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Research paper

Effect of the amphoteric properties of salbutamol on its release rate through a polypropylene control membrane

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Abstract

The permeation of salbutamol from aqueous vehicles with different pH values through the Celgard® 2500 polypropylene membrane was studied, the goal being to assess the effect of the amphoteric properties of the drug on its release by the membrane. Permeation rates were generally low, which was related to the fact that purely aqueous vehicles were not imbibed into the pores of the membrane and therefore permeation took place through the amorphous polypropylene domains. Permeability coefficients were not proportional to the fraction of uncharged drug at different bulk pH values, indicating that either a pH gradient between the bulk and the membrane surface exists and/or charged drug species can permeate the hydrophobic membrane. Calculated hypothetical pH values of the membrane surface, assuming permeation of the uncharged drug only, failed to provide a consistent explanation of the experimental permeabilities. Permeability coefficients of the different ionization forms of the drug assuming no pH gradient were calculated from a system of linear equations, each one of them corresponding to a specific bulk pH. These were for the anionic and the cationic species one to two orders of magnitude smaller than for the combined uncharged and zwitterionic species. It is possible that both, a pH difference between bulk and membrane surface and permeation of ionized molecules were simultaneously responsible for the observed permeation rates.

Keywords: Salbutamol; Amphoteric property; Membrane-controlled release; Polypropylene membrane

1. Introduction

Salbutamol (synonym: Albuterol of the United States Pharmacopeia) is a sympathomimetic agent which preferably stimulates β_2-adrenergic receptors and is used for its bronchodilating effect in reversible, obstructive airway disease such as bronchial asthma [1]. The frequency of asthma attacks exhibits a circadian rhythm with a considerable increase in the early morning hours and a maximum around 4 a.m. [2]. Following inhalation of salbutamol, the bronchodilation effect exhibits a peak within 1 to 2 h and may persist for up to 4 to 5 h. Following oral administration, the bronchodilation effect peaks within 2 to 3 h and may persist for 4 to 6 h [1]. Therefore, in order to adequately prevent nocturnal asthma with an evening administration of salbutamol, controlled sustained drug delivery into the high-risk hours is required. The transdermal route has been investigated as a means to achieve prolonged delivery of salbutamol, and it was shown that the delivered drug amounts were high enough to exert pharmacologic activity [3,4].

In a previous work [5], the Celgard® 2500 microporous polypropylene membrane was tested as a control membrane for reservoir-type drug delivery systems, such as transdermal systems. The in situ interaction of the membrane with lipid vehicles contained in the reservoir was studied as a possible way to modulate the drug release rate of the system. It was demonstrated that the vehicles were imbibed by the membrane and that membrane permeation took place practically exclusively via the vehicle-filled pores of the membrane. This provided the possibility to control the release

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rate of salicylic acid, used as a model drug, over a wide range of values by varying the viscosity of the vehicles.

In the present work, the effect of formulation parameters related to the drug vehicle on salbutamol release through the Celgard 2500 membrane is investigated; the goal being to identify methods for regulating the release rate. Purely aqueous vehicles with varying pH values are examined first. Since salbutamol has amphoteric properties [6], it can occur as a cation, an anion, a zwitterion or an uncharged molecule in proportions that depend on the pH of the solution. The pH values used in this study were appropriately chosen so as to enable assessing the role of the different ionization states of the drug in membrane permeation; the question of the permeation pathway through the membrane is also addressed. The effect of non-aqueous, water-miscible vehicles is reported in an upcoming paper [13].

2. Materials and methods

2.1. Drug and drug solutions

Salbutamol ((*RS*)-1-(4-hydroxy-3-hydroxymethylphenyl)-2-(*tert*-butylamino)ethanol) as a base and as a sulfate salt was a gift from Glaxo Group Research (Greenford, UK). The declared purity was 98.5% and 99.2% for the base and the salt, respectively, and both forms were used as received. Drug solutions were prepared with the Teorell and Stenhagen universal buffer system [7] in distilled water. This buffer system consists of 6.67 mM citrate ions, 6.67 mM phosphate ions and 11 mM borate ions, and was used to stabilize the pH of the solutions in a wide pH range. The buffer was prepared by dissolving the corresponding acids in water and adjusting the desired pH value with NaOH or HCl. (The pH was adjusted after drug was also added.) Its buffer capacity, β, was defined as the ratio of the amount of strong acid or strong base required to cause a shift of 0.3 pH units over this pH shift; the acid or base amount being expressed in equivalents per liter of buffer solution. The following buffer capacities were found: at pH 3, β (for HCl) = 0.01, β (for NaOH) = 0.009; at pH 9.5, β (for HCl) = 0.013, β (for NaOH) = 0.012; at pH 12, β (for HCl) = 0.021, β (for NaOH) = 0.037. Drug solutions at pH 7.4, in particular, were prepared with a 36.5 mM sodium phosphate buffer, which had a buffer capacity for HCl of 0.018 and for NaOH of 0.011.

Drug solubility (i.e. concentration at saturation) at different pH values was determined by adding an excess amount (roughly 2 g) of drug in 10 ml of buffer solution and equilibrating at 30°C. This temperature matched the one of the permeation experiments. The suspensions were periodically vortexed and ultrasonicated for 5 min at a time and their pH was checked and corrected, if necessary. Samples were taken at fixed time intervals and filtered through 0.45-μm filters, and the drug concentration was determined in the filtrate by HPLC after dilution. Sampling was repeated until no change in concentration was observed, typically for 12 to 24 h.

2.2. Membrane and drug solution imbibition by the membrane

The Celgard 2500 membrane (Hoechst Celanese, Charlotte, NC, USA) was used. This is a microporous flat sheet membrane consisting of polypropylene with slit-like pores of 50 × 500 nm (width × length), a thickness of 25 μm, a crystallinity of approximately 50% and a glass transition temperature of its amorphous domains of −18°C. A detailed characterization of this membrane was given previously [5,8]. The porosity of the membrane and the possible imbibition of drug solution by the membrane were determined by weight and buoyancy measurements. Based on these measurements, the total membrane volume was subdivided into three fractions: (i) the volume fraction corresponding to the polymeric material, (ii) the volume fraction of air-filled pores, and (iii) the volume fraction occupied by the solution which is imbibed into the pores of the membrane. The total porosity of the membrane is made up of the last two fractions. The methodology for these measurements has been described in detail elsewhere [5].

2.3. Permeation

Drug permeation across the Celgard membrane was determined with two-chamber glass diffusion cells which were symmetrical about the membrane. The membrane was mounted in a vertical position between the two chambers, one of which contained the donor and the other the receiver solution. The chambers had a cylindrical form, each with a length of 41.4 mm, and were furnished with a sampling and a stirrer port. The effective area of diffusion was 2.11 cm^2. Each chamber contained 9 ml of fluid which was stirred at 600 rpm with a rectangular paddle that had a height of 13 mm, a width of 7.8 mm and a thickness of 4.4 mm. Three cells were operated simultaneously with the same motor using a bead-chain to connect the stirrers, and were kept in a water-bath at 30 ± 1°C.

In the donor chamber, drug solutions with different pH values and different drug concentrations were used. The receiver solution consisted always of 36.5 mM phosphate buffer, pH 7.4, which contained variable amounts of NaCl in order to match the osmolarity of the donor solution. Osmolarities were measured based on freezing point depression with a Roebling digital micro-osmometer (Auer Bittman Soulié, Basel, Switzerland).

The duration of the permeation experiments was typically between 3000 and 5000 min. The criterion for setting this duration was that the steady-state flux should be measured in a time interval which starts at times at least twice longer than the time lag [9]. Sink conditions were maintained in the receiver solution throughout the experiment. For maintaining sink conditions, the difference of the solubility of the

drug in the donor and the receiver solutions arising from the different pH values was taking into account according to the method reported earlier [5]. No chemical degradation of the drug was detected for the duration of the permeation experiment by HPLC.

One-ml samples were drawn at predetermined time points from the receiver and replaced with fresh buffer. The donor solution was sampled in the beginning and in the end of the experiment. The pH was measured and salbutamol was assayed in the samples by HPLC. A reversed phase Spherisorb ODS2 column with the dimensions 125 × 4 mm and 5 μm particle size (Hewlett Packard, Urdorf, Switzerland) was used. The mobile phase was acetonitrile/0.02 M phosphate buffer, pH 3, in a ratio 10:90, and the flow rate was 1 ml/min. Chromatography was carried out in a Hewlett Packard 1050 system and UV detection at 229 nm was employed.

Permeability coefficients were determined from the slope of the permeating amount versus time curve according to Eq. (1).

$$M_R(t) = PAC_D(t - t_{lag}) \quad (1)$$

where $M_R(t)$ is the cumulative drug amount permeating in the receiver chamber as a function of time, P is permeability coefficient, A is diffusion surface area, C_D is drug concentration in the donor solution, t is time and t_{lag} is the time lag. Eq. (1) applies to time points $t > t_{lag}$. M_R was corrected for the drug amount withdrawn with sampling, and for C_D the average concentration between the beginning and the end of the experiment was used.

3. Results

After soaking the membrane in distilled water, its volume fraction that was occupied by air was 0.552, its volume fraction occupied by liquid was <0.001 and the volume fraction of the polymeric material was 0.448. These data show that water was not imbibed by the membrane. When using the Teorell and Stenhagen buffer at pH 3 instead of distilled water, the air fraction was 0.515, the liquid fraction was <0.001 and the fraction of the polymeric material was 0.485. The difference between distilled water and buffer is rather small, demonstrating that the presence of buffer salts did not influence water imbibition. The lack of water imbibition by the membrane is consistent with the hydrophobic nature of polypropylene that comprises the Celgard membrane. This hydrophobicity prevents water from wetting the membrane and consequently from entering into its pores. Since polypropylene is chemically inert to pH changes, no effect of the pH on water imbibition is to be expected. The porosity of the membrane found here agrees well with the manufacturer's declaration [10].

The solubility of salbutamol varied with pH as follows: pH 3, 263.5 mg/ml; pH 7.4, 261.6 mg/ml; pH 9.3, 26.96 mg/ml; pH 9.5, 26.89 mg/ml; pH 9.7, 29.6 mg/ml; pH 12, 183.4 mg/ml. Solubility values were generally in agreement with the supplier's declaration. The solubility reached a minimum in a pH region that contains the isoelectric point of the compound, which has amphoteric properties (see Section 4).

The results of salbutamol permeation through Celgard at different pH values of the donor solution are given in Table 1. Zero-order permeation was obtained in all cases and the lag-time fluctuated between 500 and 1500 min, the average being around 1000 min. No change in the donor concentration was detected in the duration of the experiment. The permeability coefficients were in general rather small, indicating that drug transport through the membrane was a slow and therefore, the rate-limiting process and consequently the effect of the diffusion boundary layer on drug permeation could be considered to be negligible. Permeability coefficients showed, however, a clear dependence on pH, with values ranging 17-fold. The standard error of the mean was quite small indicating good reproducibility. The pH of both the donor and the receiver solutions remained constant within ±0.1 pH unit during the experiments.

The donor concentration was varied at pH 3 between 173 and 57 mg/ml, at pH 9.5 between 26.8 (saturation) and 10 mg/ml, at pH 12 between 54 and 17 mg/ml, and at pH 7.4 it was approximately 90 mg/ml. No effect of the donor concentration on the permeability coefficient was observed at any pH in these concentration ranges (data not shown). This means that, within the experimental variation, the transport measurements do not notably deviate from ideality as it would be the case for an activity coefficient $\ll 1$. The different drug concentrations in the donor solution resulted in osmolarities of this solution ranging between 200 and 1120 mosmol/l. The osmolarity of the receiver solution was always adjusted at the beginning of the experiment to exactly the same value as that of the donor solution using NaCl, in order to prevent osmotic solvent flow. The transport of the drug from the donor to the receiver solution did not result in any significant osmotic effect, since the concentration in the receiver never exceeded 0.5% of the concentration in the donor because of the low permeation rate and because of the constant renewal of the receiver solution due to the drawing of samples.

To test whether the difference in chemical composition of the buffer between the donor and the receiver solution affected drug permeation, the phosphate buffer pH 7.4 typically used in the receiver solution was replaced in a selected

Table 1

Experimental permeability coefficients of salbutamol at different pH values

pH	P (mean) (cm/s)	SEM[a] (cm/s)	No. of observations
3	4.86×10^{-9}	2.2×10^{-10}	10
7.4	6.53×10^{-9}	2.64×10^{-10}	3
9.5	8.24×10^{-8}	3.35×10^{-9}	14
12	1.7×10^{-8}	9.17×10^{-10}	8

[a]Standard error of the mean.

experiment by the Teorell and Stenhagen buffer pH 7.4. In this experiment, a donor pH of 3 was used because at this pH, permeation was inherently the smallest, thus increasing the chance of detecting an effect possibly arising from the permeation of buffer salts through the membrane. No effect of the exchange of buffer on the permeability coefficient was found. Also, using salbutamol base or its sulfate salt at the different pH values was inconsequential for permeation. Therefore, data from different donor concentrations and different salts of the buffer and the drug were pooled in Table 1.

4. Discussion

Because of the lack of water imbibition by the membrane, permeation of the drug could take place solely by way of the amorphous domains of the membrane. At the temperature of the experiment, these domains are in the rubbery state since their glass transition temperature is −18°C. Crystalline membrane domains are generally considered to be practically impermeable to drug. The overall low permeability coefficients may be attributed to the high viscosity of the amorphous domains and a low partitioning of the drug into the hydrophobic membrane.

To analyze the pH dependence of the membrane permeation of salbutamol, the ionization behavior of the drug is taken into consideration. Because of the pronouncedly hydrophobic properties of polypropylene, one may expect that high levels of hydration of the molecules would adversely affect partitioning into and thus permeation through the membrane. Ionization, on the other hand, is not assumed to affect diffusivity within the polymer since the potential ionic forms exhibit no noteworthy differences in molecular size.

Salbutamol is an amphoteric compound with two ionizable groups, an acidic phenolic group and a basic secondary amine group (Fig. 1). It can be concluded based on the individual (microscopic) ionization constants that the phenolic group is more acidic than the protonated amino group, which means that the phenolic group is deprotonated at a lower pH than the amino group [6]. The deprotonation of the phenolic group leads to the zwitterionic form of the compound, while deprotonation of the amino group leads to the

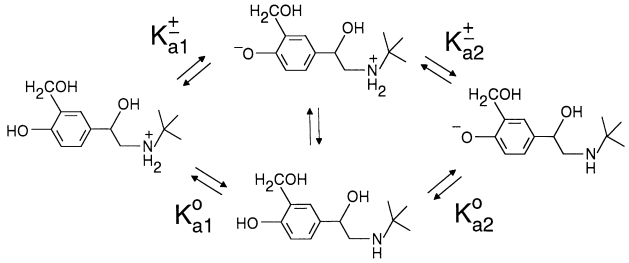

Fig. 1. Ionization scheme of Salbutamol. K_a are ionization constants. $K^{\pm}_{a1} = 6.03 \times 10^{-10}$, $K^{o}_{a1} = 2.5 \times 10^{-10}$, $K^{\pm}_{a2} = 6.03 \times 10^{-11}$, $K^{o}_{a2} = 1.45 \times 10^{-10}$ [6].

Table 2

Concentration fraction of salbutamol ionic species at different pH values

pH	F^+	$F^{\pm}$	F^o	F^-
3	0.999	6.03×10^{-7}	2.51×10^{-7}	3.63×10^{-14}
7.4	0.979	0.0148	0.00615	2.24×10^{-5}
9.5	0.246	0.469	0.195	0.0894
12	2.69×10^{-5}	0.0162	0.00676	0.977

uncharged molecule. The ratio between zwitterionic and uncharged form is given by the ratio $K^{\pm}_{a1}/K^{o}_{a1}$, which is approximately equal to 2.4. The global (macroscopic) ionization constants corresponding to the two deprotonation steps are $pK_{a1} = 9.07$ and $pK_{a2} = 10.37$ [6].

Using the ionization constants from Fig. 1, the fraction of drug occurring in the cationic, the zwitterionic, the uncharged and the anionic form at the pH values of the permeation experiment can be calculated with the following equations.

$$F^+ = \frac{1}{1 + 10^{(\mathrm{pH} - \mathrm{p}K^{\pm}_{a1})} + 10^{(\mathrm{pH} - pK^{o}_{a1})} + 10^{(2\mathrm{pH} - \mathrm{p}K^{\pm}_{a2} - \mathrm{p}K^{\pm}_{a1})}} \quad (2)$$

$$F^{\pm} = \frac{1}{10^{(\mathrm{p}K^{\pm}_{a1} - \mathrm{pH})} + 1 + 10^{(\mathrm{p}K^{\pm}_{a1} - \mathrm{p}K^{o}_{a1})} + 10^{(\mathrm{pH} - \mathrm{p}K^{\pm}_{a2})}} \quad (3)$$

$$F^o = F^+ 10^{(\mathrm{pH} - \mathrm{p}K^{o}_{a1})} \quad (4)$$

$$F^- = F^{\pm} 10^{(\mathrm{pH} - \mathrm{p}K^{\pm}_{a2})} \quad (5)$$

where F^+, $F^{\pm}$, F^o and F^- are concentration fractions of the cationic, the zwitterionic, the uncharged and the anionic species, respectively, and the pK_a constants were defined in Fig. 1. From these equations, the fractions of the different species given in Table 2 were obtained.

In view of the hydrophobicity of the Celgard membrane, it appears at first reasonable to assume that only the uncharged salbutamol molecules can account for membrane permeation. For this hypothesis to hold, the experimental permeability coefficients should be proportional to the concentration fraction of the uncharged species. This is tested in Fig. 2 where P is plotted against F^o at the different pHs, the F^o values taken from Table 2. Fig. 2 demonstrates that the data points do not obey this proportionality, since the slope of the regression line through the points deviates markedly from unity on the double-logarithmic scale. This implies that membrane permeation of salbutamol could not be explained by the sole permeation of the uncharged molecules of the drug.

The lack of proportionality seen here might, in theory, be elicited by a pH difference between the bulk of the donor solution and the surface of the membrane on the donor side. Such a difference may arise from the fact that the donor and the receiver solutions were maintained at different pH values; that is, while the donor pH varied between 3 and 12 the receiver pH was always kept at 7.4. Thus, except for a donor pH of 7.4, a pH gradient may conceivably prevail

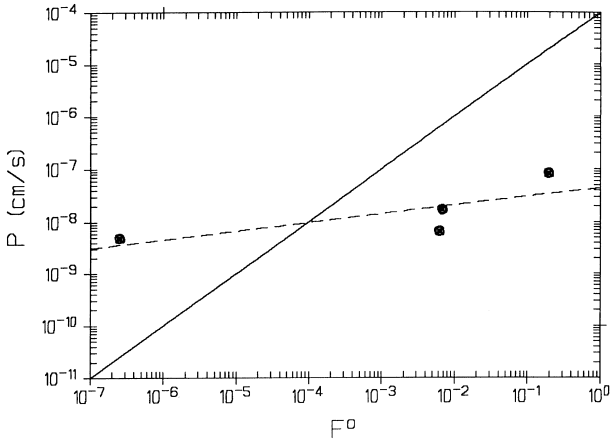

Fig. 2. Experimental permeability coefficients versus fraction of unionized drug species at different pH values. Points represent experimental data. The dashed line was drawn by linear regression through the data points after the data were subjected to double-logarithmic transformation. The equation of the dashed line is: $\log(P) = -7.3563 + 0.16457 \cdot \log(F^o)$. The solid line is a simulation with a slope equal to one on the double-logarithmic scale and thus illustrates the relationship of P being proportional to F^o. The axis intercept of the solid line depends on the proportionality constant and was chosen here arbitrarily.

within the membrane, provided that a finite permeation of hydrogen or buffer ions takes place through it. The pH gradient may also be extended between the surface of the membrane and the bulk of the solution due to the presence of a diffusion boundary layer adjacent to the membrane. The change of hydrogen ion concentration with distance within the membrane, however, is expected to be precipitous and the corresponding concentration gradient to be considerably steeper than the one in the boundary layer, since the permeation rate of ions for polypropylene must be much smaller than that for the aqueous boundary layer. A pH difference between the membrane surface and the bulk of the solution will be present on both the donor and the receiver side of the membrane. However, since sink conditions were strictly maintained in the receiver solution, the drug concentration and, as a consequence, its ionization dependence on pH in this solution have a negligible effect on the rate of permeation. Therefore, only the pH gradient in the diffusion boundary layer on the donor side is considered.

The notion that the permeation of hydrogen and buffer ions through the membrane is probably low is supported by the fact that the pH of the donor and the receiver solutions remained constant throughout the duration of the permeation experiments, that is, for up to 80 h. Yet this alone does not entirely preclude the possibility of ion permeation taking place through the membrane, since its effect on pH would be neutralized by the buffer solutions, which had a moderate buffer capacity.

Whether the lack of proportionality depicted in Fig. 2 can be explained on the grounds of a pH difference between the membrane surface and the bulk of the solution is assessed in the following. For this purpose, the pH values are calculated, which should be prevailing at the membrane surface facing the donor solution in order to obtain the measured permeability coefficients under the assumption that only the fraction of uncharged molecules can cross the membrane. At donor pH 7.4, no pH gradient between donor and receiver exists and, therefore, the membrane surface pH is equal to 7.4. At this donor pH, the permeability coefficient of the uncharged species, P^o, can be directly calculated under the above assumption using Eq. (6).

$$P = F^o P^o \tag{6}$$

where P is measured permeability coefficient and F^o is concentration fraction of uncharged species. With $P = 6.53 \times 10^{-9}$ cm/s (from Table 1) and $F^o = 0.0062$ (from Table 2), P^o comes out to be 1.05×10^{-6} cm/s. Using this value of P^o, the hypothetical fractions of the uncharged species at the membrane surface which provide the experimental permeability coefficients at donor pH 3, 9.5 and 12 are calculated with Eq. (6) (Table 3). The corresponding pH values were calculated using Eq. (4). Because solving Eq. (4) explicitly for pH is tedious, its roots were determined by numerical evaluation and are given in Table 3. Eq. (4) has two roots when solved for pH; these denote the hypothetical surface pH values.

The root 12.2 for donor pH 3 and the root 10.8 for donor pH 9.5 are impossible from a practical point of view because they lie outside the interval defined by the corresponding donor and the receiver pHs. To evaluate the validity of the other roots, the corresponding hydrogen ion concentrations were calculated and the difference of hydrogen ion concentration between membrane surface and donor bulk solution was compared to the difference of hydrogen ion concentration between donor and receiver bulk solutions. The concentration gradient of hydrogen ions in the membrane and in the diffusion boundary layer were assumed herein to be linear. For root 7.28, donor pH 3, and root 7.83, donor pH 12, the change of hydrogen ion concentration within the boundary layer (99.9% and 38% of the total change between donor and receiver, respectively) was too large to be con-

Table 3

Hypothetical parameters for the donor side surface of the membrane assuming sole permeation of uncharged molecules

pH[a]	F^o [b]	pH		%[H^+] difference[d]	
		Root 1[c]	Root 2[c]	Root 1[e]	Root 2[e]
3	0.00462	7.28	12.2	99.9	n.a.
9.5	0.0785	8.63	10.8	5	n.a.
12	0.0162	7.83	11.6	38	0.004

[a]Bulk pH of the donor solution.
[b]Deduced from Eq. (6) for the experimental permeability coefficients and $P^o = 1.05 \times 10^{-6}$ cm/s.
[c]pH values obtained from Eq. (4) for F^o given in this table.
[d]Difference in the molar concentration of hydrogen ions between bulk and membrane surface on the donor side expressed in percent of the molar concentration difference of hydrogen ions between the donor and the receiver solutions; concentrations calculated with $[H^+] = 10^{-pH}$.
[e]Numbers correspond to the two roots for pH.

sidered practically possible in view of the fact that the concentration gradient within the membrane should be steeper than within the boundary layer. In this respect, only roots 8.63 for donor pH 9.5 and 11.6 for donor pH 12 appear, in qualitative terms, to be attainable estimates of membrane surface pH. Yet the corresponding % differences of hydrogen ion concentration between bulk and membrane surface (5 and 0.004%, respectively) differ considerably. Thus, this analysis demonstrates that a pH difference between the bulk and the membrane surface in the donor solution can not consistently explain the experimental data, when it is assumed that only uncharged molecules can permeate the membrane.

Therefore, adopting the thesis that ionized drug species can also permeate the polypropylene membrane is compelling for a quantitative interpretation of the data. Permeability coefficients of all ionic species may be calculated with Eq. (7).

$$P = F^{+}P^{+} + F^{\pm}P^{\pm} + F^{o}P^{o} + F^{-}P^{-} \quad (7)$$

where P^{+}, $P^{\pm}$, P^{o} and P^{-} are permeability coefficients of the cationic, zwitterionic, uncharged and anionic species, respectively, and all other symbols were defined above.

When Eq. (7) is applied to the four bulk donor pH values used in this study, a non-homogeneous system of four linear equations results. This system has four unknowns, the permeability coefficients of the ionic species; the coefficients of the equations, F, are given in Table 2. To ascertain whether a solution of this system exists, the matrix notation for the system is employed. The matrix of the coefficients F is given below.

$$\begin{pmatrix} 0.999 & 6.03\times10^{-7} & 2.51\times10^{-7} & 3.63\times10^{-14} \\ 0.979 & 0.0148 & 0.00615 & 2.24\times10^{-5} \\ 0.246 & 0.469 & 0.195 & 0.0894 \\ 2.69\times10^{-5} & 0.0162 & 0.00676 & 0.977 \end{pmatrix} \quad (8)$$

The determinant of this matrix is found to be equal to zero, within rounding error. This is due to the ratio of the elements of the second and the third column, corresponding to coefficients $F^{\pm}$ and F^{o}, being in every row approximately equal to 2.4. This is a result of the ratio of ionization constants $K_{a1}^{\pm}/K_{a1}^{o}$, as noted above. From the fact that this determinant is equal to zero is concluded that no singular solution of this system of equations exists. In fact, a infinite number of solutions can be found in which the permeability coefficients $P^{\pm}$ and P^{o} are linearly correlated.

Since the obstacle in solving this system arises from $F^{\pm}$ and F^{o} being always present in the same ratio, a permeability coefficient $P^{\pm/o}$ corresponding to the sum of $F^{\pm}$ plus F^{o} is introduced in order to obtain a partial solution to the problem. The resulting system of four linear equations with three unknowns can be solved and the computed permeability coefficients are given in Table 4. The solution was carried out numerically (software: MINSQ, MicroMath, Salt Lake City, UT, USA) using all four equations simultaneously with a least squares minimization procedure. Since the experimental permeability coefficients were associated with an error, this procedure allowed finding the best estimates within the experimental variation and provided the standard error of the deduced permeability coefficients of the ionic species (Table 4). This standard error was maximally 8.5% and the multiple coefficient of determination, r^2, from the least squares minimization with the four equations was equal to 0.99993.

The calculated permeability coefficients of the ionic species explain adequately the experimental permeability at the different donor bulk pH values. The combined permeability coefficient of the zwitterionic and uncharged species was higher than those of the species with a net charge other than zero. This higher combined permeability coefficient is attributed to the uncharged drug species. Since this is expected to be the least solvated, i.e. least hydrated, of all ionic species, it will show the highest tendency to partition into the hydrophobic polypropylene membrane. Thus, this species should, from a thermodynamic point of view, have the highest permeability for the membrane. The zwitterionic species contains two charged groups, both of them being individually hydrated. Therefore, the zwitterionic species is expected to have a lower permeability for this membrane compared not only to the uncharged species but also to the single-charged cationic or anionic species. The fact that the net charge of the zwitterionic molecules is equal to zero, is inconsequential for this matter.

The single-charged drug species show finite permeability coefficients which are one to two orders of magnitude lower than that of the un-ionized species alone. A possible mechanism for the permeation of cationic and anionic drug molecules through the hydrophobic polypropylene membrane could be by formation of ion pairs with counter-ions from the buffer. Such ion pairs were shown to partition into organic phases, their partition coefficient being generally about 3 logarithmic units lower than that of the uncharged molecule [11]. The difference between these partition coefficients reportedly depends on the chemical structure characteristics of the molecules and can be smaller than 3 log-units for bulky, multiprotic molecules with extensive electronic delocalization. Furthermore, for amphoteric molecules, partition coefficients of oppositely charged species may differ [11]. Thus, the differences in the permeability coefficients between the different drug species deduced from the present experiments are, in semi-quantitative terms, in good agreement with what would be predicted based on the theoretical partitioning behavior of these spe-

Table 4
Permeability coefficients of ionized species of salbutamol

P^{+} (cm/s)	4.49×10^{-9}; SE[a] = 3.86×10^{-10}
$P^{\pm/o}$ (cm/s)	1.21×10^{-7}; SE = 8.31×10^{-10}
P^{-} (cm/s)	1.46×10^{-8}; SE = 5.52×10^{-10}

[a]Standard error of the calculated permeability coefficients.

cies. This, in turn, supports the notion of a partition-controlled permeation when it comes to the differences between the ionic species. Ion pair formation was invoked also by other authors for explaining drug permeation through hydrophobic polymer membranes from non-aqueous media [12].

5. Conclusion

On the condition of no pH gradient between the membrane surface and the bulk of the solution, the fraction of the uncharged drug molecules comes out to be chiefly responsible for membrane permeation; yet permeation of ionized species has to be taken into account, albeit at a comparatively smaller scale, in order to quantitatively explain the measured permeability coefficients. When sole permeation of uncharged species is assumed as an alternative, a pH difference between membrane surface and bulk solution does not afford a complete explanation of the data. It is conceivable, however, that these two situations represent extremes; reality may be more closely reflected by the intermediate situation, which is characterized by a shallow pH gradient and a larger difference of permeability coefficients between the uncharged and the single-charged drug species than the one given in this report.

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