

Relevant properties of verapamil and nicotine in relation to transdermal delivery were defined by studying their transport behavior as a function of the pH of the reservoir. The partition coefficient and diffusivity in lipid membranes are the main parameters for the transport, but the flux of drug is very sensitive to solubility when it changes as a function of pH. This shows that lipophilic drugs with high permeability coefficients are not the only candidates for transdermal delivery: hydrophilic drugs can also achieve a high permeation flux.

Introduction

Verapamil and nicotine are candidates for transdermal delivery because of their pharmacokinetic characteristics (Eichelbaum and Somogyi, 1984). Since they are ionizable molecules their permeability can be increased by iontophoretic application. Thus, the drug reservoir composition parameters such as ionic strength, pH and solvent can play a decisive role in permeation. Moreover, it is well known that nicotine is capable of forming ion pairs with some acids (Oakley and Swarbrick, 1987), thus complicating the overall mechanism of permeation.

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The aim of this work was to define the relevant properties of the two drugs in relation to transdermal delivery, by studying their transport behavior as a function of pH or the ionic strength of the reservoir.

The objectives include the identification of the effect of drug dissociation on the apparent partition coefficient and on the diffusional characteristics through artificial membranes.

Materials and Methods

The following were used: verapamil hydrochloride (Prodotti Gianni, Milan, Italy), mol. wt. 491.08, pK_a 8.9 (Ritschel and Agrawala, 1988), m.p. 143.5°C, UV max: 232 nm; (−)-nicotine (Fluka, Buchs, Switzerland), mol. wt. 163.23, $pK_{a,1}$

3.4, $pK_{a,2}$ 8.2 (Oakley and Swarbrick, 1987), $b.p._{17}$ 123°–125° C, UV max: 261 nm; (−)-nicotine bitartrate (Sigma, St. Louis, MO, U.S.A.), mol. wt. 463.41, m.p. 90° C; polypropylene membranes (Celgard® 3501, Celanese, Charlotte, NC, U.S.A.), thickness 0.0025 cm, porosity 45%, pore size 0.04 μm; cellophane membranes (2035-001; P 40 OF, Viscora, Bagnolet, France), thickness 0.0065 cm, pore size 0.0025 μm; isopropyl myristate (IPM), citric acid and disodium phosphate were of analytical grade.

Determination of apparent partition coefficient

McIlvaine buffer solutions used for the determination of apparent partition coefficients were prepared by mixing appropriate volumes of 0.1 M citric acid and 0.2 M disodium phosphate solutions. The pH of buffer solutions was 4, 5, 6 and 7 (±0.05 units of pH). Before use, IPM and buffers were saturated overnight at room temperature in a mechanical shaker and then separated. 5 ml of buffer solution containing a known concentration of drug (100 μg/ml of verapamil · HCl, 50 μg/ml of nicotine, 142 μg/ml of nicotine bitartrate) and 5 ml of IPM were equilibrated in 20 ml sealed centrifuge tubes. The centrifuge tubes were shaken mechanically at room temperature (21–22° C) for 1 h. After separating the two phases, the drug concentration in the aqueous phase was determined spectrophotometrically. The drug concentration in the organic phase was calculated from that found in the aqueous phase.

Determination of permeation parameters through artificial membranes

For membrane permeation studies, a Resomat II apparatus (Dibbern and Scholz, 1969) was employed (Desaga, Heidelberg, Germany). The donor compartment contained 50 ml of McIlvaine buffer drug solution and the receptor compartment 200 ml of the same buffer. The initial drug concentration in the donor solution was chosen at 10 or 20% of its solubility in the buffer. The apparatus was thermostated at 37° C and two magnetic stirrers eliminated the boundary layer effects in each compartment. The amount of drug which permeated the membrane was calculated from the drug concentration in the receptor phase, by continuously monitoring with a UV spectrophotometer. As barriers, polypropylene membranes, impregnated with IPM according to Barthélémy et al. (1986) or cellophane membranes for donor to receptor separation were used. At the end of each experiment the integrity of the impregnated membrane was tested by evaluating its impermeability to ions. To this end, 1 g of sodium chloride was added to the donor compartment and the absence of chloride ions in the receptor phase compartment was checked over a period of 30 min.

Determination of solubility

An excess amount of drug was added to phosphate-citrate buffer and incubated over 24 h at 37° C. After centrifugation, the concentration of the drug in the supernatant was determined spectrophotometrically. The time (24 h) was sufficient for equilibration.

Results and Discussion

IPM / water apparent and true partition coefficients

As lipophilic phase in partitioning experiments, isopropyl myristate (IPM) was chosen because its polar and non-polar properties can simulate skin lipids (Barry, 1983). The measured apparent partition coefficients as a function of pH, for the examined drugs, are shown in Fig. 1. The profiles obtained indicate an expected increase in the lipophilicity of the two basic drugs by increasing the pH value. The curves of nicotine and its bitartrate are practically superposed, indicating no influence of the tartrate ion on the partitioning of the base. The values of the apparent partition coefficients in the range of pH 6–7, where the drugs are mainly present in undissociated form, show that the lipophilicity of verapamil is higher than that of nicotine.

The measured partition coefficients were found to be independent of the ionic strength of the aqueous solution used, in the range of 0.15–0.45 M. The apparent partition coefficient describes the partitioning characteristics of a molecule without separating the effects of drug association or dissociation. Determination of the true partition coefficient, which considers only the parti-

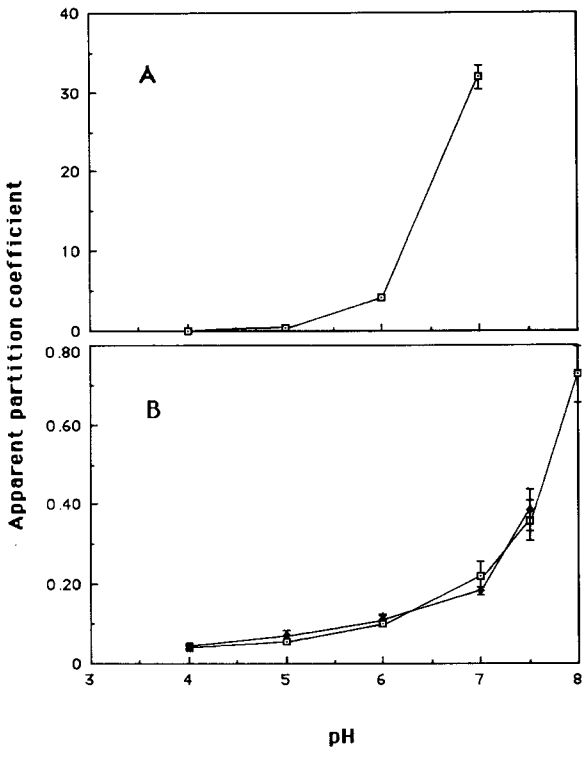

Fig. 1. Apparent partition coefficient (±SE) as a function of pH value: (A) verapamil·HCl; (B) nicotine (□) and nicotine bitartrate (◆).

tioning of the unionized base between the two phases, also makes it possible to obtain information on ion pair formation.

According to the model of Irwin and Li Wan Po (1979), modified by Oakley and Swarbrick (1987), the true partition coefficients of nicotine and verapamil were calculated from the measured apparent coefficients. The equation used in the pH range of 4.0–7.5 is:

$$K\left(1+\frac{K_a}{[H_3O^+]}\right)=K_{ip}+K_u\frac{K_a}{[H_3O^+]} \qquad (1)$$

where K is the apparent partition coefficient, K_{ip} denotes the partition coefficient of the ion pair, K_u represents the true partition coefficient, and K_a is the dissociation constant.

For nicotine the $K_{a,2}$ value was used as K_a. By plotting $K(1+K_a/[H_3O^+])$ vs $K_a/[H_3O^+]$, a straight line is obtained whose slope corresponds to the true partition coefficient and the intercept to the ion pair partition coefficient.

TABLE 1

True partition coefficient, K_u and ion pair partition coefficient, K_{ip}, values (mean value ± SE; in parentheses the significance level of the intercept (K_{ip}))

Drug	True partition coefficient	Ion pair partition coefficient
Verapamil·HCl	2585 ±54	0.361±0.344 (N.S.)
Nicotine bitartrate	1.96± 0.15	0.074±0.014 (95%)
Nicotine	1.77± 0.06	0.076±0.016 (95%)

The true partition coefficient (K_u) and ion pair partition coefficient (K_{ip}) values, obtained with nicotine, nicotine bitartrate and verapamil·HCl, are listed in Table 1. The true partition coefficient found for nicotine is evidently lower than the value of 31.1 reported by Oakley and Swarbrick (1987). This disparity may be attributed to the different buffers employed in the partitioning experiments. Phosphate buffer, instead of citric acid and disodium phosphate buffer, was employed by those authors.

Comparing the values obtained, the true partition coefficient of nicotine is approximately three orders of magnitude smaller than that of verapamil, indicating a higher hydrophilicity of nicotine. The ion pair partition coefficients are very low in comparison with the partition coefficient of the unionized form, and are not statistically different from zero for verapamil.

In order to confirm this result, the adherence to the pH-partition theory of IPM/water distribution of the drugs studied was verified. Again according to Oakley and Swarbrick (1987), Eqn 2 was employed:

$$\log\frac{K}{K_u}=\log\left(\frac{K_a}{K_a+[H_3O^+]}\right) \qquad (2)$$

By using Eqn. 2, it is possible to draw the curve describing the theoretical profile of variation of log (K/K_u) as a function of pH. This profile expresses the partitioning of molecules adhering to the pH-partition hypothesis. In Fig. 2 the variations of log (K/K_u) found with nicotine, nicotine

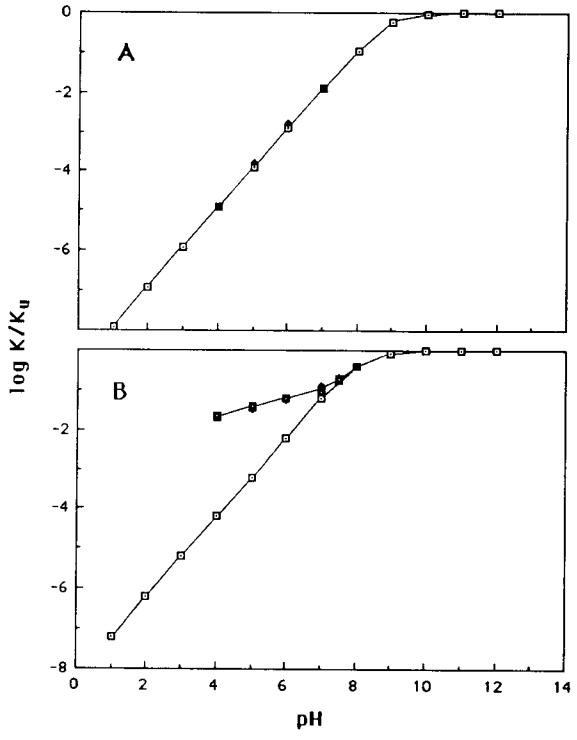

Fig. 2. Variation of log K/K_u as a function of pH: (⊡) theoretical, (■) and (◆) observed values. (A) Verapamil·HCl, (B) nicotine and nicotine bitartrate (the respective values are practically superposed).

bitartrate and verapamil · HCl, compared with the theoretical profiles, are presented vs pH. In the range of pH examined, verapamil strictly follows the pH-partition theory, whereas nicotine deviates in both its base and bitartrate form. The experimental profile of nicotine differs from the theoretical one up to pH 7, indicating ion pair formation at pH values lower than 7 (Fig. 2B). Above this pH value, the theoretical and experimental curves superpose. An identical pattern was found by Oakley and Swarbrick (1987) employing excised stratum corneum as a lipophilic phase. In contrast, they found that nicotine adhered to the pH-partition hypothesis when IPM was employed as a lipophilic phase. Again, this disparity must be attributed to the buffer employed in this study, which contains citric acid. It effectively means that citric acid forms ion pairs with nicotine which distribute in IPM.

The incidence of ion pair formation on the partitioning of the nicotine can be evaluated by means of Eqn 3 (Oakley and Swarbrick, 1987), where the left hand expression is the ratio of the concentration of the ion pair to the free base in the organic phase:

$$\log\left(\frac{[\mathrm{Nic}^+]}{[\mathrm{Nic}]}\right)_{org} = (\log K_{ip} - \log K_u) - (\mathrm{pH} - \mathrm{p}K_a) \quad (3)$$

where $[Nic^+]$ and [Nic] represent the concentrations of ionized and unionized nicotine in the organic phase, respectively. The contribution of ion pair formation to the nicotine concentration in the organic phase is 99% at pH 5, 87% at pH 6 and 41% at pH 7.

Permeation parameters

In order to verify the influence of partitioning on the 'in vitro' absorption of the drugs studied, permeability experiments through IPM-impregnated polypropylene and cellophane membranes were carried out. Fig. 3 shows the amount of nicotine transported at the examined pH values of drug solution. According to the model describing permeation from the donor at nonconstant source to the receptor compartment containing fluid of the same composition, the permeation curves obtained at different pH values were analyzed. The

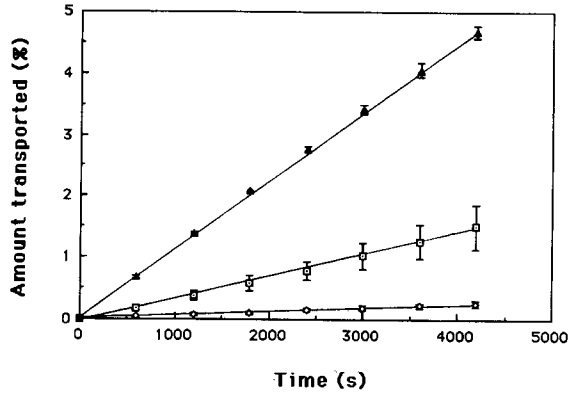

Fig. 3. Amount of nicotine transported (±SE) as a function of time, through polypropylene membranes impregnated with IPM. (◇) pH 5; (⊡) pH 6; (▲) pH 7.

equation of the model, which assumes pseudo-steady state conditions, is (Baker and Londsale, 1974):

$$M_t = \frac{M_\infty}{V_1 + V_2}\left(V_2 \exp\left[-\frac{DAK(V_1 + V_2)}{hV_1V_2}t\right] + V_1\right) \quad (4)$$

where M_t is the mass of drug in the donor compartment at time t, M_∞ is the total mass of drug, D is the diffusion coefficient, A is the area of the surface available for the permeation, h is the thickness of the membrane, V_1 is the volume of the donor solution, V_2 is the volume of the receptor solution, t is the time, and K is the apparent partition coefficient.

By plotting $\ln[(M_t/M_\infty) - (V_1/\{V_1 + V_2\})]$ against time, in all cases a straight line was obtained (Fig. 4). The slope of this line allows the calculation of the permeability coefficient (P), where $P = DK/h$. From the permeability coefficient through the membrane (P) and drug solubility (C_s) at the pH value examined, the limiting or maximal achievable flux from saturated solution, J_{lim}, can be determined by means of the relationship $J_{lim} = PC_s$. The drug diffusion coefficient (D) was also calculated.

The results are presented in Tables 2 and 4; for the sake of comparison, J_{lim} was reported as flux normalized to the membrane thickness.

Examining the permeation through impregnated polypropylene membranes as a function of pH (Table 2), verapamil shows permeability coefficient values increasing with pH. However, its value levels off in the pH range 6–7. Because the partition coefficient evidently increases, this result is determined by a decrease of verapamil diffusion coefficient in the lipid membrane from pH 5 to 7. In particular from pH 6 to 7 the diffusion coefficient decreases significantly. This behavior of permeability coefficient as a function of pH, together with the decrease of verapamil solubility by increasing the pH value of donor solution, determines the highest verapamil flux at pH 6 and the lowest at pH 7. In the case of nicotine, permeability, diffusion coefficients and flux increase significantly with the pH of the donor solution.

The comparison between nicotine and verapamil transport shows that the flux of verapamil

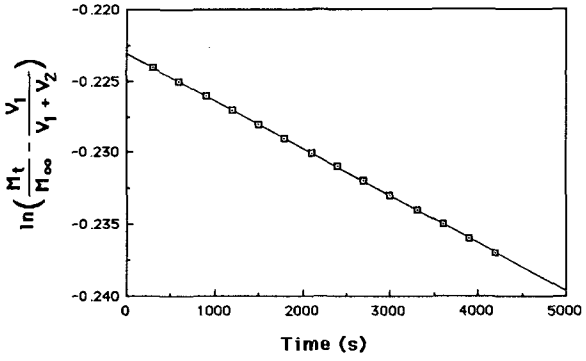

Fig. 4. Nicotine bitartrate permeation data from pH 6 solution through an IPM impregnated polypropylene membrane, represented according to Eqn 4.

TABLE 2

Permeation parameters through polypropylene membranes impregnated with IPM (mean value ± SE)

pH	Solubility (C_s) (g cm^{-3})	Partition coefficient (K)	Permeability coefficient (P) (cm s^{-1})	Diffusion coefficient (D) (cm^2 s^{-1})	Limiting flux (J_{lim}) (g cm^{-1} s^{-1})
Verapamil · HCl					
5	0.165	0.436	$0.26 \times 10^{-4} \pm 8.0 \times 10^{-7}$	1.49×10^{-7}	1.07×10^{-8}
6	0.025	4.29	$1.92 \times 10^{-4} \pm 1.0 \times 10^{-5}$	1.12×10^{-7}	1.20×10^{-8}
7	0.010	31.9	$1.99 \times 10^{-4} \pm 6.0 \times 10^{-6}$	0.16×10^{-7}	0.50×10^{-8}
Nicotine bitartrate					
5	0.240	0.074	$0.30 \times 10^{-5} \pm 3.6 \times 10^{-7}$	1.02×10^{-7}	0.18×10^{-8}
6	0.294	0.119	$1.74 \times 10^{-5} \pm 3.0 \times 10^{-6}$	3.66×10^{-7}	1.28×10^{-8}
7	0.288	0.183	$5.24 \times 10^{-5} \pm 4.0 \times 10^{-6}$	7.16×10^{-7}	3.77×10^{-8}

from a pH 5 donor solution is higher than that of nicotine. The opposite results are obtained at pH 7.

At all the pH values for nicotine and at pH 5 for verapamil, the solubility of drug compensates the low permeability coefficient contribution on overall transport. In the case of nicotine, whose solubility is practically unaffected by pH value, the substantial increase of flux is due to the increase of partition and diffusion coefficients with the pH.

It is interesting to note that the diffusion coefficient, in relation to the pH value of the donor solution, behaves in the opposite way for verapamil and nicotine: in the case of verapamil the remarkable increase in partition coefficient value, by increasing the pH of drug solution, reduces the molecule diffusibility because of the drug accumulation in the lipid membrane. The drug accumulated in the membrane exhibits a concentration dependent diffusivity. In the case of nicotine, the contrasting diffusion coefficient behavior with respect to the pH, is due to lower increase of partition coefficient values and the proven formation of ion pair in the lipid phase under pH 7 (cf. Fig. 2).

In order to show the dependence of drug transport on ionization, the contribution to the total flux of the ionized and unionized species, according to Swarbrick et al. (1984), was calculated (Table 3). The permeability coefficients through the model membrane of the unionized form is two orders of magnitude higher than the ionized one for both the drugs; however, the limiting flux values of the ionized form, in the pH range studied, are generally higher or equivalent to those of the unionized form. The presence in the donor solution of a high concentration of the ionized form (more than 95 and 90% at pH 7 for verapamil and nicotine, respectively) compensates for its reduced permeability contribution to the flux.

The differences existing between the total flux reported in Table 2 and the sum of the contributions of the two species (Table 3), show the varia-

TABLE 3

Limiting fluxes of ionized and unionized species

pH	Limiting flux of unionized form ($g\ cm^{-1}\ s^{-1}$)	Limiting flux of ionized form ($g\ cm^{-1}\ s^{-1}$)
Verapamil · HCl		
5	4.30×10^{-10}	4.09×10^{-8}
6	6.47×10^{-10}	6.20×10^{-9}
7	2.69×10^{-9}	2.45×10^{-9}
Nicotine bitartrate		
5	1.80×10^{-10}	4.48×10^{-9}
6	3.03×10^{-9}	5.45×10^{-9}
7	6.59×10^{-9}	5.30×10^{-9}

Permeability coefficients: unionized form: 8.78×10^{-3} cm s^{-1} for verapamil, 7.33×10^{-4} cm s^{-1} for nicotine; ionized form: 9.93×10^{-5} cm s^{-1} for verapamil, 7.47×10^{-6} cm s^{-1} for nicotine.

TABLE 4

Permeation parameters through cellophane membranes (mean value ± SE)

pH	Solubility (C_s) ($g\ cm^{-3}$)	Permeability coefficient (P) ($cm\ s^{-1}$)	Diffusion coefficient (D) ($cm^2\ s^{-1}$)	Limiting flux (J_{lim}) ($g\ cm^{-1}\ s^{-1}$)
Verapamil · HCl				
5	0.165	$1.63\times10^{-5}\pm7.6\times10^{-7}$	1.06×10^{-7}	1.75×10^{-8}
6	0.025	$1.39\times10^{-5}\pm2.1\times10^{-7}$	0.90×10^{-7}	0.23×10^{-8}
7	0.010	$2.60\times10^{-5}\pm4.1\times10^{-7}$	1.69×10^{-7}	0.17×10^{-8}
Nicotine bitartrate				
5	0.240	$4.88\times10^{-5}\pm7.6\times10^{-6}$	3.17×10^{-7}	7.61×10^{-8}
6	0.294	$4.19\times10^{-5}\pm7.7\times10^{-7}$	2.72×10^{-7}	8.00×10^{-8}
7	0.288	$4.19\times10^{-5}\pm8.0\times10^{-7}$	2.72×10^{-7}	7.84×10^{-8}

bility in the application of the model applied (Swarbrick et al., 1984).

With cellophane membranes (Table 4) the flux of verapamil decreases on increasing the pH value, whereas permeability and diffusion coefficients do not vary substantially. In this case, the inert membrane acts as a porous barrier: the solubility, and thus the concentration gradient through the membrane, is the only factor affecting the flux.

The higher diffusion coefficient of nicotine in comparison with verapamil found with this membrane must be attributed in this case to the difference in the molecular weight of the two drugs.

In conclusion, the flux of a drug through lipid membranes is not totally dependent on the partitioning of the molecule. In our experiments this is particularly evident with verapamil, where the decrease in solubility and in diffusion coefficients, as the pH values increase, reduces the contribution of partition coefficient for the flux.

On the other hand, when the solubility is unaffected by the pH value, as in the case of nicotine, the increase in the partition coefficient value with pH gives rise to an evident increase in the maximum flux attainable. In this case, the concentration of donor solution equalizes the importance of partitioning for drug transport through the membrane. Therefore, lipophilic drugs with high permeability coefficient are not the only candidates for transdermal delivery; hydrophilic drugs can also reach high permeation flux.

These results confirm the conclusions obtained by Okumura et al. (1989), where the authors affirmed that 'some water-soluble drugs with low molecular weight and high solubility in water might possess high skin permeation even though the partition of the drug to skin is low'.

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