Pore Charge Distribution Considerations in Human Epidermal Membrane Electroosmosis

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Abstract □ The aim of this study was to assess the extent to which a model with pores having only net negative charges would adequately describe transdermal electroosmosis in human epidermal membrane (HEM) at neutral pH. Such information would enhance the predictive value of the modified Nernst–Planck model for transdermal iontophoresis, in addition to providing insights regarding the likelihood of significant pore charge distribution in HEM. Baseline results (the control) obtained from 0.1 to 0.4 V anodal and cathodal electroosmosis experiments with synthetic polycarbonate membranes (Nuclepore membranes), using radiolabeled urea and mannitol as the model permeants, demonstrated that such a membrane system can be modeled by the electrokinetic (electroosmosis) theory with the assumption of the pores possessing only negative charges. The studies with HEM were carried out at low voltage ($\leq$0.5 V) where alterations in the barrier properties of HEM were minimal and at higher voltages ($\gtrsim$1.0 V) where significant field-induced pore formation in HEM occurred. In both the low and high voltage studies, radiolabeled urea, mannitol, and water were employed as permeants in cathodal and anodal iontophoresis experiments. The results of the low voltage iontophoresis experiments suggest significant pore charge distribution in HEM (a significant deviation between the predictions from the single pore charge type assumption and the experimental data). Under the higher applied voltage conditions ($\gtrsim$1.0 V), results from anodal and cathodal electroosmosis studies were consistent with the model in which the HEM has only pores that are net negatively charged.

Introduction

There has been important progress in our understanding of the mechanisms of transdermal iontophoresis with human epidermal membrane (HEM). The modified Nernst–Planck model (Nernst–Planck equation with corrections for convective solvent flow due to electroosmosis) has been tested with HEM under low voltage iontophoresis conditions,[1,2] and semiquantitative agreement between the experimental results and predictions from the model has been generally observed. In other studies with HEM, the effective sizes of the pores involved in passive permeation and of the pores induced during low to moderate voltage iontophoresis have been deduced from the hindered transport theory and found to be in the range of ~6 to 25 Å.[3,4] More recently, a method based on square-wave alternating current (ac) iontophoresis was developed to study the induction of pores (electroporation) in HEM during iontophoresis at low to moderate voltages without interference from electroosmosis.[5] With this method, direct evidence of new pore induction as an iontophoretic flux enhancing mechanism was presented, and the effective pore sizes of the induced pores were assessed.

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As part of an effort to establish a more comprehensive understanding of the mechanisms of iontophoresis in HEM and to maximize the predictive value of the modified Nernst–Planck model, some independent quantitative assessment of electroosmosis in HEM is necessary. Burnette and Ongpipattanakul[6] previously examined the permselectivity of human cadaver skin at current densities of ~0.08–0.2 mA/cm^2 and showed that skin has an apparent net negative charge at neutral pH. Later studies by Pikal and by Pikal and Shah[7–9] on iontophoretic transport across hairless mouse skin at current densities $\gtrsim$0.3 mA/cm^2 have suggested that negatively charged pores dominate at neutral pH, but the presence of positively charged and neutral pores was also hypothesized. In these hairless mouse skin studies, pore size, pore charge, and their distributions were estimated with a theoretical model using experimental iontophoretic fluxes and electrical resistance measurements made as a function of pH, NaCl concentration, and current density. In an HEM study conducted in our laboratory,[10] the direction of the net electroosmotic convective solvent flow at neutral pH was anode-to-cathode; this direction is consistent with the HEM pores being net negatively charged. However, we have not previously examined the question of the importance of possible positively charged and/or neutral pores in HEM electroosmosis.

The aim of the present study was to examine the extent to which HEM electroosmosis data obtained at neutral pH could be described/predicted by the presence of only negatively charged pores (i.e., that convective solvent flow in the pores would be only in the anode-to-cathode direction). Electroosmotic flux enhancements of urea and mannitol in both anodal and cathodal iontophoresis and iontophoretic water fluxes were determined under different applied voltage conditions (in pH 7.4 and 0.1 M ionic strength phosphate buffered saline) for model analysis. These results were compared with theory predictions assuming a single pore charge (negative), and an assessment was made as to the need to involve positively charged and/or neutral pores in interpreting HEM electroosmosis data. A point of particular interest in this study is the comparison of results obtained with pores newly induced in HEM (induced by applied electric fields) with those from the preexisting pores.

Experimental Section

Materials—[^{14}C]Urea, [^{3}H]mannitol, and [^{3}H]water were obtained from New England Nuclear (Boston, MA) and American Radiolabeled Chemicals (St. Louis, MO). Human epidermal membrane (HEM) was provided by TheraTech, Inc. (Salt Lake City, UT). The epidermal membrane was prepared by heat separation as previously described[10] and immediately frozen for later use. Millipore GVWP filters (0.22 μm pore diameter) were obtained from Millipore Corp. (Bedford, MA). Nuclepore polycarbonate membranes with a nominal pore radius of 7.5 nm and porosity of

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0.001 were purchased from Costar Scientific Corporation (Pleasanton, CA). Phosphate-buffered saline with 0.02% sodium azide (PBS), ionic strength 0.1 M and pH 7.4 (0.077 M NaCl and 0.0074 M phosphate buffer), was prepared from reagent grade chemicals and distilled deionized water.

Theory and Model Analysis—The steady-state iontophoretic flux ($J_{\Delta\psi}$) of a nonionic permeant across a homogeneous porous membrane can be described by the modified Nernst−Planck model:[3]

$$J_{\Delta\psi} = \epsilon\left(-HD\frac{dC}{dx} + W'vC\right) \quad (1)$$

where v is the average velocity of the convective solvent flow (positive v denotes flow from donor to receiver and negative v denotes flow from receiver to donor), ϵ is the combined porosity and tortuosity factor for the membrane; C, x, and D are the concentration, the position in the membrane, and the diffusion coefficient of the permeant, respectively; and H and W' are the hindered transport factors for passive diffusion and for transport due to electroosmosis, respectively. Assuming a single pore size (radius, R_p) and a cylindrical pore geometry in the membrane and when the ratio of solute radius to pore radius (r/R_p) is small (i.e., $r/R_p < 0.4$), the hindrance factor for Brownian diffusion (H) and the hindrance factor for pressure-induced parabolic convective solvent flow (W) can be expressed by:[11]

$$H = (1-\lambda)^2(1 - 2.104\lambda + 2.09\lambda^3 - 0.948\lambda^5) \quad (2)$$

$$W = (1-\lambda)^2(2-(1-\lambda)^2)(1 - 0.667\lambda^2 - 0.163\lambda^3) \quad (3)$$

where $\lambda = r/R_p$. For convenience, W' is assumed to be equal to W as discussed previously.[3] The diffusion coefficients and Stokes−Einstein radii of the permeants in the present study were taken from literature.[4,12] Integrating eq 1 results in

$$J_{\Delta\psi} = \frac{C_D\epsilon Wv}{1 - \exp[-Wv(\Delta x)/(HD)]} \quad (4)$$

where C_D is the donor concentration and Δx is the effective thickness of the membrane. At the convection limit (electroosmotic transport ≫ passive diffusion), eq 4 reduces to

$$J_{\Delta\psi} = \epsilon WvC_D \quad (5)$$

The total flux enhancement (E_{total}) is defined as the ratio of iontophoretic flux to the passive flux at the same donor concentration:

$$E_{total} = \frac{J_{\Delta\psi}}{J_{passive}} \quad (6)$$

where

$$J_{passive} = \frac{DC_D\epsilon H}{\Delta x} \quad (7)$$

Under an applied potential of ≳1 V, when the electrical resistance of HEM decreases due to pore induction, the total transport enhancement can be expressed by eq 8:

$$E_{total} = E_{\Delta R}E_v \quad (8)$$

where $E_{\Delta R}$ is the enhancement due to pore induction and is defined as the ratio of the porosity-tortuosity factor in iontophoretic transport to the porosity-tortuosity factor in passive transport:

$$E_{\Delta R} = \frac{\epsilon_{ion}}{\epsilon_{pass}} \quad (9)$$

E_v is the transport enhancement due to electroosmosis as described by

$$E_v = \frac{Pe}{1 - \exp\{-Pe\}} \quad (10)$$

where Pe is the Peclet number, which characterizes the contribution of convective transport due to electroosmosis. Pe is expressed as

$$Pe = \frac{Wv\Delta x}{HD} \quad (11)$$

Previous studies[5,13] have demonstrated a proportional relationship between the HEM electrical conductance and HEM permeability under the iontophoresis conditions of a few volts when the sizes of the conducting ions in the solution are comparable to those of the permeants. Thus, the $E_{\Delta R}$ values will be estimated from changes in HEM conductance during iontophoresis in the present study. For the Nuclepore membranes, $E_{\Delta R}$ is equal to unity, and E_{total} equals E_v. Effective pore radii for HEM during iontophoresis and in passive diffusion experiments will be estimated by the flux ratios of the model permeants as described previously,[2−5] with the assumption that the permeant pairs follow the same polar transport pathway in a given run.

In the present study, eq 10 will be used with experimental data obtained from both the anodal and cathodal configurations. The analysis should provide an assessment of the extent to which the model with only negatively charged pores may hold.

Transport Experiments: General Procedure—Transport experiments with HEM and with Nuclepore membranes were conducted using a side-by-side diffusion cell (with a diffusional area of ~0.75 cm^2 and cell volume of 2 or 4 mL) and a four-electrode potentiostat system (JAS Instrument Systems, Inc., Salt Lake City, UT) with Ag-AgCl counter electrodes as described previously.[3] Square-wave ac iontophoresis experiments were carried out with a waveform programmer (JJ 1276, JAS Instrument Systems, Inc., Belmont, NC) with the four-electrode potentiostat setup just described. The electrical resistance of the membranes was calculated with Ohm's law and the current was measured by the potentiostat system in direct current (dc) iontophoresis experiments or with an oscilloscope (Model 2211, Tektronix Inc., Beaverton, OR) in the ac experiments. Transport runs were conducted at 37 °C, which was maintained with a circulation water bath. Before each run, the receiver and donor chambers were filled with PBS and PBS premixed with appropriate amounts (tracer levels) of radiolabeled permeants, respectively. Urea/mannitol and urea/water were the permeant pairs employed. At predetermined time intervals, a 1-mL sample was withdrawn from the receiver chamber and replaced with fresh PBS. At the same time, a 10 μL sample was taken from the donor. The samples were then mixed with 10 mL of scintillation cocktail (Ultima Gold, Packard, Meriden, CT) and assayed in a liquid scintillation counter (1900 TR Liquid Scintillation Analyzer, Packard, Meriden, CT). The permeability coefficients for iontophoretic transport ($P_{\Delta\psi}$) and for passive transport ($P_{passive}$) were calculated by

$$P_{\Delta\psi} \text{ or } P_{passive} = \frac{1}{AC_D}\frac{dQ}{dt} \quad (12)$$

where A is the membrane surface area, t is time, Q is the cumulative amount of permeant transported into the receiver chamber, and

$$P_{\Delta\psi} = J_{\Delta\psi}/C_D \quad (13)$$

$$P_{passive} = J_{passive}/C_D \quad (14)$$

Experiments with Nuclepore Membranes—Nuclepore membranes were presoaked and sonicated in PBS to remove any entrapped air in the membranes. Then, 50 of the membranes were assembled into a single composite membrane in the diffusion cell. The Nuclepore membrane studies were divided into two stages: passive permeation experiments and iontophoresis experiments. Anodal and cathodal iontophoresis experiments were conducted at 0.1, 0.2, 0.4, and/or 0.75 V dc. Passive permeation runs were carried out before and after each iontophoresis run. The same sets of Nuclepore membranes (from the same lot) were used without disassembling to avoid variabilities arising from membrane-to-membrane variations.

Experiments with HEM—HEM was equilibrated in PBS for 12 to 24 h at 37 °C before starting a transport experiment. The

initial electrical resistances were 41 ± 26 kΩ cm^2, average ± SD, $n = 50$. HEM studies were divided into two parts: Studies I and II.

Study I was a baseline study with low applied voltage iontophoresis (0.25 and/or 0.5 V dc) where alterations in the barrier properties of HEM were minimal (generally within 15% of the original HEM electrical resistance). Experiments in Study I were divided into five stages. With each HEM sample, passive permeability coefficients were determined in Stages I, III, and V. Stage II was an iontophoresis run at an applied potential of 0.25 or 0.5 V dc. In Stage IV, the iontophoresis run was carried out with the same applied voltage as in Stage II but with the opposite electrode polarity. Anodal iontophoresis was conducted first (Stage II) with about half of the HEM samples and cathodal iontophoresis was first with the other half.

Study II involved higher applied voltage conditions where significant pore induction occurred in HEM. The initial experimental design in this study was a 4.0 V dc prepulse for 1 min followed by 1.0, 2.0, or 3.0 V dc exposure during which transport experiments were conducted. The 1-min 4.0 V dc prepulse preceding the 1.0, 2.0, or 3.0 V dc iontophoresis run was an attempt (with only variable success) to enhance the extent of pore induction and to minimize differences in the pore conditions between the anodal and cathodal runs. With this experimental protocol, cathodal iontophoresis generally induced a greater extent of pore induction than anodal iontophoresis at the same applied voltage and duration (>3 times in some cases; data not shown). Also, the $E_{\Delta R}$ values in some experiments were <10, suggesting a >10% contribution from preexisting pores to transport in these experiments. Thus, this initial protocol was problematic as we desired simultaneously (a) to compare the anodal and cathodal electroosmotic fluxes under conditions of comparable electroporation and (b) not to have significant flux contributions from preexisting pores compromising data analysis. A new protocol was introduced at this point that was designed to overcome or minimize these problems. This protocol involved superimposing 12.5 Hz square-wave ac onto cathodal or anodal dc iontophoresis with the aim to maintain comparable HEM electrical conductance (pore induction) during the anodal and cathodal electroosmosis runs and to achieve a high extent of pore induction ($E_{\Delta R} > 10$). In this arrangement, 0.5, 1.0 or 2.0 V dc was employed as the driving force for electroosmosis, and the 12.5 Hz square-wave ac with adjustable voltage between 0 and 3 V was used to manually control the extent of pore induction ($E_{\Delta R}$). The outcome of this arrangement was that there was always a >10-fold increase in HEM electrical conductance during iontophoresis (relative to the initial conductance) and a <30% variation in conductance during the combined anodal and cathodal iontophoresis runs. The particular ac frequency of 12.5 Hz was chosen only because of our previous experience with it in the dc/ac superposition iontophoresis.[5]

The experiments of Study II with this new protocol were divided into four stages. Similar to Study I, Stage I was a passive permeation run before the application of the electric field. Stage II was the iontophoresis run superimposing 12.5 Hz square-wave ac (0−3 V) with 0.5, 1.0, or 2.0 V dc for electroosmosis. Stage II was followed by rinsing the receiver chamber two to three times with fresh PBS. In Stage III, the same protocol of superimposing ac and dc was carried out as in Stage II but with opposite electrode polarity. HEM electrical resistance in Stages II and III was regulated by adjusting the ac voltage to obtain a >10-fold increase in electrical conductance (relative to the initial resistance in Stage I) and a <30% variation in resistance throughout the anodal and cathodal iontophoresis runs (Stages II and III). Stage IV was a passive diffusion run after iontophoresis.

Results and Discussion

Analysis of the Urea/Mannitol Dual-Permeant Data—Figure 1 presents the experimental results with the Nuclepore membrane, where the enhancement factor (E_v) for cathodal iontophoresis is plotted against that for anodal iontophoresis. The predictions from the electroosmosis theory (eq 10) with the assumption of a single pore charge are given by the curved line. It is evident that transport data obtained in the experiments with the Nuclepore membrane are consistent with the ideal, single pore charge

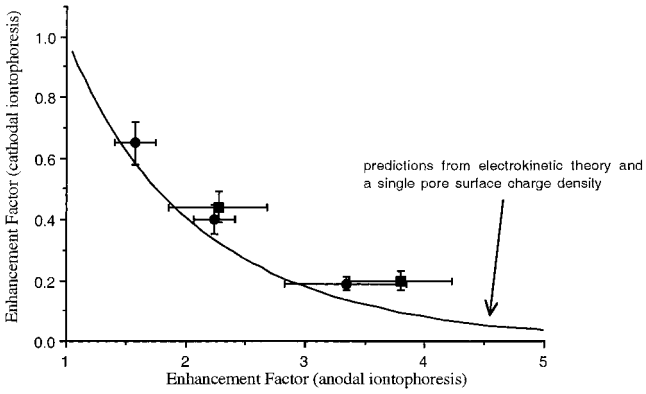

Figure 1—Relationship between cathodal and anodal electroosmotic flux enhancement for Nuclepore membranes at 0.1, 0.2, and 0.4 V dc. The curve represents predictions from electrokinetic theory with the assumption of a single pore surface charge density: E_v for anodal transport is plotted against E_v for cathodal transport (eq 10). Key: (circles) urea; (squares) mannitol. Each data point represents the mean and standard deviation of $n \geq 3$.

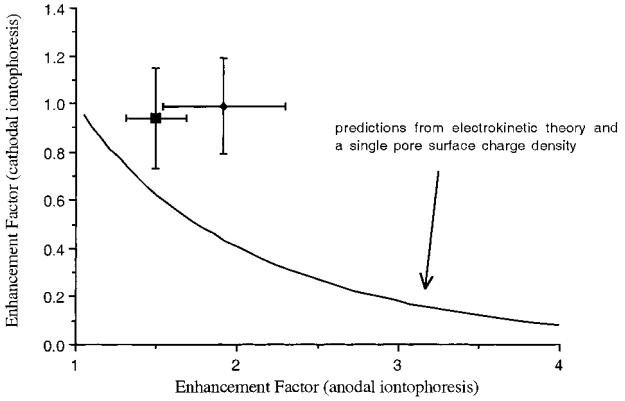

Figure 2—Relationship between cathodal and anodal electroosmotic flux enhancement for urea with HEM at 0.25 and 0.5 V dc. The curve represents predictions from electrokinetic theory with the assumption of a single pore surface charge density. Key: (square) 0.25 V; (diamond) 0.5 V. Each data point represents the mean and 90% confidence interval ($n = 6$).

theory. This result validates the theory and demonstrates that the Nuclepore membrane (a synthetic hydrophilic, poly(vinylpyrrolidone)-coated, polycarbonate membrane) can be modeled accordingly.

Figure 2 presents the low voltage (0.25 and 0.5 V dc) results with HEM (Study I) for urea. The changes in HEM electrical resistance in these low voltage experiments were generally <15% relative to the initial resistance before the iontophoresis runs; thus, these results may be interpreted as being essentially for the preexisting pores of HEM. Significant deviations can be noted between the predictions from electrokinetic theory with a single pore charge type assumption (the curve) and the experimental data with HEM (diamonds and squares). These deviations between the actual transport behavior of HEM and the theory predictions (in the positive direction from the theory) demonstrate that the preexisting pores of HEM, although predominantly negatively charged, may include some positively charged and/or some neutral pores.

Figure 3 summarizes the results of Study II where significant pore induction was involved and when the ac/dc superimposition protocols (0.5, 1.0, and 2.0 V dc plus ac) were employed to control the extent of pore induction ($E_{\Delta R}$) during electroosmosis transport experiments. In Figure 3, the enhancement factors due to electroosmosis (E_v) for urea were calculated from eq 8. An important outcome here is that the experimental results are consistent with theoretical predictions based on a single pore

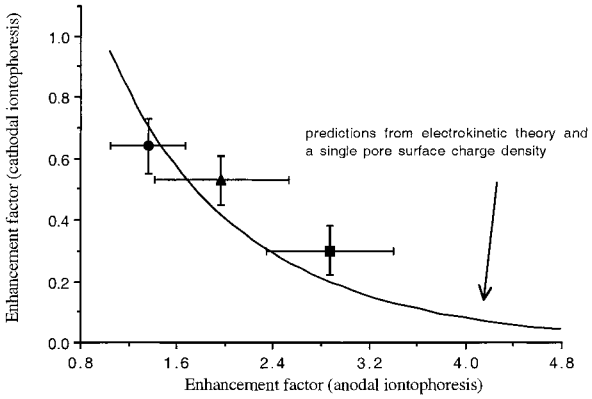

Figure 3—Relationship between transport enhancement due to cathodal and anodal electroosmosis for urea with HEM in 12.5 Hz square-wave ac and dc superposition iontophoresis experiments (Study II). The curve represents predictions from electrokinetic theory with the assumption of a single pore surface charge density. Key: (circle) 0.5 V dc plus 1–3 V ac; (triangle) 1.0 V dc plus 0–3 V ac; (square) 2.0 V dc plus 0–3 V ac. Each data point represents the mean and 90% confidence interval ($n = 7$).

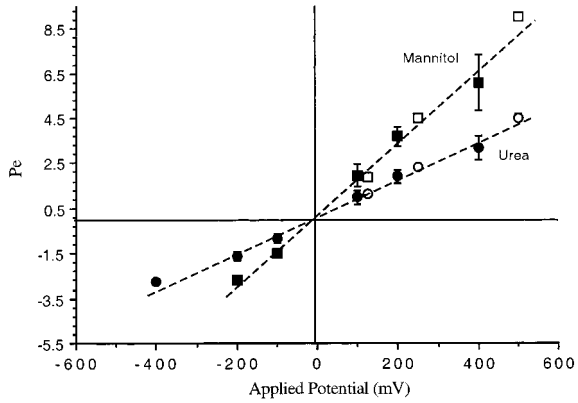

Figure 4—Relationship between the apparent *Pe* and the applied voltage for Nuclepore membranes. Key for closed (present study) and open (data from Peck et al.[2]): (circles) urea; (squares) mannitol. Each data point represents the mean and standard deviation of $n \geq 3$.

(charge) model, suggesting that newly induced pores are essentially all net negatively charged. Although preexisting positive and/or neutral pores may have still been present, the high $E_{\Delta R}$ values in these higher voltage experiments likely reduced their importance during 0.5, 1.0, and 2.0 V electroosmosis to a very small or negligible level.

Figures 4 and 5 present the relationships between the apparent *Pe* and the applied voltage obtained with the Nuclepore membrane and with HEM, respectively. The *Pe* values in Figure 4 were calculated from the urea and mannitol flux data with eq 10. Figure 4 includes data from a previous Nuclepore membrane study.[2] The linear relationship of *Pe* and the applied voltage and the close to zero ordinate intercept in Figure 4 for the Nuclepore membrane system are consistent with what would be expected from electrokinetic theory assuming an effective single pore charge type and density. The greater slope for the mannitol results than for the urea results reflects the smaller diffusion coefficient of mannitol (see eq 11). The *Pe* data in Figure 5 for HEM were determined from the urea flux and HEM electrical resistance data in Studies I and II by eqs 8 and 10. There are likely two separate relationships between *Pe* and the applied voltage in Figure 5. One relationship is for the situation at low voltages (0.25 and 0.5 V dc) where there are predominantly preexisting pores. The second relationship is for the situation at the higher voltages (0.5, 1.0, and 2.0 V dc for electroosmosis plus 0−3 V ac to maintain a high extent of pore induction) where

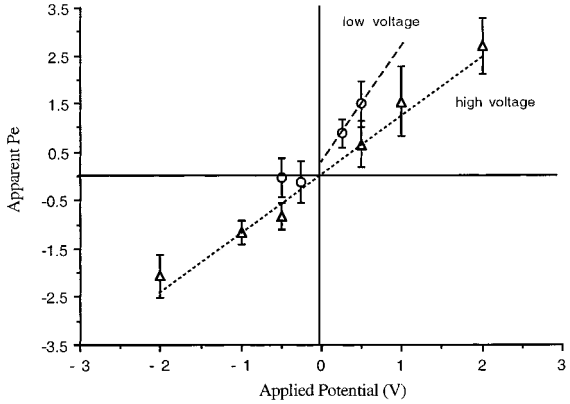

Figure 5—Relationship between the apparent *Pe* for urea as the permeant and the applied voltage with HEM. Key: (circles) low voltage, ≤0.5 V dc; (triangles) high voltages, ≥0.5 V dc plus 0–3 V ac. Each data point represents the mean and 90% confidence interval ($n \geq 6$).

the newly induced pores dominate electroosmosis. The steeper slope under the low voltage anodal conditions (0.25−0.5 V dc) relative to that under the higher voltage anodal and cathodal conditions (−2.0, −1.0, −0.5, 0.5, 1.0, and 2.0 V dc plus ac) is consistent with the interpretation that the effective pore charge density of the negatively charged induced pores is less than that of the preexisting pores. This interpretation is in agreement with the previously observed smaller than expected (based on low voltage studies) electroosmotic enhancement with 2.0 V dc iontophoresis.[3] The approximately linear relationship in Figure 5 for the low voltage anodal electroosmosis without pore induction (0.25−0.5 V dc) is consistent with what will be expected from the electrokinetic theory and the preexisting pores being dominated by negatively charged pores. The deviation from this linear relationship of the data for cathodal iontophoresis (especially at −0.5 V) is a departure, however, from the idea of preexisting pores being only negatively charged (as was also concluded from the data in Figure 2). This low voltage behavior contrasts with the high voltage results (of newly induced pore) that are essentially linear in Figure 5 in both the anodal and cathodal regions and consistent with the induced pores being essentially only negatively charged.

An alternate explanation for the lower slope of the high voltage data in Figure 5 might be based on pore sizes of the newly induced pores being significantly different from those of the preexisting pores. However, electroosmotic flux ratios of urea and mannitol determined as part of the present study at ≥1.0 V have yielded (from the hindered transport theory) pore sizes comparable to those found in similar experiments conducted at low voltages (∼8 Å). Therefore, it appears unlikely that pore size differences can contribute importantly to the slope differences in Figure 5.

Although the results from the higher voltage cathodal/anodal HEM electroosmosis studies are consistent with a model based on only negatively charged pores, one obviously needs to be cautious in making any detailed mechanistic conclusions. Considering the complicated morphology of the stratum corneum, it is only safe to state that HEM electroosmosis behavior under these circumstances is effectively equivalent to that of a membrane with only negatively charged pores. Nonetheless, this information is quite useful in assessing and in correcting for electroosmosis effects in various iontophoretic situations involving HEM.

Analysis of the Water/Urea Dual-Permeant Data—The passive permeability coefficient ratio, tritiated water-to-urea, for the Nuclepore membrane (average ± SD: ratio

$= 2.1 \pm 0.2$, $n = 5$) is consistent with what would be expected (ratio = 1.8) from the urea and water molecular diffusion coefficients with a small correction arising from hindered diffusion effects and with there being no significant difference between the diffusion coefficients (or the effective diffusion coefficients) of ordinary water and tracer tritiated water under the present experimental conditions.[12,14,15] During 0.75 V anodal dc iontophoresis with the Nuclepore membranes, water and urea fluxes were essentially the same at the same donor concentration (permeability coefficients of urea and water $= 1.1 \pm 0.4 \times 10^{-5}$ and $1.2 \pm 0.3 \times 10^{-5}$ cm/s, respectively, average ± SD, $n = 3$), demonstrating that the water velocity was essentially the same as the average velocity for urea and that the electroosmotic transport was at the convection limit.[2]

Tritiated water has been used to study the barrier properties of skin[16–18] and the electroosmosis behavior of skin.[19] The study of water transport across HEM during iontophoresis was expected to provide some independent insights into the mechanism of electroosmosis because liquid water is the carrier in electroosmotic transport. In the present study, the water passive permeability coefficients for HEM (average ± SD: $1.2 \pm 0.3 \times 10^{-6}$ cm/s, $n = 9$; HEM electrical resistance $= 30 \pm 19$ kΩ cm^2, ranging from 12 to 74 kΩ cm^2) are consistent with the permeability coefficients in a previous study.[20] The relatively high passive permeability coefficients of water for HEM compared with that of urea may be due to the accessibility of water to both the pore and the lipoidal pathways of HEM. This hypothesis is supported by present and previous studies: (a) the transport of water across HEM in the present study does not demonstrate an inverse proportionality between HEM permeability (P) and HEM electrical resistance (R) (slope of log P versus log R plot $= -0.26$ with $r^2 = 0.293$) as observed for urea in the present study and literature;[3,5,21] (b) the diffusional activation energy for water transport in HEM was reported to be ~17 kcal/mol,[22] which contrasts with the value of 7 kcal/mol for the transport of polar permeants across HEM[21] and the value (~4 kcal/mol) for unhindered diffusion in an aqueous medium; and (c) transport of water across lipid bilayers has been observed to be much higher than that for polar nonelectrolytes and ions.[23]

No significant difference between the transport rates of water across HEM during 0.25 V dc iontophoresis and during passive diffusion was found [ratios of iontophoretic flux to passive flux during anodal and cathodal iontophoresis (average ± SD) were 1.08 ± 0.05 ($n = 3$) and 1.03 ± 0.07 ($n = 3$), respectively]. This result is direct evidence of the lipoidal pathway being the dominant transport pathway for water in HEM in the absence of pore induction (i.e., low voltage iontophoresis). Under higher voltage conditions (e.g., a prepulse of 4.0 V dc for 1 min followed by 2.0 V dc iontophoresis) when significant pore induction occurred ($E_{\Delta R} > 10$), the pore pathway became dominant for water transport across HEM.

The permeability coefficient of the pore pathway for water in passive diffusion was estimated by the urea passive transport data of each individual HEM sample and eq 7:

$$P^{\text{pore}}_{\text{water,p}} = \frac{H_{\text{water}} D_{\text{water}}}{H_{\text{urea}} D_{\text{urea}}} P_{\text{urea,p}} \qquad (15)$$

The permeability coefficient of the pore pathway in HEM for water during 2.0 V dc iontophoresis was estimated by a parallel pore and lipoidal pathway model:

$$P^{\text{pore}}_{\text{water,i}} = P^{\text{total}}_{\text{water,i}} - (P^{\text{total}}_{\text{water,p}} - P^{\text{pore}}_{\text{water,p}}) \qquad (16)$$

where the subscripts "p" and "i" represent the passive and the iontophoresis experiments, respectively; the superscripts "*total*" and "*pore*" represent the total permeability coefficient and the permeability coefficient of the pore pathway, respectively; and $P_{\text{urea,p}}$ is the experimental permeability coefficient for urea in passive transport. Equations 15 and 16 assume (a) that transport via the lipoidal pathway is the same in passive transport and during iontophoresis, (b) an effective pore radius of 12 Å (the average R_p value deduced in the present passive transport experiments in the *Analysis of the Urea/Mannitol Dual-Permeant Data* section) for the calculation of H_{water} and H_{urea}, and (c) independent lipoidal and pore pathways in HEM. It should be noted that, with eq 16, water transport via the lipoidal pathway was generally small compared with the total iontophoretic flux of water with the application of 2.0 V dc.

With eq 16 and correcting for pore induction with eq 8, the enhancement due to electroosmosis (E_v) for water was estimated to be 2.0 ± 0.7 (average ± SD, $n = 5$) during 2.0 V anodal electroosmosis and 0.4 ± 0.2 (average ± SD, $n = 5$) during cathodal electroosmosis. This result corresponds to water Pe of ~1.6 and −1.5 and apparent water volumetric flow rates (ϵWvA) of $6 \pm 3 \times 10^{-6}$ and $-5 \pm 2 \times 10^{-6}$ cm^3/s across 1 kΩ cm^2 HEM at 2.0 V dc (average ± SD, $n = 5$; calculated using eq 4 and the experimental water P values normalized to 1 kΩ cm^2 HEM) during anodal and cathodal iontophoresis, respectively. These results are consistent with significant convective solvent flow and a net negatively charged HEM under the 2.0 V dc conditions. With the assumption that water and urea follow the same pore pathway in HEM and a R_p value of 8 Å (the average R_p calculated from the present anodal electroosmosis experiments in the *Analysis of the Urea/Mannitol Dual-Permeant Data* section), the water velocity and the velocity of urea convective transport across the pore pathway were compared using eq 4. The ratio of water velocity to that for urea was estimated to be ~1.2. This close-to-unity ratio is direct evidence of water being the carrier for urea during electroosmosis. The slightly higher water velocity than that for urea estimated here (the factor of 1.2) is consistent with differential hindered transport effects if pore radii of ~6 Å is assumed. This 6 Å value is of the same order of magnitude as that deduced from urea/mannitol flux data (i.e., the average R_p of 8 Å). Results from this analysis independently show that water is the carrier in electroosmotic transport during iontophoresis.

Conclusion

By conducting both anodal and cathodal electroosmosis experiments with polar nonionic permeants, the predictivity of a model of pores with a single charge was examined for the preexisting pores and the newly induced pores (due to applied electric fields) in HEM. The electrokinetic model was first checked with a synthetic polycarbonate membrane system, and the experimental results with this model membrane system were consistent with what would be expected of pores possessing only negative charges. Results from experiments with HEM at low voltages (little or no electroporation) showed that although negative pore charges dominate, there were effectively some positive or positive and neutral pores at neutral pH. At higher voltages (>1.0 V), where new pores dominated, there seemed to be contributions only from negatively charged pores at neutral pH. Results from the water transport experiments provide further support that water is the carrier in electroosmotic transport during iontophoresis.

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