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Research Article

Facilitated Transport of Basic and Acidic Drugs in Solutions Through Snakeskin by a New Enhancer—Dodecyl *N,N*-Dimethylamino Acetate

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The permeation characteristics of two model drugs, clonidine (basic) and indomethacin (acidic), were studied by determining their penetration fluxes through hydrated shed snakeskins (*Elaphe obsoleta*) at 32°C. The drugs were formulated in buffers of different pH's, ranging from 3 to 7. The total penetration fluxes at pH 7.0 for both compounds using skins pretreated with dodecyl *N,N*-dimethylamino acetate were at least 11 times higher than those of the control runs without enhancer treatment. Equations were derived to calculate the permeability coefficients (K_i) and fluxes (J_i) for the ionized and the nonionized species to allow for comparison of their penetration ability through the model membrane. The permeability coefficient of clonidine is 2.50×10^{-3} cm/hr for the nonionized form and 2.41×10^{-4} cm/hr for the protonated form. This result indicates that the nonionized form penetrates the skins better than the ionized form. Both permeability coefficient values are 11 times larger than the corresponding values obtained from the control skins. The total flux of clonidine is dependent on its initial concentration in the donor cell but is independent of the ionic strength of the solution formulations. The penetration characteristics of indomethacin are similar to those of clonidine, with a higher permeability coefficient of the nonionized form (3.90×10^{-3} cm/hr) than of the ionized form (7.97×10^{-4} cm/hr) using pretreated skins. While the enhancer shows 24 times penetration enhancement of the ionized form of indomethacin, it does not enhance the penetration of the nonionized species.

KEY WORDS: clonidine; indomethacin; transdermal penetration enhancer; permeability coefficient; pH effect.

INTRODUCTION

In the previous communication (1), we reported the development of a series of alkyl N,N-disubstituted amino acetates as excellent transdermal penetration enhancers for the transport of indomethacin through shed snakeskin (*Elaphe obsoleta*) from either a semisolid dosage form or a solution formulation. Of particular interest is the excellent penetration of the ionized form of indomethacin from a buffer solution of pH 7.2 using skins with or without pretreatment with dodecyl *N,N*-dimethylamino acetate. This result led us to investigate the effect of pH on penetration fluxes of acidic and basic drugs in the presence of dodecyl *N,N*-dimethylamino acetate.

A contribution of flux from an ionogenic species to the total flux may be due to its predominant concentration at a specific pH, although its permeability coefficient may be small compared with that of its counterpart. For a basic ionogenic drug in solutions, the equilibrium of ionization can be given as

$$BH^+ \overset{K_a}{\rightleftharpoons} B + H^+$$

$$K_a = \frac{a_B a_{H^+}}{a_{BH^+}} \quad (1)$$

where a_{BH^+} and a_B are the activity for the protonated form of the base and the nonionized form of the base, respectively, and K_a is the thermodynamic dissociation constant of the base. Taking the logarithm of Eq. (1) and rearranging gives

$$\log \frac{a_{BH^+}}{a_B} = pK_a - pH$$

For calculation simplicity, concentration instead of activity is used.

$$[BH^+] = \frac{10^{(pK_a - pH)}}{1 + 10^{(pK_a - pH)}} \times [T]$$

where $[T]$ is the total concentration of both species, B and BH^+, i.e.,

$$[T] = [B] + [BH^+]$$

$$[B] = [T] - [BH^+]$$

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The transdermal steady-state rate of penetration (2) is given as

$$\frac{dQ}{dt} = \frac{PD_s}{h} C_v A$$

where dQ/dt is the steady-state rate of penetration, P the effective partition coefficient of drug between skin barrier and vehicle, C_v the concentration of drug dissolved in the vehicle, D_s the average diffusion constant of the drug in the skin barrier, A the area of skin to which the drug is applied, and h the effective thickness of the skin barrier.

The permeability coefficient (3) of penetrant is defined as

$$K = \frac{PD_s}{h} \text{ (cm/hr)}$$

and Fick's first law of diffusion as

$$J = \frac{dQ}{dt} \cdot \frac{1}{A}$$

where J is the amount of material passing perpendicular to a reference plane of unit area in unit time and D is the diffusion coefficient.

Since

$$\frac{dQ}{dt} = K C_v A$$

therefore,

$$\text{flux} = J = \frac{dQ}{dt} \times \frac{1}{A} = K C_v$$

For an ionogenic drug at various pH values, there will be a simultaneous presence of both ionized and nonionized species in solution. The total transdermal flux of a ionogenic drug is the sum of the flux contribution from the two species based on two assumptions: (i) each species moves through the skin by diffusion down its own concentration gradient across the skin membrane, and (2) the fluxes of the species are linearly additive.

$$J_{total} = J_B + J_{BH^+}$$
$$J_B = K_B [B]$$
$$J_{BH^+} = K_{BH^+} [BH^+]$$
$$J_{total} = K_B [B] + K_{BH^+} [BH^+]$$

where J_{total} is the total flux, J_B is the flux of the nonionized species, J_{BH^+} is the flux of the ionized species, and K_B and K_{BH^+} are the permeability coefficients of the nonionized and ionized species, respectively. Several conditions could arise as follow:

(i) when pK_a = pH, [B] = [BH^+], J_B and J_{BH^+} may both contribute significantly to J_{total}, if $K_B \approx K_{BH^+}$;
(ii) when pH $\gg$ pK_a, [B] $\gg$ [BH^+], $J_{total} \approx K_B$ [B], if $K_B \approx K_{BH^+}$; and
(iii) when pH $\ll$ pK_a, [B] $\ll$ [BH^+], $J_{total} \approx K_{BH^+}$ [BH^+], if $K_B \approx K_{BH^+}$.

The outcomes from the above conditions stand only if K_B and K_{BH^+} are similar in magnitude. To calculate K_B and K_{BH^+}, two penetration profiles are required preferably at pH values which are several pH units away from the pK_a of the drug. An approximation approach, discussed under Results and Discussion, can be employed to obtain the corresponding permeability coefficient values. Another method, probably a better one, to calculate the K_B and K_{BH^+} values is to obtain two total flux equations from two penetration profiles at different pH values and solve for the permeability coefficients values simultaneously. This method of calculation does not assume both K_B and K_{BH^+} values to be similar in magnitude. Both methods of calculation are used in this study.

MATERIALS AND METHODS

Reagents and Buffers

Dodecyl *N,N*-dimethylamino acetate was synthesized as described previously (1). Indomethacin and clonidine hydrochloride were both purchased from Sigma and used without further treatment. Buffer solutions were prepared using phosphoric acid (0.002 *M*) and monosodium phosphate (0.00778 *M*) for pH 3.0, sodium acetate (0.025 *M*) for pH 4.6, and monopotassium phosphate (0.02 *M*) for pH 5.7 and 7.0. The ionic strength of the buffer was maintained at 0.15 *M* with sodium chloride. Deionized water obtained from a Nanopure apparatus was used in the preparation of solutions.

Shed Snakeskin

The hydration treatment of shed snakeskin has been reported previously (1,4). A whole snakeskin from a single snake was used for each set of penetration experiments and was cut into small pieces (approximately 3 × 3 cm). After hydrating the skins, they were treated with 8 mg of the new enhancer by applying the enhancer evenly over a circular area of the skin with a diameter of 1.5 cm. The treated skins were left separately for 5 hr in small plastic pans in which several drops of water were placed to keep the skins moisturized. The skins without enhancer treatment were used in the control experiments.

Solution Formulations

Clonidine. Clonidine hydrochloride (2%) was dissolved in 6 ml of buffers at pH 3.0 and 7.0. The pH of the solutions was measured with a Corning pH meter 145 equipped with a glass electrode.

Indomethacin. Appropriate amounts of indomethacin were suspended in 6 ml of buffers at pH 3.0 and 7.0. The solubility of indomethacin at various pH values can be estimated from a report by Chien *et al.* (5).

Analysis

Indomethacin was analyzed by a high-performance liquid chromatographic (HPLC) procedure reported in previous communications (1,4). The concentration of clonidine was determined by HPLC analysis using a Perkin–Elmer ISS-100 HPLC apparatus with a Perkin–Elmer LC90UV spectrophotometric detector, a Perkin–Elmer ISS-100 auto-

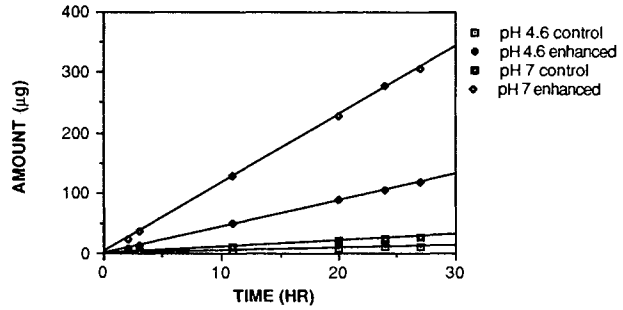

Fig. 1. Time-course penetration profiles of clonidine through shed snakeskin at 32°C for aqueous solution formulations of pH 4.6 and 7.0. The lines labeled enhanced are for skin pretreated with dodecyl *N,N*-dimethylamino acetate.

matic sampler, and a Perkin–Elmer LCI-100 integrator with the detection wavelength set at 240 nm. A reverse-phase column, RP-8 Spheri-5, 4.6 × 100 mm, and a guard column, OD-GU (both purchased from Brownlee Labs), were used in conjunction with the HPLC system. The solvent system used was a mixture of a phosphate buffer (pH 3.0) (25%) and acetonitrile (75%), and the flow rate was 2.0 ml/min. The retention time of clonidine was 2.85 min.

Penetration Study

The procedure for the penetration study has been reported previously (1). After mounting the skin on top of the receptor cell of a Franz-type diffusion-cell assembly, followed by an O-ring, the donor cell was placed on top of the receptor cell. Appropriate buffer solutions with the same pH as in the donor cell were used in the receptor cell to eliminate pH and buffer effects on the penetration flux. The cell assembly was clamped together and placed in a water bath of 32°C. A volume of 0.4 ml of the clonidine solution or indomethacin suspension was pipetted into the donor cell. Samples were withdrawn at regular time intervals and the experiment was done in quadruplicate.

Concentration Effect of Clonidine on Penetration Flux

Three solution formulations of clonidine were prepared by dissolving 0.5, 1.0, and 2.0% of clonidine hydrochloride in 6.0 ml of buffer at pH 4.6. The permeation experiments were carried out with skins pretreated with dodecyl *N,N*-dimethylamino acetate and with 0.4 ml of the appropriate clonidine solution in the donor cells. Buffer at pH 4.6 was used in the receptor cells.

Effect of Ionic Strength on Penetration Flux

Three solution formulations of clonidine were prepared by dissolving 2% of clonidine hydrochloride in 6.0 ml of buffer at pH 4.6 with ionic strengths of 0.05, 0.10, and 0.15 *M*, respectively. A volume of 0.4 ml of the appropriate solution formulations was transferred into the donor cells, and the skins used were pretreated with dodecyl *N,N*-dimethylamino acetate. Buffer at pH 4.6 was used in the receptor cells.

Solubility of Indomethacin

Indomethacin was suspended in 6 ml of buffers at pH 2.9, 5.7, and 7.0 in 10-ml screw-cap vials. The suspensions were stirred at 32°C for 3 days. The saturated solubility in each buffer solution was determined from standard curves obtained by plotting the peak heights of a series of standard indomethacin solutions obtained from an HPLC analysis for indomethacin described earlier (1) using a Kratos Spectroflow 783 programmable absorbance detector with a BioRad HPLC Model 1330 pump.

RESULTS AND DISCUSSION

Skin Permeation of Clonidine from Solutions of Different pH

The penetration profiles of clonidine in solution formulations of different pH values are shown in Fig. 1. Low penetration fluxes of clonidine were obtained for the control runs at pH 4.6 and 7.0, where the snakeskins were not pretreated with the new enhancer, dodecyl *N,N*-dimethylamino acetate. Since the concentration of clonidine hydrochloride in the donor cells was 4000 μg for all the experimental runs, the penetration fluxes obtained from formulations at different pH values were comparable. The total flux at pH 7.0 was 2.7 times higher than that at pH 4.6. This is due to the increase in the concentration of the nonionized form of clonidine since the total concentration of clonidine in all the donor cells for both pH values was the same (Table I). The lag times for the penetration profiles are short, indicating that clonidine penetrated snakeskins readily under the facilitated

Table I. Penetration Parameters of Clonidine Using Shed Snakeskins as the Model Skins at 32°C

				Enhanced			Control			Enhanced		Control					
pH	Conc. (μg)[a]	$[BH^+]$ (μg)	[B] (μg)	J_{total} (μg/cm²-hr)	J_B	J_{BH^+}	J_{total}	J_B	J_{BH^+}	K_B (cm/hr)	K_{BH^+}	K_B	K_{BH^+}	$\frac{K_B(enh)}{K_B(cont)}$	$\frac{K_{BH^+}(enh)}{K_{BH^+}(cont)}$	$\frac{J_{total}(enh)}{J_{total}(cont)}$ pH 4.6	pH 7.0
4.6	4000	3996	4	2.43	0.025	2.407	0.208	0.0023	0.206	0.0025	2.41×10^{-4}	2.30×10^{-4}	2.06×10^{-5}	10.9	11.7	11.7	11.1
7.0	4000	3322	678	6.24	4.24	2.00	0.563	0.390	0.171	0.0025[b]	2.41×10^{-4b}	2.30×10^{-4b}	2.06×10^{-5b}				

[a] Four-tenths milliliter of 2% of a clonidine solution was applied to the donor cells.
[b] Values calculated by the simultaneous equations approach.

conditions. When the skins were pretreated with the enhancer, the total fluxes of clonidine increased by 11.7 times for pH 4.6 and by 11.1 times for pH 7.0.

As shown in the introduction of this communication, the penetration flux of an ionogenic drug through the skin is the sum of penetration fluxes of the drug species present in the solution formulations, ionized and nonionized. The flux of each species is the product of its permeability coefficient and its concentration at the particular pH. The pK_a value of clonidine was calculated as 7.69 from a literature value (8.05) (6). At pH 4.6, 99.9% of the total clonidine is in protonated form. To a first approximation, the K_{BH^+} can be calculated from the relationship

$$J_{total}\ (\text{pH } 4.6) = K_{BH^+}\ [BH^+]$$

where $[BH^+]$ and J_{total} (pH 4.6) are known values (Table I). Then K_{BH^+} can be used to calculate K_B at pH 7.0.

$$J_{total}\ (\text{pH } 7.0) = K_B\ [B] + K_{BH^+}\ [BH^+]$$

where J_{total} (pH 7.0), [B], K_{BH^+}, and $[BH^+]$ are known values (Table I). After obtaining K_B, we can fit K_B back to the total flux at pH 4.6 to calculate K_{BH^+} by a second approximation,

$$J_{total}\ (\text{pH } 4.6) = K_B\ [B] + K_{BH^+}\ [BH^+]$$

where J_{total} (pH 4.6), K_B, [B], and $[BH^+]$ are known values. K_{BH^+} (second approximation) can be fitted in an iterative calculation for K_B. By doing this iterating calculation, it takes only two approximations to obtain constant K_B (2.50×10^{-3} cm/hr) and K_{BH^+} (2.41×10^{-4} cm/hr) values (Table I). Now we can compare the permeation ability of the nonionized and the protonated species. The permeability coefficient of the nonionized species is about 11 times higher than that of the protonated species for both pH 4.6 and pH 7.0. This means that the nonionized species permeated better than the protonated form under either the control or the enhanced conditions. The fluxes calculated for the nonionized species (J_B) and the protonated species (J_{BH^+}) are shown in Table I. At pH 4.6, the contribution to the total flux is predominated by the flux of the protonated species owing to its predominant concentration in the donor cells. However, at pH 7.0, the flux of the nonionized form surpasses the flux of the ionized form by a factor of 2, contributing more than 60% to the total flux although its concentration is only 17% of the total clonidine concentration.

Table II. Concentration Effect on Penetration Parameters of Clonidine at pH 4.6 and 32°C with Skins Pretreated with Dodecycl *N,N*-Dimethylamino Acetate

Conc. (μg)[a]	J_{total} (μg/cm²-hr)	K_{BH^+} (cm/hr) $\times 10^4$
1000 (0.5%)	2.85	11.4 ± 0.10
2000 (1%)	4.83	9.67 ± 0.69
4000 (2%)	10.0	10.0 ± 0.4
Average		10.4

[a] Four-tenths milliliter of an appropriate concentration of clonidine solutions was applied to the donor cells.

To rationalize the approximation approach of calculation, we also manipulate the penetration data of clonidine using multiple total flux equations to obtain K_B and K_{BH^+} values. From two penetration profiles we can obtain two total flux equations:

$$J_{total}\ (\text{pH } 4.6) = K_B\ [B]\ (\text{pH } 4.6) + K_{BH^+}\ [BH^+]\ (\text{pH } 4.6)$$
$$J_{total}\ (\text{pH } 7.0) = K_B\ [B]\ (\text{pH } 7.0) + K_{BH^+}\ [BH^+]\ (\text{pH } 7.0)$$

Since J_{total} (pH 4.6), J_{total} (pH 7.0), [B] (pH 4.6), [B] (pH 7.0), $[BH^+]$ (pH 4.6), and $[BH^+]$ (pH 7.0) are known, we can calculate K_B and K_{BH^+} by solving the two equations simultaneously, giving 2.5×10^{-3} and 2.41×10^{-4} cm/hr, respectively. These values are exactly the same as those obtained from the approximation approach (Table I).

This study shows the importance of obtaining these microscopic constants (permeability coefficients) for the species present in the solution formulations in order to compare their permeation ability and respective contribution to the total flux. Most work reported in the literature (5,7,8) has not taken this into consideration, and thus it is not safe to state that one species permeates better than the other, although superficially the contribution of flux to the total flux may seem to be predominated by a species at a specific pH. A study of the permeation of ephedrine, scopolamine, and chlorpheniramine also shows that the nonionized forms penetrated human skin much better than the ionized forms (9) by comparing their permeability coefficients.

By treating the control penetration data of clonidine in a similar fashion, we can obtain the J_{total}, J_B, J_{BH^+}, K_B, and K_{BH^+} values (Table I). It is interesting to note that the J_{total}, J_B, and J_{BH^+} values at pH 4.6 and pH 7.0 are all about 11

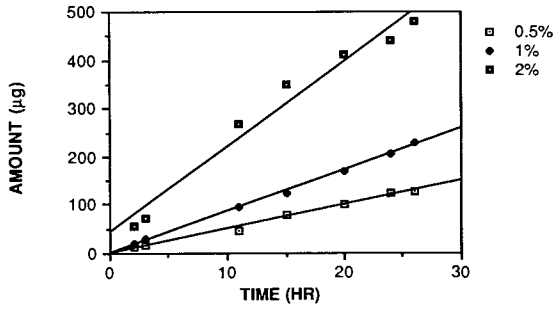

Fig. 2. Effect of concentration on the penetration of clonidine at 32°C.

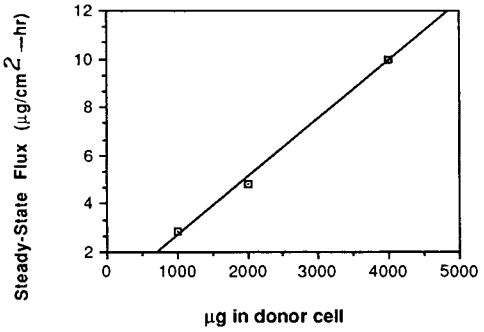

Fig. 3. Linear relationship between the steady-state flux and the initial concentration of clonidine.

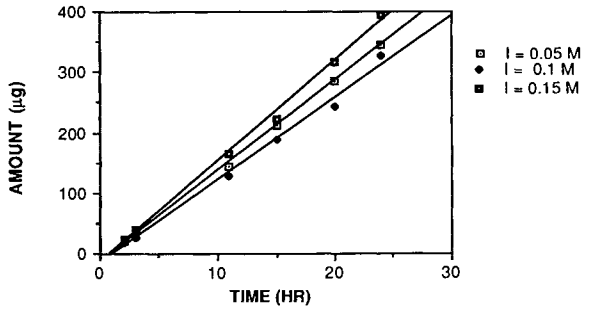

Fig. 4. Effect of ionic strength on penetration of clonidine at 32°C.

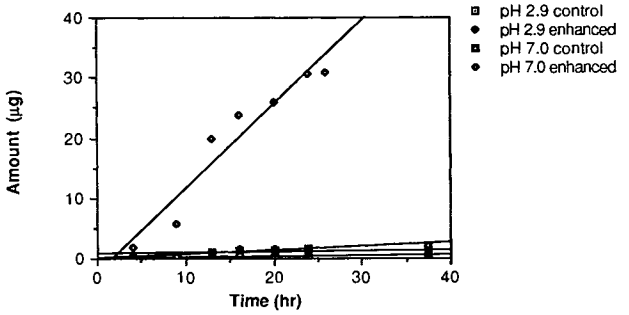

Fig. 5. Time-course penetration profiles of indomethacin through shed snakeskin at 32°C for aqueous suspension formulations of pH 2.9 and 7.0. The lines labeled enhanced are for skins pretreated with dodecyl *N,N*-dimethylamino acetate.

times smaller than the corresponding values obtained for skins pretreated with the enhancer. The fact that all these penetration parameters are proportionally increased from the untreated skins to the enhancer-pretreated skins indicates that the enhancer reduces the resistance barrier of the skin, possibly by a mechanism of facilitated diffusion (10). It is likely that the enhancer acts by changing the electronic structure of the skin, whereby the ion-exchange characteristics of the skin is altered. The change in electronic structure of skin requires a significant difference in the penetration parameters for the ionized and the nonionized species. However, this has not been observed for clonidine.

To examine how concentration can affect the penetration flux of clonidine, a permeation experiment was carried out by applying different concentrations (0.5, 1, and 2%) of clonidine hydrochloride at pH 4.6 to the donor cells. The penetration profiles of this experiment are given in Fig. 2, and the penetration parameters in Table II. The J_{total} values are linearly concentration dependent (Fig. 3 and Table II), and this indicates that the total fluxes obey Fick's first law of diffusion. The K_{BH^+} values calculated by the first-approximation approach discussed earlier are also given in Table II, showing close agreement with an average of 1.04×10^{-3} cm/hr. This relative small variation in K_{BH^+} values indicates that the skin characteristics and the thickness of the skins are similar within a whole shed snakeskin obtained from a single snake since the pieces of skin were chosen randomly for the diffusion cells.

Since the solution formulations are ionic in nature, it would be interesting to know whether ionic strength would have any effect on penetration flux. To determine this we studied the penetration flux of clonidine in solutions with a constant concentration of clonidine at variable ionic strengths (0.05, 0.1, and 0.15 *M*). The penetration profiles are shown in Fig. 4 and the results of this study are given in Table III. The total flux is the highest at $I = 0.15$ *M*, while it is the lowest at $I = 0.1$ *M*. This trend is also observed for 5-fluorouracil studied under similar conditions (11).

The pK_a value of 3-(hydroxymethyl)-5,5-diphenylhydantoin ester with *N,N*-diethyl β-alanine has been determined to be 6.6 at 25°C and $I = 0.5$ *M* by a kinetic method (12). The *N,N*-diethylamino propionate group of this prodrug is analogous to the *N,N*-dimethylamino acetate group of the enhancer, dodecyl *N,N*-dimethylamino acetate, which, therefore, is expected to have a pK_a value in the range around 7.0. At pH 7.0 and 2.9, the enhancer is expected to be in a mixture of nonionized and cationized form.

The present treatment of data assumes that one form of the ionogenic drug did not enhance or inhibit the transport of the other form. Theoretically, it is possible that one form could interfere the transport of the other, but it is difficult to verify experimentally.

Table III. Effect of Ionic Strength on Penetration Parameters of Clonidine at pH 4.6 and 32°C

I (*M*)	Conc. (μg)[a]	[BH^+] (μg)	J_{total} (μg/cm²-hr)	K_{BH^+} (cm/hr) $\times 10^4$
0.05	4000	3996	8.38	8.39 ± 0.10
0.1	4000	3996	7.71	7.72 ± 0.77
0.15	4000	3996	9.36	9.37 ± 0.88
Average				8.49

[a] Four-tenths milliliter of an appropriate solution formulation of clonidine was applied to the donor cells.

Skin Permeation of Indomethacin from Solutions of Different pH's

The penetration profiles for indomethacin in aqueous solution formulations are shown in Fig. 5. At pH 7.0 the flux of indomethacin is about 25 times higher for skins pretreated with the enhancer than the control. However, the fluxes for both pretreated skins and the control are similar at pH 2.9. This may be due to the low solubility of indomethacin at this pH. A study was carried out to determine indomethacin solubility at various pH's (Fig. 6). The saturated solubility at 32°C are 2.35 μg/ml (pH 2.9), 138.3 μg/ml (pH 5.7), and 916.6 μg/ml (pH 7.0). The penetration data were treated in the

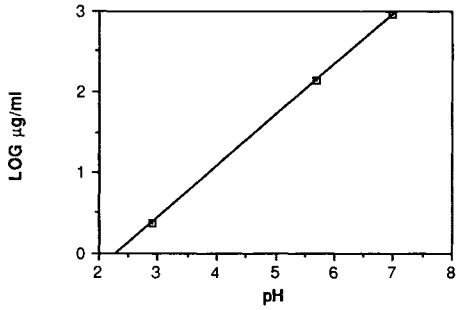

Fig. 6. pH–solubility profile for indomethacin.

Table IV. Penetration Parameters of Indomethacin Using Shed Snakeskins as the Model Skins at 32°C

				Enhanced			Control			Enhanced		Control				$\frac{J_{total}(enh)}{J_{total}(cont)}$	
pH	[Ind] (μg)	[A^-] (μg)	[HA] (μg)	J_{total} (μg/cm²-hr)	J_{A^-}	J_{HA}	J_{total}	J_{A^-}	J_{HA}	K_{HA} (cm/hr)	K_{A^-}	K_{HA}	K_{A^-}	$\frac{K_{HA}(enh)}{K_{HA}(cont)}$	$\frac{K_{A^-}(enh)}{K_{A^-}(cont)}$	pH 2.9	pH 7.0
2.9	2.35	0.056	2.29	8.98 × 10^{-3}	4.5 × 10^{-5}	8.93 × 10^{-3}	8.3 × 10^{-3}	0	8.3 × 10^{-3}	3.9 × 10^{-3}	7.97 × 10^{-4}	3.62 × 10^{-3}	2.19 × 10^{-5}	1.0	36.4	1.0	24.7
7.0	916.6	913.9	2.7	0.738	0.728	0.010	0.0298	0.02	0.0098	3.9 × 10^{-3a}	7.97 × 10^{-4a}	3.62 × 10^{-3a}	2.19 × 10^{-5a}				

[a] Values calculated by the simultaneous equations approach.

same way as described previously for clonidine and the results are given in Table IV.

The permeability coefficient for the ionized species (K_{A^-}) and nonionized species (K_{HA}) for both enhanced and control experiments were obtained by the approximation and simultaneous equations calculation (see Table IV). K_{HA} (3.9 × 10^{-3} cm/hr) and K_{A^-} (7.97 × 10^{-4} cm/hr) values for the enhanced experiments are the same for both types of calculation. For the control experiments the K_{HA} (3.62 × 10^{-3} cm/hr) and K_{A^-} (2.19 × 10^{-5} cm/hr) values are also the same for both types of calculation. While for the enhanced experiment, the nonionized species of indomethacin permeated the skin five times faster than its ionized counterpart, the difference in permeation ability is more than a hundred times that of the control experiment. It is interesting to note that the K_{HA} values for the enhanced and control experiments are identical, indicating that the enhancer has no effect on the permeability of the nonionized species of indomethacin. This phenomenon is different from that of clonidine discussed earlier, where the transport of both ionized and nonionized species of clonidine was enhanced by 11 times. The reason why the enhancer had no effect on the nonionized form of indomethacin is not clear. The effect of enhancer on the permeability is more dramatic on the ionized species as evident from the ratio (36.4) of the permeability coefficient of the ionized form [K_{A^-}(enh)] to that [K_{A^-}(cont)] (Table IV). Therefore, the enhancing effect of dodecyl *N,N*-dimethylamino acetate is very low or nonexistent at lower pH's due primarily to the low solubility of indomethacin. At higher pH's, the higher concentration of indomethacin becomes a very important factor responsible for the total flux of indomethacin.

We have applied our methods of calculation to the penetration data for indomethacin reported by Chien *et al.* (5). These penetration experiments were done using hairless mouse skins as the model and their parameters are given below.

	Approximation approach (2nd appr.)	Simultaneous equations approach
K_{A^-} (cm/hr)	0.00346	0.00346
K_{HA} (cm/hr)	1.04	1.04

These permeability coefficients indicate that hairless mouse skin is much more permeable to indomethacin than is snakeskin, particularly for the nonionized species. This result shows the same trend that the nonionized indomethacin has a much higher permeability coefficient than the ionized form, as found in our penetration experiments using snakeskin as the skin model.

Conclusion

The present study has demonstrated the importance of obtaining the microscopic constants (permeability coefficients) for ionogenic drugs. They could lead to insight on the mechanism of the permeation phenomenon.

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