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Iontophoretic transdermal permeation of verapamil (III): Effect of binding and concentration gradient on reversibility of skin permeation rate

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Summary

This study was undertaken to determine if the prolonged reversibility time (i.e., time required for the skin permeation rate to return to that of passive diffusion after an iontophoresis treatment) of verapamil HCl was related to an increased concentration gradient across the skin and binding of the drug in the skin. The concentration gradient across the stratum corneum, determined by stripping the skin, was significantly greater after an iontophoresis treatment than that obtained under passive diffusion; furthermore, the concentration of drug in the viable skin was also significantly greater with iontophoresis. Verapamil was found to bind with both the stratum corneum and the viable skin. The increased concentration gradient and binding of drug in the skin could be responsible for a prolongation of the enhanced permeation rate observed after iontophoresis treatment.

Introduction

A number of theoretical models for describing electrodiffusion predict that the concentration of an ion in a membrane in presence of an electric field (of the same sign as the charge on the ion) will be greater than the concentration obtained during passive diffusion (Finklestein and Mauro, 1977; Garrido, 1985; Keister and Kasting, 1986; Kasting and Keister, 1989). If these models can be applied to iontophoretic transdermal transport, then after the iontophoresis treatment is terminated, the permeation rate of drug may remain elevated until sufficient drug has desorbed from the skin and the concentration gradient becomes equivalent to that of the passive diffusion profile. The length of time for desorption to occur depends on the diffusivity, binding affinity, and (if binding does occur) the concentration of drug in the skin. For drugs with very low diffusivity or high binding affinity, desorption may take many hours (Tojo et al., 1988).

In previous studies on the iontophoretic transdermal transport of verapamil (Wearley et al., 1989a,b), it was observed that the reversibility time, that is, the length of time required for the iontophoresis-facilitated permeation rate to return to that of passive diffusion (after a 10 min iontophoresis treatment) was a function of concentration. Generally, the time required for re-

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versibility to occur closely followed the time required for 50% of the drug to desorb from the skin, when the donor solution was removed immediately after iontophoresis treatment. In addition, when the polarity of the electrodes was reversed after an iontophoresis treatment in an attempt to drive the drug out of the skin, the reversibility time did not change significantly. These observations are indicative of possible binding of verapamil in the skin.

This investigation was undertaken to determine the actual concentration profile of verapamil in the skin following iontophoresis treatment, and to assess the extent of binding in the skin.

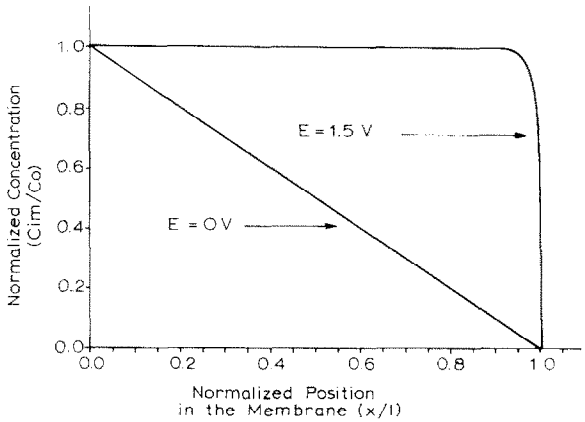

Fig. 1. Theoretical concentration profile of an ion across a homogeneous membrane in the presence of a uniform electric field of 1.5 and 0 V (replotted from Keister and Kasting (1986)).

Theory

Concentration profiles predicted from the Nernst-Planck equation

Two approximate solutions to the Nernst-Planck equation which have been used to descibe electrodiffusion through membranes are the Goldman approximation and the electroneutrality approximation. The assumptions of each and their application to transdermal iontophoretic transport have been thoroughly reviewed by Kasting and Keister (1989). The exact solution to the Nernst-Planck equation requires numerical analysis and Garrido (1985) describes a general numerical solution which includes a term for convective flow. In most cases, the steady-state concentration profile predicted for an ion in the presence of an applied electric field (where ionic charge and electric field are of the same sign) is greater than that predicted for passive diffusion. Fig. 1 is the concentration profile predicted from the Goldman approximation with $E = 0$ and $E = 1.5$ V (where the concentration inside the membrane, C_{im}, at $x/l = 1$ is zero, and at $x/l = 0$ is C_0). If the upper profile represents the concentration profile of a drug in the stratum corneum during iontophoresis treatment, it is obvious that the permeation rate immediately after termination of treatment would remain elevated for a period of time (since the flux is proportional to the concentration gradient at the membrane/receptor interface), until desorption of sufficient drug occurs to make the two profiles equal.

In addition to differences in concentration gradient, the partitioning of an ion into a lipid membrane may be different in the presence of an electric field compared to the passive condition. The presence of an electric field may raise or lower the free energy barrier for partitioning of an ion into a lipid membrane, like the skin (Bard and Faulkner, 1980; Kasting and Keister, 1988). Thus, under certain iontophoresis conditions, the surface concentration (at $x/l = 0$) of an ionic drug may be greater than that under passive diffusion conditions. This will also cause a prolongation of the enhanced permeation rate after the iontophoresis treatment is terminated.

Dual sorption model for binding

If a drug binds in the skin, the concentration gradient at the stratum corneum/viable skin interface or the viable skin/receptor interface may remain elevated for a longer period of time than when no binding occurs. Using the 'dual sorption' model, Michaels et al. (1963) and Paul (1969) showed that binding will affect both the diffusional and desorption lag times. The dual sorption model assumes that binding follows the Langmuir isotherm with a single layer of adsorbate occupying the binding sites. This type of binding may

occur in chemisorption and physical adsorption (Branauer, 1938). Without binding, the diffusional and desorption lag times are functions of the permeant diffusivity and thickness of the membrane. However, Frisch (1957) and Paul (1969) have shown that when a diffusant binds in a membrane the diffusional and desorption lag times are nonlinear functions of permeant concentration, as well as diffusivity and membrane thickness. Thus, one method to determine whether or not binding occurs in the skin is to ascertain whether the diffusion or desorption lag times vary with concentration.

In order to quantitate the binding constants of a drug in the skin, Chandresakaran et al. (1976) utilized a method based on the dual sorption model. The method consists of equilibrating weighed skin specimens with known concentrations of drug. Assuming Langmuir-type binding is operative, the total concentration of drug in the skin may be expressed as follows:

$$C_T = K_d C + C_l^* bC/(1 + bC) \quad (1)$$

where C_T represents the total concentration of drug in the stratum corneum, K_d is the stratum corneum/water partition coefficient of free drug, C denotes the concentration of drug in solution, C_l^* is the maximum concentration of drug bound (Langmuir constant) and b is the Langmuir equilibrium binding constant. At high concentrations, Eqn. 1 reduces to

$$C_T = K_d C + C_l^* \quad (2)$$

Therefore, a plot of C_T vs. C will have a slope of K_d and intercept of C_l^*. At low concentrations Eqn. 1 reduces to

$$C_T = K_d C + C_l^* bC \quad (3)$$

Using the values for K_d and C_l^* obtained from Eqn. 2 and then plotting $(C_T - K_d C)$ as a function of C, the typical Langmuir binding profile may be obtained. Analysis of this function by a linear transformation will allow determination of the value for b.

Experimental

Materials and equipment

Equipment and materials used were the same as previously described (Wearley et al., 1989a). Briefly, in vitro permeation studies were carried out in Valia-Chien skin permeation cells, which have a cross-sectional area of 0.636 cm^2. A pair of Ag/AgCl electrodes were immersed in the donor and receptor solutions through the sampling port of each half-cell. Pulsed current (0.1 mA, square wave form with frequency of 2 kHz and on/off ratio of 1 : 1) was delivered via a constant current power source (Transdermal Periodic Iontotherapeutic System, developed in this Research Center). Verapamil HCl (Knoll Pharmaceuticals, Whippany, NJ) and [*N-methyl*-^{3}H]verapamil HCl (Dupont NEN Research, Boston, MA) were used as obtained.

In vitro skin permeation studies

The procedure for these studies was the same as previously described (Wearley et al., 1989a). Briefly, freshly excised abdominal skin from the male fuzzy rat was secured between the donor and receptor half-cells. The donor half-cell was filled with verapamil HCl in water and the receptor half-cell with normal saline. For iontophoresis experiments, pulsed current (0.1 mA, 0.157 mA/cm^2) was applied for the specifed time. The receptor solution was sampled at predetermined intervals over the length of the experiment. For desorption studies, the donor solution was removed immediately after the iontophoresis treatment, the skin was first rinsed and the stratum corneum surface was covered with foil for mechanical strength and clamped back in place between the half-cells; then the receptor solution was sampled at regular intervals. The diffusional lag time was determined by extrapolating the steady-state portion of the passive permeation profile to the time axis (x-axis). The desorption lag time after iontophoresis was determined by the intersection of the two extrapolated linear portions of the desorption profile.

Stratum corneum stripping studies

In order to determine the concentration profile of verapamil across the skin, layers of the stratum

corneum were stripped off with adhesive tape (Scotch Magic Tape, 3M Co., St. Paul, MN) and the amount of radioactivity in each strip was counted. Specifically, 0.1 mA of pulse current was applied for a period of time (10–130 min), the skin was then removed from the cell, rinsed with approx. 5 ml of water, patted dry and trimmed to the cross-sectional area of the cell opening. Immediately after trimming, layers of the stratum corneum were removed with tared pieces of adhesive tape and reweighed, so that the weight of each stratum corneum strip could be determined. For comparison, the drug concentration profile following passive diffusion was also assessed by repeating the skin permeation study without the intophoresis treatment for the same duration and stripping the stratum corneum in the same manner as in the iontophoresis experiments. Each strip was then placed in 10 ml of Biofluor (Dupont NEN), extracted for 24 h and the amount of radioactivity determined by liquid scintillation counting (RackBeta 1214-001, Pharmacia LKB, Gaithersburg, MD). From the specific activity of the donor solution and the weight of each strip, the concentration of drug in each skin layer was determined. The concentration of the donor solution was 0.09 mg/ml of verapamil in water (no buffer). The receptor was normal saline.

Although other investigators have shown that 20 or more strippings may be necessary to remove completely the stratum corneum (Pinkus, 1951) as determined microscopically, 15 strippings of the stratum corneum were used to determine the concentration profile in this study for two reasons: (a) The potential difference across the skin, measured according to Wearley et al. (1989), was observed to reach a minimum in less than 10 strippings (Fig. 2) and (b) The amount of stratum corneum removed after approx. 10–15 strippings was so low that it was difficult to obtain an accurate determination of weight. For the purposes of comparing the passive and iontophoretic concentration profiles, removal of 10–15 layers appeared to be sufficient. The thickness of each strip was calculated from the following expression:

$$h_s = w_s/(A\delta) \qquad (4)$$

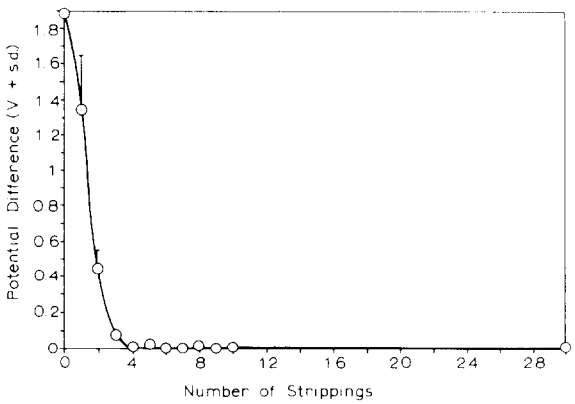

Fig. 2. Potential difference across the skin during iontophoresis treatment (0.1 mA of pulse current) as a function of layers of stratum corneum stripped.

where h_s and w_s denote the thickness and weight of the strip removed, respectively, A is the cross-sectional area of the skin specimen (0.636 cm^2) and δ is the density of the stratum corneum. The density was determined in a separate experiment by measuring the weight and thickness of the skin before and after stripping. The thickness was determined by placing the skin specimen between two glass slides and measuring with calipers.

Binding studies

The Langmuir isotherms of verapamil in the stratum corneum and viable skin were determined by equilibrating known weights of each with known concentrations of drug. The stratum corneum was separated from the viable skin of male fuzzy rat by placing the freshly excised abdominal skin with viable skin side down on filter paper soaked with 5% trypsin and incubation for approx. 16 h at room temperature (Knutson et al., 1985). After separation and rinsing, weighed samples of stratum corneum or viable skin were equilibrated at 37 °C with 3 ml of verapamil HCl in acetate buffer (0.1 M, pH 5.7) with concentration ranging from 0.27 to 90 mg/ml (triplicate skin specimens were used for each concentration). After 24 h, the skin specimens were removed from the verapamil solutions, thoroughly rinsed and placed in 5 ml of methanol to extract the drug from the skin. After 24 h, the skin was removed and placed

in a fresh aliquot of methanol and the extraction continued for an additional 24 h. This procedure was repeated until no additional radioactivity was detected in the extraction solution. The concentration in the stratum corneum or viable skin was plotted vs. that in the donor solution according to Eqn. 1. The slope and y-intercept of the isotherm at high concentration was determined in order to calculate K_d and C_I^*, respectively, according to Eqn. 2. Then $(C_T - K_dC)$ was plotted as a function of C to obtain the Langmuir binding profile. A Lineweaver-Burk transformation was then used to determine the value for b. Specifically, $C/(C_T - K_dC)$ was plotted as a function of C and b was determined from the intercept which is equal to $1/(bC_I^*)$.

Results and Discussion

Concentration profiles

Fig. 3 indicates that with an iontophoresis treatment of 0.1 mA of pulse current, the steady-state value of flux is reached after approx. 50 min

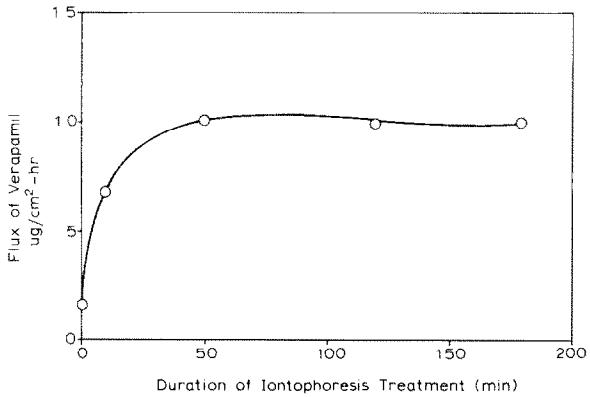

Fig. 3. Relationship between the skin permeation flux of verapamil and the duration of iontophoresis treatment (pulsed current, 0.1 mA).

iontophoresis. The concentration profiles across the stratum corneum obtained under passive conditions and with an iontophoresis treatment of increasing duration are compared in Fig. 4. The concentration of drug in the stratum corneum under these particular conditions is much greater with iontophoresis than for passive diffusion, even for the shortest duration of iontophoresis treat-

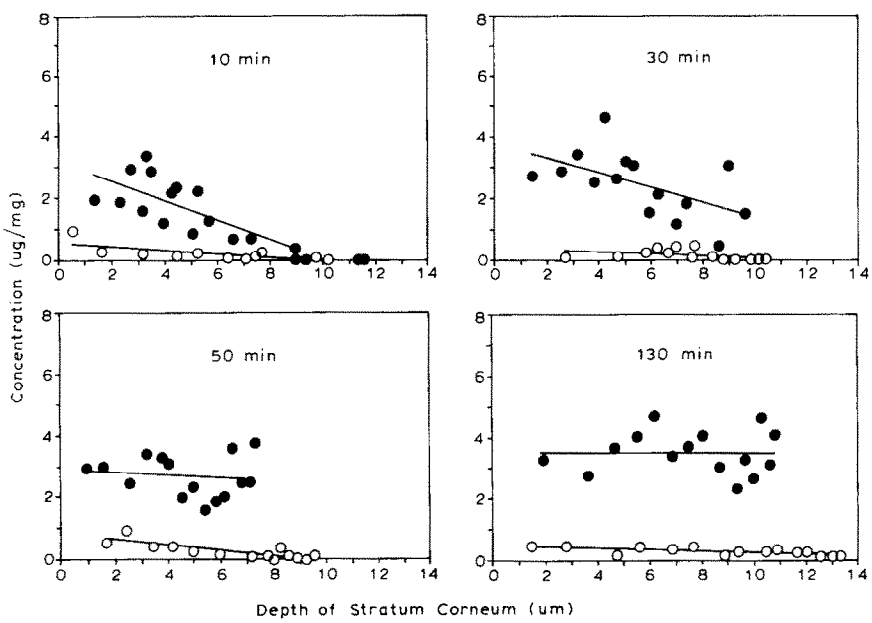

Fig. 4. Concentration profile of verapamil across the stratum corneum after an iontophoresis treatment of 10, 30, 50 and 130 min (●). The concentration profiles under passive diffusion after exposure to donor and receptor solution for the same amount of time are shown for comparison (○). (———) Regression line.

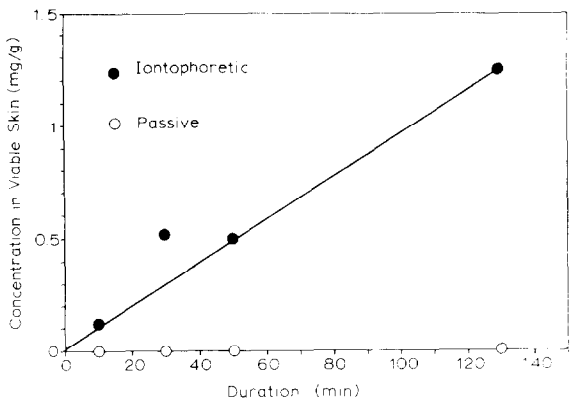

Fig. 5. Concentration of verapamil in the viable skin as a function of duration of iontophoresis treatment (●) and duration of exposure to the donor solution, under passive diffusion (○). Donor concentration contained 0.09 mg/ml of verapamil.

ment (10 min). Note that the concentration profile shows very little increase after 50 min of iontophoresis treatment, which correlates with the time at which steady-state flux is reached with iontophoresis (Fig. 3).

Another interesting feature shown in Fig. 4 is the difference in surface concentration between the passive diffusion and iontophoretic profiles. On average, the concentration in the first two strips of stratum corneum is 4.9 (S.D. $= \pm 1.8$) times greater for iontophoresis treatment than under passive diffusion. It may be that partitioning of the charged verapamil molecule into the stratum corneum is more favorable, or that equilibrium is approached more rapidly in the presence of an electric field compared to passive diffusion. Alternatively, the differences in surface concentration may be a result of variations in desorption between the iontophoretic and passive concentration profiles. It should be noted that the stripping technique does not differentiate between the ionic and neutral species of verapamil (which is predominantly protonated at physiological pH (Wearley et al., 1989a)). The iontophoresis treatment probably enhances the movement of both species into the skin whereas, under passive conditions, the neutral species would more readily partition into the skin.

Fig. 5 shows that the concentration of drug in the viable skin is also significantly greater with iontophoresis than under passive diffusion.

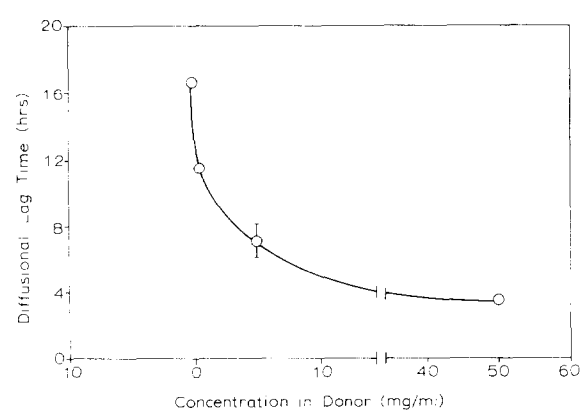

Fig. 6. Diffusion lag time calculated from passive skin permeation profiles of verapamil as a function of donor concentration.

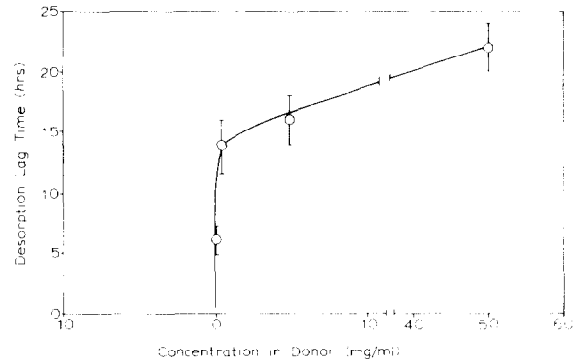

Fig. 7. Desorption lag time calculated from the desorption profiles of verapamil after an iontophoresis treatment (0.1 mA, applied for 10 min) as a function of donor concentration.

Binding studies

Fig. 6 indicates that the diffusional lag time obtained under passive diffusion conditions is a

TABLE 1

Partition coefficients and Langmuir binding constants [a]

Skin tissue	K_d	C_f^* (mg/g)	b (g/mg)
Stratum corneum	0.191	9.54	0.282
	(0.065)	(1.24)	(0.048)
Viable skin	0.186	2.91	0.261
	(0.025)	(0.52)	(0.14)

[a] Determined from Eqns. 2 and 3 and expressed as the mean ($\pm$ S.D.) of three determinations.

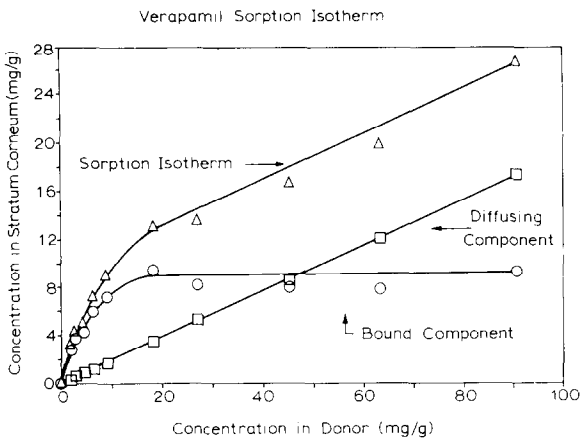

Fig. 8. Sorption isotherm (△) for verapamil in the stratum corneum with the diffusing component (□) and the bound component (○) plotted according to Eqns. 2 and 3.

function of concentration. The desorption lag time obtained after iontophoresis is also a function of concentration as indicated in Fig. 7. Both of these observations are strong indications of binding as previously discussed. The sorption isotherm for verapamil in the stratum corneum has been plotted in Fig. 8, with the diffusing and bound components separated according to Eqns. 1–3. A similar plot for the viable skin is shown in Fig. 9, and the Langmuir binding constants for verapamil in the stratum corneum and viable skin have been calculated and compared in Table 1.

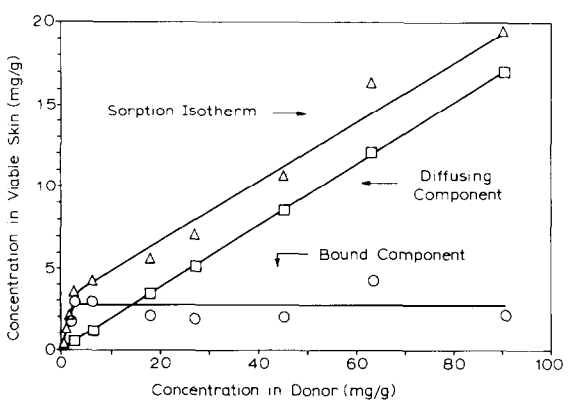

Fig. 9. Sorption isotherm (△) for verapamil in the viable skin with the diffusing component (□) and the bound component (○) plotted accoding to Eqns. 2 and 3.

Conclusion

The results outlined above may help to explain the lack of immediate reversibility of verapamil permeation rate after iontophoresis. The iontophoresis treatment under these particular conditions increases the concentration of verapamil in the skin. The partitioning of the drug from donor into the stratum corneum may also be enhanced and the concentration gradient at the stratum corneum/viable skin interface is greater than under passive diffusion. Since verapamil is extensively bound in both the viable skin and stratum corneum, the concentration gradient may be sustained for a number of hours as desorption of bound drug occurs and this would result in a prolongation of reversibility time.

References

Bard, A. and Faulkner, L., *Electrochemical Methods*, Wiley, New York, 1980, pp. 86–108.

Branauer, S., Emmett, P. and Teller, E., Adsorption of gases in multimolecular layers. *J. Am. Chem. Soc.*, 60 (1938) 309–315.

Chandrasekaran, S., Michaels, A., Campbell, P. and Shaw, J., Scopolamine permeation through human skin in vitro. *AIChE J.*, 22 (1976) 828–832.

Finklestein, A. and Mauro, A., Physical principles and formalisms of electrical excitability. In: Geiger, S. (Ed.), *Handbook of Physiology*, Section I: The Nervous System, Vol. 1, Am. Physiol. Soc., Bethesda, 1977, pp. 162–163.

Frisch, H., The time lag in diffusion. *J. Phys. Chem.*, 61 (1957) 93–95.

Garrido, J., Mafe, S. and Pellicer, J., Generalization of a finite-difference numerical method for the steady-state and transient solutions of the Nernst-Planck flux equations. *J. Membrane Sci.*, 24 (1985) 7–14.

Kasting, G. and Keister, J., Theoretical models for iontophoretic transport of drugs through skin. *Pharm. Res.*, 5 (1988) S81.

Kasting, G. and Keister, J., Application of electrodiffusion theory for a homogeneous membrane to iontophoretic transport through skin: a review. *J. Controlled Rel.*, 8 (1989) 195–200.

Keister, J. and Kasting, G., Ionic mass transport through a homogeneous membrane in the presence of a uniform electric field. *J. Membrane Sci.*, 29 (1986) 155–167.

Knutson, K., Potts, R., Guzek, D., Golden, G., McKie, J., Lambert, W. and Higuchi, W., Macro- and molecular physical-chemical considerations in understanding drug

transport in the stratum corneum. *J. Controlled Rel.*, 2 (1985) 67–87.

Michaels, A., Vieth, W. and Barrie, J., Solution of gases in polyethylene terephthalate. *J. Appl. Phys.*, 34 (1963) 1–12.

Paul, D., Effect of immobilizing adsorption on the diffusion time lag. *J. Polym. Sci.*, 7 (1969) 1811–1818.

Pinkus, H., Examination of epidermis by the strip method of removing horny layers. *J. Invest. Dermatol.*, 16 (1951) 383–386.

Tojo, K., Chiang, C.C., Doshi, U. and Chien, Y.W., Stratum corneum reservoir capacity affecting dynamics of transdermal delivery. *Drug Dev. Ind. Pharm.*, 14 (1988) 561–567.

Wearley, L., Liu, J.C. and Chien, Y.W., Iontophoretic transdermal permeation of verapamil HCl: (I) in-vitro evaluation and mechanistic studies. *J. Controlled Rel.*, 8 (1989a) 237–250.

Wearley, L., Liu, J.C. and Chien, Y.W., Iontophoretic transdermal permeation of verapamil (II): factors affecting the reversibility of skin permeability. *J. Controlled Rel.* 9 (1989b) 231–242.