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Iontophoretic transport of acetate and carboxylate ions through hairless mouse skin. A cation exchange membrane model

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Summary

As models for transdermal iontophoretic drug delivery, carboxylate anions were electrochemically driven from aqueous solutions across excised hairless mouse skin into aqueous buffer solutions. The flux of ^{14}C-anions was measured. Using [^{14}C]acetate, the flux increased as the current increased, as the acetate concentration in the donor solution increased, as the pH of the donor solution decreased, or if the donor solution was not stirred. The composition of the receptor solution had little effect on the acetate flux, as did turning the skin backwards. Under the same conditions, a comparison of the efficiencies of carboxylate delivery showed acetate > hexanoate > dodecanoate. Acetate delivery through the perfluorosulfonic acid cation-exchange membrane, Nafion, was studied for comparison. The mouse skin results are interpreted in terms of iontophoretically driven acetate delivery through essentially aqueous pathways with similarities to transport through cation-exchange membranes.

Introduction

Passive transdermal delivery of drugs provides an effective method for controlled release at a constant rate. A more sophisticated device can be envisaged in which the delivery rate is controlled electrochemically. Such a device would provide the possibility of varying the rate since the rate would be controlled by the current flowing through an electrochemical circuit. Specifically, it is possible to drive ions through the skin by the use of an applied electrochemical potential. Therefore, ionic drugs, which otherwise do not permeate skin very well, could be delivered at controlled rates. Numerous publications over several decades describe experiments in which this approach allowed drugs to be "iontophoresed" through skin (Comeau et al., 1973; Csillik et al., 1982; Gangarosa et al., 1977, 1980; Gordon and Weinstein, 1969; Okabe et al., 1986; Park et al., 1978; Ragelis 1981a and b; Warwick et al., 1985).

There is, however, little understanding of the iontophoretic process, and we have undertaken a project to collect fundamental data. In so doing, it is anticipated that results revealing the nature of ion transport in skin will be obtained which will have significance beyond the present goal. Precedent for the study reported here is most closely provided by a study of benzoate delivery with a very similar experimental protocol (Bellantone et

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al., 1986). The iontophoretic delivery of the carboxylate, salicylate, for plantar wart treatment has also been reported (Gordon and Weinstein, 1969). We have reported preliminary results on acetate delivery (Miller et al., 1987) as well as electrochemical studies of release of several biomedically significant ions from polymer films (Miller et al. 1987; Blankespoor et al. 1985; Zinger, 1984).

Materials and Methods

Materials

Donor and receptor solutions were prepared from analytical grade or better reagents with no further purification (Mallinkrodt, Paris, KY & Aldrich, Milwaukee, WI). Water to make stock solutions was distilled and deionized with resistance > 18 MΩ/cm. The donor solutions were "spiked" with ^{14}C labeled sodium salt of appropriate donor compound (Research Products International Corp., Mt. Prospect, IL) to give activities of 4–45 μCi/mmol. The receptor solution was Sorensen buffer (0.027 M NaH_2PO_4, 0.040 M Na_2HPO_4, 0.079 M NaCl with pH 7.0). The salt bridges contained 1.0 M NaCl/3% w/v agar (Bacto-Agar, Difco, Detroit, MI) with the isolated carbon electrodes immersed in 0.1 M NaCl. This insured that the ^{14}C anion would not be affected by electrolysis, and insulated the cells against pH changes by H_2O electrolysis. The diffusion cells were custom-made, but are almost identical with commercially available cells (model VC-100, Crown Glass, Somerville, NJ) and were placed in a cell holder with magnetic synchronous stirrers (similar to model VCDU-1, Crown Glass) and maintained at 37°C with a circulating water bath (Model 80, Fisher Scientific). Current was supplied by a galvanostat (Model 173, Princeton Applied Research, Princeton, NJ). Nafion membrane was type 117, non-reinforced film of 1100 equivalent weight perfluorinated copolymer with a thickness of 0.2 mm (DuPont Co., Wilmington, DE). Aliquots for scintillation counting were made with a Pipetman (Model P-200, Rainin, Woburn, MA). Scintillation cocktail was a xylene based mix (Safety-Solve Research Products International, Mt. Prospect, IL). Samples were counted on a liquid scintillation counter (Mark III Model 6881, Tracor Analytical, Elk Grove Village, IL).

Methods

Hairless mice (female, 8–12 wks, SIM : HRS/hr · hr, Simonson, Gilroy, CA) were sacrificed by cervical dislocation. Whole-thickness skin was removed and divided along the sagittal plane into two pieces (left and right sides) with excess adipose tissue removed by gentle scraping. The skin pieces were soaked in the receptor buffer solution for approximately 45 min prior to placing in the cells. Immediately after skin was placed in the cell, the appropriate volume of receptor solution (2.5 or 3.5 ml) was pipetted into the cell followed immediately with 3.5 ml of donor solution into the cell. (Solutions were preheated in a water bath.) Electrodes were then placed into the cells, and current with the appropriate polarity was switched on.

The initial concentration of ^{14}C labelled material was determined from an aliquot of the donor solution. Aliquots were withdrawn periodically from the receptor solution and replaced with buffer to maintain the volume. The aliquots diluted with premixed scintillation cocktail (12 ml) were analyzed with a scintillation counter after completion of the experiment. All samples in flux calculations were corrected for background counts (approximately 30 cpm). The computational program accounted for the small depletion of the donor solution (less than 1% of ^{14}C label was transported), and the dilution of the receptor ^{14}C concentration due to aliquot replacement. Samples were typically two or more times background within 10–20 min after the beginning of experiment. Flux rates were determined by plotting cumulative flux (concentration at designated time plus amounts removed in previously samples) as a function of elapsed time and performing a simple linear regression of the points in the steady-state range. Typically, the experiment was conducted over a period of 3 h; and a sequence 0.1, 0.3 and 0.5 mA/cm^2 of skin area was used. The use of constant current delivery insured that there was a constant total flux of ions driven across the skin by the applied potential.

Results

Acetate delivery

A series of experiments was designed to test various experimental variables, one at a time. A typical experiment involved the use of 0.1 M sodium acetate as the donor solution and Sorensen buffer as the receptor solution. The cumulative amount of acetate delivered per cm^2 of skin area was measured at 3 current levels. Fig. 1 shows typical results. At each current level the cumulative amount of acetate delivered increased linearly during the time interval utilized, and the flux increased with increased current as expected. The passive flux ($I = 0$) was very low; only 1–3% of the flux measured at 0.1 mA/cm^2.

The correlation coefficients for plots of acetate delivered vs time were always at least 0.99. The slope of the lines gives the flux, J_A. The reproducibility of J_A from one piece of skin to the next was typically better than $\pm 10\%$. This variation was also observed with skin sections from the same animal. In an experiment in which I was stepped from 0.1 to 0.3 to 0.5 to 0.3 to 0.1 mA/cm^2, the J_A values at each current level were within experimental error at the two times. This indicates that the results do not depend significantly on the usual protocol of only using 0.1 then 0.3 then 0.5 mA/cm^2.

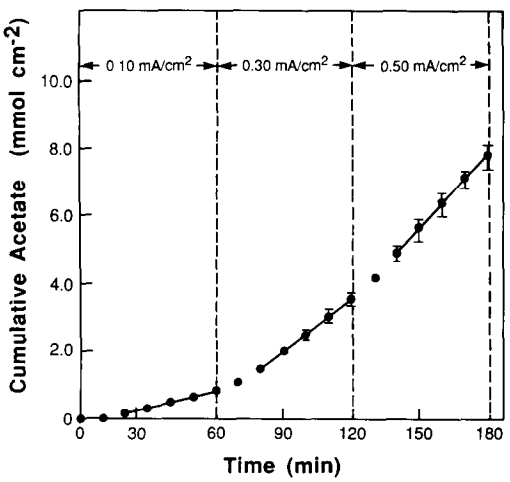

Fig. 1. Cumulative amounts of acetate delivered from 0.15 M sodium acetate into Sorensen buffer. Two pieces of skin from each of two mice.

TABLE 1

J_A for delivery into Sorensen buffer [a] at 3 currents

Donor [NaAc] (M) [b]	J_A (μmol/cm^2/h)		
	0.1 mA/cm^2	0.3 mA/cm^2	0.5 mA/cm^2
0.025 [c]	0.33	0.78	1.02
0.05 [d]	0.68	1.6	2.3
0.075 [c]	0.86	2.4	3.5
0.10 [d]	0.94	2.3	3.5
0.15 [d]	1.02	3.1	4.4

[a] Sorensen buffer 0.19 M in Na^+.
[b] Sodium acetate concentration, unbuffered.
[c] Average of two runs.
[d] Average of 4 runs.

Table 1 and Fig. 2 shows average J_A values at each of the three current levels at donor acetate concentrations varying from 0.025 to 0.15 M. It can be seen from the figure that J_A is not strictly proportional to current or to concentration.

An important aspect of membrane transport is consideration of the effects of concentration polarization near the membrane. This was tested by conducting an experiment without stirring the donor solution to enhance the problem. Using 0.025 M acetate the J_A values were higher (Table 2) rather than lower as they would have been if

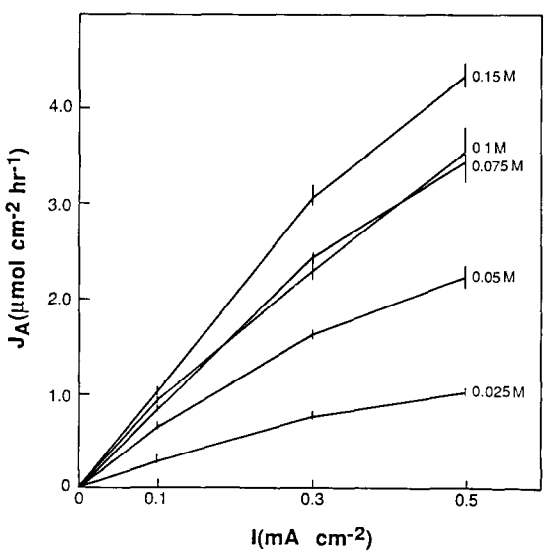

Fig. 2. Dependence of J_A on I and acetate concentration in the donor solution.

TABLE 2

J_A at 3 currents under various conditions [a]

Donor concn (M) [b]	Donor conditions [c]	Receptor solution [d]	J_A (μmol/cm²/h)		
			0.1 mA/cm²	0.3 mA/cm²	0.5 mA/cm²
0.1		Sor	0.94	2.3	3.5
0.1		0.1 M NaAc	0.99	2.6	4.5
0.1	pH 4.7	Sor	1.17	3.9	6.4
0.1	pH 4.7	pH 4.7	1.6	6.8	9.5
0.1		19 mM Sor	0.56	1.54	2.2
0.1		1.9 mM Sor	0.57	1.43	2.1
0.025		Sor	0.33	0.78	1.02
0.025		0.025 M NaAc	0.14	0.36	1.16
0.025	pH 6.0	Sor	0.67	1.46	2.4
0.025	pH 4.7	Sor	0.99	2.4	3.4
0.025	No Stir	Sor			3.3

[a] J_A are averages of two or more runs.
[b] Designates total acetate concentration.
[c] No entry means stirred 0.1 M sodium acetate.
[d] Sor = Sorensen buffer, NaAc = sodium acetate, pH 4.7 = 0.1 M acetate buffer.

depletion was a determinant, but consistent with polarization effects. A second concern was with effects due to the asymmetry of the skin. Therefore, the skin was turned around, so that acetate was delivered from the inside of the skin, instead of the epidermal side. This lowered the J_A values by about 15%, only slightly more than experimental error.

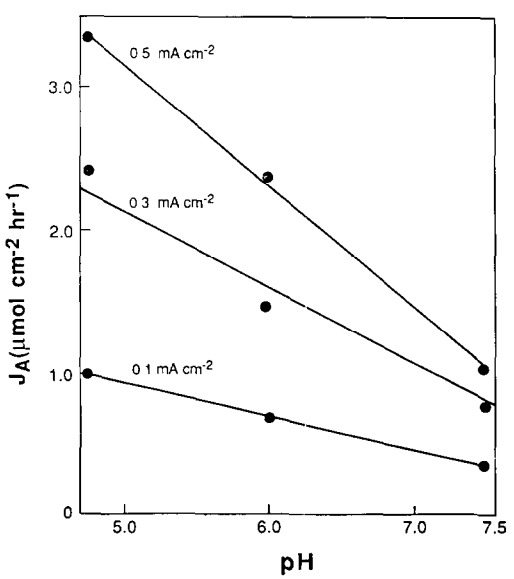

Fig. 3. Dependence of J_A on pH. Delivery from 0.025 M total acetate.

TABLE 3

J_A for delivery through Nafion [a]

Donor concn (M)	Donor pH	J_A (μmol/cm²/h)
0.025	7.52	0.12
	4.75	1.3
0.10	7.86	0.36
	7.86 [b]	0.37
	4.75	3.8

[a] At 0.5 mA/cm using identical donor and receptor solutions.
[b] Sorensen buffer in receptor.

Because acetate is a weak base, and because the permselectivity of the skin could depend on the pH, donor acetate buffer solutions of pH 6.0 and 4.75 were used to look for pH effects. Using donor solutions which were 0.025 and 0.1 M in *total* acetate, the flux values at each current were substantially enhanced at lower pH. Data (Table 2) are displayed in Fig. 3. Again at pH 4.75, the passive delivery rates were enhanced somewhat, but were still negligible.

The effect of changing the composition of the receptor solution was also investigated (Table 2). Replacing Sorensen buffer with sodium acetate

had no appreciable effect. Similarly diluting the Sorensen buffer by as much as a factor of 100, led only to a small decrease in J_A. Only when the donor solution was pH 4.75, 0.1 M acetate buffer was any substantial effect noted. In that one case, changing the receptor solution from Sorensen buffer to 0.1 M pH 4.75 acetate buffer raised J_A.

It was of interest to see if a cation exchange membrane would behave like mouse skin for acetate delivery. A Nafion membrane (a sulfonated fluoropolymer) was selected for this comparison. Data are shown in Table 3. The J_A values are smaller for Nafion; but as with mouse skin, an increase in donor acetate concentration increases J_A; 0.1 M sodium acetate in the receptor behaves like Sorensen buffer, and lower pH enhances J_A.

Carboxylate ion delivery

An understanding of the relative permeability of various compounds in passive transdermal experiments have shown lipid solubility of the drug to be an important attribute for good transport. In iontophoretic delivery this might also be a factor; but since the ion is being driven by the electrochemical potential, it could also be true that smaller (hydrophilic) ions would be transported faster. Since the constant current experiment requires a constant total rate of ion transport, the test of relative rate is the efficiency. Thus, it is expected that an ion which is more rapidly transported will compete better with other processes and give a larger number of mol delivered per Faraday of charge passed.

We selected the series acetate, hexanoate and dodecanoate to examine the relationship between size (lipophilicity) and efficiency. The experiments were all conducted using Sorensen buffer as the receptor solution and 0.1 M solutions of the carboxylate salts as donor solutions. In each case steady state behavior was rapidly achieved at each of the three current levels. Flux values are reported in Table 4.

The passive rate ($I = 0$) for hexanoate was negligible under these conditions. The passive rate for dodecanoate was not negligible compared to that at 0.1 mA/cm^2 so that the actual iontophoretic rate is even lower. Dodecanoate results may also differ because the concentration exceeds the critical micelle concentration. It is not known if this affects the comparison or not.

TABLE 4

Flux of carboxylate ions

Ion	J(μmol/cm^2/h)		
	0.1 mA/cm^2	0.3 mA/cm^2	0.5 mA/cm^2
Hexanoate	0.29	0.79	1.47
Dodecanoate	0.11	0.48	0.58

[a] Donor solution 0.1 M sodium carboxylate. Receptor solution Sorensen buffer.

Discussion

The results confirm that simple anions can be electrochemically driven across skin at controlled rates. These rates are controlled by the electrochemical current, the pH and anion concentration of the donor solution, and to a limited extent, the composition of the receptor solution and the stirring of the donor solution. The results can be understood, in part, from a consideration of an ion-exchange membrane model. Indeed, the correlation of results for mouse skin and Nafion make this model attractive.

Acetate delivery

Consider the results in light of the standard theory for ionic diffusion through membranes (Bungay et al., 1986; Meares et al., 1968, 1972), which has been developed to understand the transport of simple inorganic ions through such materials such as ion exchange membranes. One expects that the electrochemical current will be carried by a combination of anion transport from donor to receptor, and cation transport in the other direction. The efficiency of acetate transport is then determined by this competition. In the experiments reported here, the efficiency, mol of acetate per Faraday of electricity, ranged from 0.1 for 0.025 M sodium acetate as the donor solution to 0.4 for 0.1 M pH 4.75 acetate buffer as the donor solution.

These efficiencies, which are less than 0.5, indicate that sodium cations are transported preferentially to acetate anions. Permselectivity of skin favoring cation transport has been suggested long

ago (Rein, 1924) and has been demonstrated for iontophoretic NaCl transport (Burnette and Ongpipattanakul, 1987). It presumably results because the pathways utilized for iontophoretic transport are like those in a cation exchange membrane. At pH 7 the skin is expected to have an excess of anionic sites and a high concentration of sodium cations. This is why skin acts like Nafion in the present study.

The cation exchange membrane model allows qualitative understanding of the concentration effects on J_A as well as the stirring effect on J_A. In such a membrane the relative flux rates of acetate and sodium will depend on their activities in the membrane as well as concentration polarization in the solutions near the membranes. As donor acetate concentrations increase, membrane acetate concentrations also increase and J_A should increase as observed. There is also a concentration polarization due to the permselectivity which raises the acetate concentration near the stratum corneum. This polarization is enhanced in the nonstirred experiment and leads to the larger J_A which is observed. Because the skin at pH 7 has a relatively high sodium ion concentration, variations in the (more dilute) receptor solution sodium ion concentration should have only minor effects on J_A, as observed. Again, concentration polarization will take place in the receptor solution, but in this case it will be mediated by the higher interstitial fluid content of the dermis structure.

Turning now to pH effects, the data show that as the pH of the donor solution goes down, so that acetate is replaced by acetic acid, J_A increases (Fig. 3). A qualitatively similar pH effect was noted for hexanoate. Before considering the mechanistic implications of these observations, it is noted that the results in Fig. 2 using varying concentrations of unbuffered sodium acetate may in part reflect the varying pH of these donor solutions. Thus, 0.1 M sodium acetate has a pH of 7.6 and 0.025 M has a pH of 7.4. Second, we note that a similar pH effect has been discovered in studies of thyrotropin-releasing hormone (TRH) iontophoresis through hairless mouse skin (Burnette et al., 1986). TRH is a protonated amine and in more basic donor solutions, where the amine will be deprotonated, the iontophoretic rate increased. The authors attributed this iontophoretic transport of the neutral TRH to convective flow induced by electro-osmosis of water.

TABLE 5

Concentrations at various pH for 0.025 M total acetate

pH	$[Ac^-]$ (mM)	[HAc] (mM)	$[H^+]$ (mM)	$[OH^-]$ (mM)
7.5	25	3×10^{-5}	3×10^{-5}	3×10^{-4}
6.0	24	1	1×10^{-3}	1×10^{-5}
4.75	12.5	12.5	5×10^{-5}	2×10^{-7}

In the case of acetate delivery from solutions of constant total acetate, the higher flux at lower pH is inconsistent with a simple picture where acetate ion is iontophoresed and neutral acetic acid is not transported. That picture would lead to the opposite trend.

The pH effect on J_A could be qualitatively explained if OH^- was a competing ion being transported in a parallel process. However, as the data in Table 5 show the relative mobility of OH^- compared to acetate would have to be variable and 10^4–10^8 times larger than that of acetate in order to explain the pH dependence. Thus a parallel process involving OH^- is unreasonable. Also unreasonable is a simple parallel process in which acetic acid is passively delivered while acetate is iontophoretically delivered. The passive J_A is too small and would not respond to the change in current in the observed manner.

Two types of mechanisms can, however, qualitatively explain to pH effect. One involves the effect of solution pH on the skin properties, and the second type includes mechanisms in which acetic acid, proton, and acetate transport are coupled. Consider the former. Since a change in pH from 7.6 to 4.75 only increases J_A by a factor of 3, this change could be due to a change in the permselectively of the skin (Rein, 1924), acting like cation-exchange membrane. In this model the permselectivity of the skin results from the net negative charge of the static skin components which line narrow pathways through which the ions are transported. As the pH is lowered, it is expected that the net charge on these components (which could be protein carboxylates and amines)

will change becoming more positive. This would tend to favor transport of the anionic acetate, as observed.

The second set of possibilities involves not the skin, but coupled transport. One specific explanation is that there is iontophoretically driven acetate transport with acetic acid being carried along. It is known that in the ion-exchange membranes (Meares et al., 1972) and across skin (Gangarosa, et al., 1980; Burnette et al., 1987) there is a substantial transport of water (electro-osmosis) as well as ions and that this water transport can carry along neutral molecules. In the present case, there would have to be a net transport of water from donor to receptor in order to get acetic acid carried to the receptor. However, the flow is expected to go in the other direction, as it does for NaCl (Burnette et al., 1987; Rein, 1924) since it appears that the efficiency of acetate transport is less than 0.5. Furthermore, when 0.025 M acetate at pH 6.0 is used in the donor so that the acetic acid concentration is 1 mM (Table 5), it can be calculated that at 0.5 mA/cm^2 about 1 ml of this donor solution would have to be transported to carry along the necessary $\sim 1\ \mu$mol of acetic acid. This volume change would have been easily detected had it occurred. Finally, the data in Table 5 suggests that this mechanism should give a special enhancement of J_A only near the pK_a of acetic acid.

A more satisfactory type of coupled transport involves the proton flux as well as acetate and acetic acid. The data are compatible with the involvement of iontophoretically driven proton transport, and it is known that certain membranes will favor proton transport. For example, the competition between alkali metal ion and proton transport across ion exchange membranes in an electrochemical cell has been studied. Bipolar ion exchange membranes (cation and anion exchange membranes back-to-back) can give high proton fluxes compared to alkali metal cation fluxes (Frilette, 1956, Grossman, 1976). If there is a substantial proton flux in the mouse skin experiment this complicates the interpretation of J_A. Thus, the pH gradient will affect the acetate, acetic acid equilibrium as the membrane is traversed and this will in turn affect J_A.

We conclude that there is a similarity of acetate transport in skin and in Nafion, and hypothesize that in iontophoresis an ion-exchange membrane is a good model for skin. An accurate description of transport during iontophoresis of acetate requires an understanding of skin protonation and proton transport. This is not available and not easily attained when solutions with some buffer capacity like acetate are employed.

Carboxylate ion delivery

The data on carboxylate ion delivery (Table 4) are limited in detail but informative on the point of interest. They demonstrate that using solutions of their sodium salts, acetate is iontophoresed more efficiently than hexanoate and that dodecanoate has the lowest efficiency. The smaller, more hydrophilic ion is transported faster under these conditions than the larger ions. Indeed, the ratio of efficiencies are quite consistent with the differing diffusion coefficients expected for ions in water based upon their size.

This is a most interesting and important result. It is consistent with the cation exchange model and suggests that during iontophoretic delivery, the ions travel along pathways that are essentially aqueous. These pathways could involve hair follicles, pores, or breaks in the stratum corneum, but they could also be the hydrophilic domains of the stratum corneum. The hydration of the stratum corneum is well known, and it would appear that this hydration may provide pathways of electrochemically driven ion transport. This hypothesis will be tested in future studies.

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References

Bellantone, N.H., Rim, S., Francoeur, M.L. and Rasadi, B., Enhanced percutaneous absorption via iontophoresis. I.

Evaluation of an in vitro system and transport of model compounds. *Int. J. Pharm.*, 30 (1986) 63–72.

Blankespoor, R.L. and Miller, L.L., Polymerized 3-methoxythiophene. A processable material for the controlled release of anions. *Chem. Commun.*, (1985) 89–92.

Bungay, P.M., Lonsdale, H.K. and dePinho, M.N. (Eds.), *Synthetic Membranes: Science, Engineering and Applications,* Reidel, Dordrecht, The Netherlands, 1986.

Burnette, R.R. and Ongpipattanakul, B., Characterization of the permselective properties of excised human skin during iontophoresis, *J. Pharm. Sci.*, 76 (1987) 765–772.

Burnette, R.R. and Marrero, D., Comparison between the iontophoretic and passive transport of thyrotropin releasing hormone across excised nude mouse skin *J. Pharm. Sci.*, 75 (1986) 738–743.

Comeau, M., Brummett, R.B. and Vernon, J., Local anesthesia of the ear by iontophoresis. *Arch. Otoalaryngol.*, 98 (1973) 114–120.

Csillik, B., Knyihar-Csillik, E. and Szacs, A., Treatment of chronic pain syndrome with iontophoresis of *Vinca* alkaloids to the skin of patients. *Neurosci. Lett.*, 31 (1982) 87–90.

Frilette, V.J., Preparation and characterization of bipolar ion exchange membranes, *J. Phys. Chem.*, 60 (1956) 435–439.

Gangarosa, L.P., Park, N.H. and Hill, J.M., Iontophoretic assistance of 5-iodo-2′-deoxyuridine penetration into neonatal mouse skin and effects on DNA synthesis. *Proc. Soc. Exp. Biol. Med.*, 154 (1977) 439–443.

Gangarosa, L.P., Park, N.H. Wiggins, C.A. and Hill, J.M., Increased penetration of nonelectrolytes into mouse skin during iontophoretic water transport (iontohydrokinesis). *J. Pharm. Exp. Ther.*, 212 (1980) 377–381.

Gordon, A.H. and Weinstein, M.V., Sodium salicylate iontophoresis in the treatment of plantar warts. *Phys. Ther.*, 49 (1969) 869–870.

Grossman, G., Water dissociation effects in ion transport through composite membrane. *J. Phys. Chem.*, 80 (1976) 1616–1625.

Meares, P., Thain, J.F. and Dawson, D.G., In G. Eisenman (Ed.), *Membranes Vol. 1,* Dekker, New York, 1972, Chap. 2.

Meares, P. and Sutton, A.H., Electrical transport phenomena in a cation-exchange membrane. I. The determination of transport numbers and the ratios of tracer fluxes. *J. Colloid Interface Sci.* 28, (1968) 118–133.

Miller, L.L., Smith, G.A., Chang, A.C. and Zhou, Q.X., Electrochemically controlled release. *J. Controlled Release,* in press.

Miller, L.L. and Zhou, Q.X., Poly-(*N*-methylpyrrolium)/polystyrenesulfonate. A conductive, electrically switchable cation exchanger cathodically binds and anodically releases dopamine. *Macromolecules,* 20 (1987) 1594.

Okabe, K., Yamaguchi, H. and Kawai, Y., New iontophoretic transdermal administration of the beta-blocker metroprolol. *J. Controlled Release,* 4 (1986) 79–85.

Park, N.H. Gangarosa, L.P., Known, B.S. and Hill, J.M, Iontophoretic application of adenine arabinoside monophosphate to herpes simplex virus type I-infected mouse skin. *Antimicrob. Agents Chemother.,* 14 (1978) 6005–6008.

Ragelis, S.I., Penetration into tissues of penicillin and streptomycin administered with modified method of electrophoresis. *Antibiotiki,* 26 (1981a) 696–699.

Ragelis, S.I. Penetration into tissues of tetracycline administered with modified methods of electro- and phonophoresis. *Antibiotiki* 26 (1981b) 699–703.

Rein, H., Experimentally Studien über Elektro-endosmose an überlebender menschlicher Haut. *Z. Biol.* 81 (1924) 125–40.

Warwick, W. et al., Evaluation of a Cystic Fibrosis Screening System Incorporating a Miniature Sweat Stimulation and Disposable Chloride Sensor, *Clin. Chem.* 32 (1985) 850–853.

Zinger, B. and Miller L.L., Timed released of chemicals from polypyrrole films. *J. Am. Chem. Soc.*, 106 (1984) 6861–6863.