Published in final edited form as:

J Pharm Sci. 2008 December ; 97(12): 5186–5197. doi:10.1002/jps.21368.

Mechanistic Study of Electroosmotic Transport Across Hydrated Nail Plates: Effects of pH and Ionic Strength

JINSONG HAO and S. KEVIN LI

Division of Pharmaceutical Sciences, College of Pharmacy, University of Cincinnati, Cincinnati, Ohio 45267

Abstract

The objective of this study was to investigate the effects of pH and ionic strength on electroosmotic transport in transungual iontophoresis. Transungual iontophoretic transport of model neutral permeants mannitol (MA) and urea (UR) across fully hydrated human nail plates in phosphate-buffered saline of different pH and ionic strengths were investigated *in vitro*. Two protocols were involved in the transport experiments with each protocol divided into stages including passive and iontophoresis transport at 0.1 and/or 0.3 mA. Nail plate electrical resistance and water uptake of nail clippings were measured at various pH and ionic strengths. In the pH study, electroosmosis enhanced the anodal transport of MA at pH 9 and cathodal transport at pH 3. The Peclet numbers of MA were more than two times higher than those of UR under these conditions. No significant electroosmosis enhancement was observed for MA and UR at pH 5. In the ionic strength study, a decrease in solution ionic strength from 0.7 to 0.04 M enhanced electroosmotic transport. Nail electrical resistance increased with decreasing the ionic strength of the equilibrating solution, but reached a plateau when the ionic strength was less than approximately 0.07 M. Solution pH and ionic strength had no significant effect on nail hydration. Under the studied pH and ionic strength conditions, the effects of electroosmosis were small compared to the direct-field effects in transungual iontophoretic transport of small to moderate size permeants.

Keywords

transungual; iontophoresis; human nail plate; electroosmosis

INTRODUCTION

Many nail diseases are difficult to cure owing to the intrinsic nail barrier and the “deep-seated” target site underneath the nail plate.¹ Treatment of resistant nail diseases remains challenging despite the efforts pharmaceutical scientists have put on improving the therapy.^{2–10} Iontophoresis is a method to deliver a compound across a membrane with the assistance of an electric field.¹¹ During iontophoresis, transport enhancement of neutral permeants can be due to electroosmosis while transport enhancement of ionic permeants is a result of both electroosmosis and electrophoresis. In transungual drug delivery, iontophoresis has been demonstrated as a promising method to enhance drug delivery through nail plates.^{12–14} In our previous study,¹⁵ electrophoresis (direct-field effect) was shown to be the dominant driving force in transungual iontophoretic transport of small permeants across fully hydrated nail plates with small contribution from electroosmosis. The average contribution of electroosmosis to

transport enhancement was less than 10% for small permeants at pH 7.4 and ionic strength of 0.16 M.

The role of electroosmotic flow in transdermal iontophoresis has been thoroughly investigated,^{16,17} but is not mechanistically studied in transungual iontophoresis. Electroosmosis is related to both the net charge of the transport pathway in a membrane and the electric field across the membrane. The magnitude of electroosmotic flux enhancement is a function of permeant physicochemical properties and membrane and solution electrical properties. The nail plate is primarily composed of highly disulfide-linked keratins and thus has an isoelectric point (pI) of 4.9–5.4.⁵ The hydrated nail plate behaves like a hydrogel with a network of aqueous pores. Changes in solution pH and ionic strength in transungual iontophoresis are expected to affect pore charge and ion distribution in the pores in the nail plate and subsequently electroosmosis contribution to permeant transport during iontophoresis. Although transungual flux enhancement due to electroosmosis was previously shown to be marginal at pH 7.4 and ionic strength of 0.16 M,¹⁵ the effect of electroosmosis can be significant under different pH and ionic strength conditions. Electroosmosis can enhance or retard drug delivery depending on the conditions during iontophoresis. Understanding the extent of electroosmosis, its contribution to iontophoretic transport, and its influencing factors will allow effective development of formulations for transungual iontophoretic delivery. For example, what is the extent of transungual electroosmosis (compared with electrophoresis) under different pH and ionic strength conditions commonly encountered in pharmaceutical settings? Will the contribution of electroosmosis to iontophoretic transport be important at a certain pH and ionic strength?

The objectives of the present study were to investigate the effects of solution pH and ionic strength on electroosmosis across hydrated nail plates and to quantify transungual electroosmotic transport. As hydration would affect the nail effective pore size and thus transungual transport, nail hydration study was first performed to assess the pH and ionic strength effects upon nail water uptake. In the transport experiments, two polar neutral permeants of different molecular sizes, mannitol (MA) and urea (UR), were chosen as the model permeants. Anodal (anode in the donor) and cathodal (cathode in the donor) iontophoretic transport experiments of MA and UR were conducted with constant direct current at 0.1 and 0.3 mA. The conditions examined were pH 3–9 and ionic strengths of 0.04–0.7 M. The effects of pH and ionic strength on transungual electroosmotic transport and their importance in transungual iontophoretic delivery were determined. Nail electrical resistance in solutions of different pH and ionic strengths was also measured.

EXPERIMENTAL SECTION

Materials

Phosphate-buffered saline (PBS) of pH 7.4 was prepared by dissolving PBS tablets (Sigma-Aldrich, St. Louis, MO) in deionized, distilled water. The total ion concentration of PBS solution was approximately 0.15 M, consisting of 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride. Total ion concentration was defined as the sum of the concentrations of all the components in the solution. Solutions of pH 3, 5, and 9 were prepared by pH adjustment of adding concentrated sodium hydroxide or hydrochloric acid solutions to PBS. Solutions of 0.01, 0.03, 0.06, 0.09, 0.3, 0.45, and 0.6 M total ion concentrations were prepared by either diluting 0.15 M PBS with deionized, distilled water or adding an appropriate number of PBS tablets into 0.15 M PBS. Sodium azide (99% purity, Acros, Morris Plains, NJ) of 0.02% (w/v) was added as a bacteriostatic agent. The solution pH and conductivity were checked with a pH/conductivity meter (PC510, Oakton Instruments, Vernon Hills, IL). Unless otherwise stated, the solutions are described by their ionic strengths rather than total ion concentrations in this paper. For example, the ionic strengths of the

solutions were calculated to be 0.16, 0.16, and 0.18 M for 0.15 M pH 3, 5, and 9 solutions and 0.04, 0.16, and 0.7 M for 0.03, 0.15, and 0.6 M pH 7.4 solutions, respectively. ^3H -mannitol (1-3H(N)-, 10–30 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). ^{14}C -urea (50–60 mCi/mmol) was purchased from Moravak Biomaterials and Radiochemicals (Brea, CA). Both radiolabeled chemicals had purity of at least 97%. All materials were used as received.

Preparation of Nail Samples

Human fingernail plates (male, age 68–83) were obtained from Science Care Anatomical (Phoenix, AZ). The frozen nail plates were thawed in PBS solutions at room temperature. Adhering tissues on the nail plates were removed with a pair of forceps. The nails were then rinsed with and soaked in PBS solutions for 24 h to allow complete hydration before the transport experiments. The thickness of the hydrated nail plates was from 0.4 to 0.7 mm measured using a micrometer (Mitutoyo, Kawasaki, Japan) at the end of the experiments. Nail clippings were obtained from healthy volunteers (male and female, age 30–50) using nail clippers. The nail clippings were cleaned with a pair of forceps, rinsed with PBS solutions, blotted dry with Kimwipes®, and air dry before the hydration study. The use of human tissues was approved by the Institutional Review Board at the University of Cincinnati, Cincinnati, OH.

Hydration of Nail Clippings

The clean nail clippings were weighed and then soaked in 1 mL of PBS solutions of different pH and ionic strengths contained in a screw-capped vial at room temperature ($20 \pm 2^\circ\text{C}$) for 48 h. After hydration, the nail clippings were removed, blotted dry with Kimwipes®, and quickly weighed (i.e., wet weight). The wet nail clippings were allowed to oven dry at 60°C for 24 h to constant weights (i.e., dry weight). The percentage water content in the nail clippings was expressed as the percentage of decrease in the weight of nail clippings from hydration to after oven dry divided by the dry weight, that is $(\text{wet weight} - \text{dry weight}) / \text{dry weight} \times 100\%$.

Transport Study Protocols

In our preliminary studies, it was found that the nail plates, even from the same human donor, showed nail-to-nail variability in permeability results. Our previous study¹⁵ also demonstrated that the fully hydrated nail plates were stable for up to 3 months in transport experiments. Therefore, the same nail plates were used consecutively in all transport experiments in the present study, and a dual permeant strategy (i.e., concurrent MA and UR delivery) was employed. Together, this experimental design minimized the influence of inter-sample variability on data interpretation. With the trace amounts of radiolabeled permeants used in the experiments, the presence of one permeant was found not to affect the results of the other (unpublished data). Two protocols were involved in the present transungual iontophoresis study: Protocols 1 and 2. Protocol 1 was designed to study the effect of solution pH (i.e., pH 3, 5, and 9) at relatively constant ionic strength (0.16, 0.16, and 0.18 M for pH 3, 5, and 9, respectively) on electroosmotic transport. Protocol 1 included five consecutive stages: passive transport at pH 7.4 (P1), second passive transport at pH 3, 5, or 9 (P2), anodal iontophoresis of 0.3 mA at the same pH as in P2, cathodal iontophoresis of 0.3 mA at the same pH as in P2, and third passive transport at pH 7.4 (P3). Protocol 2 was to investigate the effect of solution ionic strength (i.e., 0.04 and 0.7 M) at constant pH of 7.4 on electroosmotic transport. Protocol 2 had four consecutive stages: passive transport at 0.16 M (P1), second passive transport at ionic strength of 0.04 or 0.7 M (P2), anodal iontophoresis of 0.3 mA at 0.7 M or 0.1 mA at 0.04 M, and third passive transport at 0.16 M (P3). Ionic strength less than 0.04 M was not studied because of the difficulty in maintaining constant ion concentrations in the donor and receptor solutions under such conditions during iontophoresis. In addition, it was found in the

present study that the nail counterions control the nail electrical properties below 0.07 M, so the effect of ionic strength on electroosmosis is expected to be minimal in this ionic strength region. On the other hand, ionic strength higher than 0.7 M was not investigated because most drug compounds for transungual delivery have solubilities less than this value. Experiments of pH 7.4 and ionic strength 0.16 M at 0.1 and 0.3 mA were performed previously¹⁵ and therefore were not repeated in the present study. Figure 1 summarizes the experiments and treatment protocols of the nail plates. All the nail plates were subjected to treatment of Protocol 1 first and then Protocol 2. A passive transport experiment of MA and UR at pH 7.4 and 0.16 M was also carried out with each nail plate before Protocol 1. However, these results were not included in the analysis because the nail plates in the first passive transport experiment usually had lower permeability coefficients than those in the later stages, possibly due to incomplete nail hydration.¹⁵

Transport Study Experiments

Transport experiments were conducted similar to those described previously.¹⁵ After 24 h of hydration in PBS, the nail plate was mounted between two side-by-side diffusion half-cells (Dana Enterprise, West Chester, OH) with a custom-made silicone nail adapter. The adapter with an opening of 0.64 cm² in the center was constructed with silicone elastomer (MED-6033, NuSil Silicone Technology, Carpinteria, CA) to fit the nail plate curvature, and was tested to show no noticeable permeant-to-adapter binding. The dorsal side of the nail plates faced the donor chamber, and the ventral side faced the receptor chamber. The diffusion cells have an effective diffusion area of approximately 0.64 cm² and a cell volume of 2 mL. The receptor solutions were 2 mL PBS solutions of the studied pH and ionic strength, and the donor solutions were the same 2 mL solutions but containing trace amounts of radiolabeled permeants (2 μ Ci of ³H-MA and 1 μ Ci of ¹⁴C-UR, i.e., approximately 0.1 and 20 nmol, respectively) added immediately before the transport experiments. The symmetric donor and receptor system avoided a time-dependent pH or ionic strength gradient in the nail that could make mechanistic interpretations of the data difficult due to the transport of the buffer ions and NaCl across the membrane. In addition, the symmetric and equilibrium conditions allowed the examination of the maximum effects of pH and ionic strength. The transport experiments were performed at room temperature (20 $\pm$ 2°C) and the durations of the experiments were 15 h for iontophoresis and 46–48 h for passive transport. Between the transport experiments of each stage in the protocols, the diffusion cells on both sides of the nail plates were rinsed with fresh solution of the pH and ionic strength to be studied at least three times by replacing the donor and receptor solutions over at least 12 h unless otherwise stated. Anodal iontophoretic transport experiments were immediately conducted at the end of the second passive permeation experiments without replacement of donor solutions. In anodal iontophoretic transport experiments at 0.04 M, the donor solution was replaced with fresh donor solution every 3 h to prevent depletion of ions during iontophoresis. Cathodal iontophoretic transport experiments were performed at least 12 h after the end of anodal iontophoretic transport experiments with fresh donor and receptor solutions. The third passive permeation experiments after iontophoresis were performed with fresh donor solutions immediately after rinsing both diffusion cells with PBS at the end of the iontophoresis experiments. A constant direct current was applied with a constant current iontophoretic device (Phoresor II Auto, Model PM 850, Iomed, Inc., Salt Lake City, UT) using Ag and Ag/Cl as the driving electrodes. The voltage drop across the nail plates was monitored using a multimeter (Fluke 73III, Everett, WA) during iontophoresis and the electrical resistance of the nail plates was measured using Ohm's law. The electrical resistance of the nail plates before the iontophoresis experiments was also measured by applying 0.1 mA current across the nail for 30 s and using Ohm's law. Ten microliters of donor solution and 1 mL of receptor solution were withdrawn at predetermined time intervals and 1 mL fresh PBS was added to the receptor to maintain a constant volume in the receptor. The samples were mixed with 10 mL of liquid scintillation cocktail (Ultima Gold™, PerkinElmer Life and Analytical Sciences,

Shelton, CT) and assayed by a liquid scintillation counter (Beckman Counter LS6500, Fullerton, CA).

The cumulative amount of permeant transported through the nail plate (ΔQ) was plotted against time (t), and the steady-state flux of permeant (J) was calculated from the slope of the linear portion of the plot.

$$J = \frac{\Delta Q}{A \Delta t} \quad (1)$$

where A is the diffusional surface area and $\Delta Q/\Delta t$ is the slope of the cumulative amount against time plot. The steady-state permeability coefficient, P (cm/s), is defined as the flux divided by the concentration of permeant in the donor chamber. Enhancement factor (E_v) is defined as the ratio of the permeability coefficient during iontophoresis to that of passive transport.

Resistance Measurements

The effects of pH and ionic strength on nail resistance were determined in solutions of 0.01–0.7 M at pH 5, 7.4, and 9. The experimental setup was the same as that in the transport experiments except that a fixed resistor and a variable resistor were connected in series and in parallel with the diffusion cells, respectively, to form the electric circuit. The fixed resistor had a resistance of 0.98 k Ω and the resistance of the variable resistor was from 0 to 4.6 k Ω . Before the resistance measurements in each solution, the diffusion cells were rinsed with fresh solution of the pH and ionic strength to be studied, and the nail plates were equilibrated with the solution for 24 h. Electric current of 0.1, 0.2, and 0.3 mA was then applied across the system with the constant current iontophoretic device in the anodal configuration for 30 s. The voltage across each resistor was measured with a multimeter. The total resistance of the nail and solutions were determined by plotting the measured voltage against electric current across the diffusion cell system using Ohm's law. The solution resistance was measured using the same experimental setup but without the nail plate sandwiched between the diffusion cells. The nail resistance was calculated by subtracting the solution resistance from the total resistance.

Theory and Equations

The steady-state iontophoretic flux ($J_{\Delta\psi}$) of a permeant through a homogeneous porous membrane can be described by the modified Nernst–Planck model as described previously:^{18,19}

$$J_{\Delta\psi} = -\varepsilon_p \left\{ HD \left[\frac{dC}{dx} + \frac{CzF}{R_{\text{gas}}T} \frac{d\psi}{dx} \right] \pm WvC \right\} \quad (2)$$

where ψ is the electric potential in the membrane, F is the Faraday constant, R_{gas} is the gas constant, T is the temperature, v is the average velocity of the convective solvent flow, ε_p is the combined porosity and tortuosity factor of the membrane, and C , x , z , D are the concentration, position in the membrane, charge number, and diffusion coefficient of the permeant, respectively. H and W are the hindrance factors for Brownian diffusion and for pressure induced parabolic convective solvent flow, respectively.

For the iontophoretic transport of a neutral permeant, the Peclet number (Pe) can be determined from the enhancement factor by:²⁰

$$E_v = \frac{Pe}{1 - \exp(-Pe)} \quad (3)$$

Assuming a single pore size and cylindrical pore geometry in the porous membrane, Pe is related to v by:²¹

$$Pe = \frac{W_v(\Delta x)}{HD} \quad (4)$$

where Δx is the thickness of the membrane. When the electrical double layer thickness $1/\kappa \ll$ pore radius R_p the velocity of convective solvent flow due to electroosmosis can be expressed as:²²

$$v = \left(\frac{\sigma}{\kappa\eta} \right) \left(\frac{\Delta\psi}{\Delta x} \right) \quad (5)$$

where σ is the surface charge density, η is the viscosity of the bulk solution, and:

$$\kappa = \left(\frac{2F^2 I_s}{\epsilon R_{\text{gas}} T} \right)^{1/2} \quad (6)$$

where I_s is the ionic strength in molar and ϵ is the permittivity. The surface charge density, σ , is related to the fraction of the ionized functional groups on the surface and is a function of pH.

Statistical Analysis

Student's *t*-test for two-tailed distribution was used to evaluate the significance of transport and hydration parameters between different groups of various pH and ionic strengths. Differences were considered to be significant at a level of $p < 0.05$.

RESULTS

Hydration Studies

Nail clippings were equilibrated in solutions of different pH and ionic strengths for 48 h before subjected to oven drying in the present study. The hydration results are shown in Figure 2. There was no significant difference in water content among each treated group ($p > 0.05$). The average water content in the nail clippings equilibrated in different pH and ionic strengths was $41 \pm 5\%$. This value was similar to that obtained previously at pH 7.4 and 0.16 M of PBS ($36 \pm 5\%$). Further, experiments were performed to provide a closer examination of the pH or ionic strength effect on nail hydration for the two cases showing the largest water content difference in Figure 2 (pH 7.4 and 9). In these nail hydration experiments, the nail clippings were equilibrated in PBS at pH 7.4 (0.16 M), weighed, dried, and weighed and then equilibrated in PBS at pH 9, weighed, dried, and weighed again. These nail clippings showed essentially the same water content at pH 7.4 and 9 ($p > 0.05$). It should be noted that the amounts of salts retained in the sample after water evaporation should not affect the results with the PBS solutions used. A preliminary study has shown no significant difference in water content between nail water uptake in deionized water and in PBS (unpublished data), supporting the argument that the error from the weight of the salts is negligible in this type of studies.

The results in the present study indicate that solution pH and ionic strength have no effect on the hydration of the nail clippings. The acidic and basic solutions (pH 3 and 9) did not cause degradation of nail keratins. The results also imply that the transungual transport experiments in the present study under different pH and ionic strength conditions would not be due to nail hydration alteration. Factors other than hydration likely contributed to the differences in transungual transport observed at different pH and ionic strengths during iontophoresis.

Effect of pH on Iontophoretic Transport

Figure 3 presents the permeability coefficients of the nail plates for MA and UR in the passive transport experiments P1, P2, and P3 under Protocol 1. The data show relatively small pH

effects upon the passive diffusion of MA and UR across the hydrated nail plates. In addition, the relatively constant passive permeability coefficient ratios of MA and UR at different pH suggest essentially the same hindrance transport in the nail under these conditions (i.e., the transport pathways have essentially the same effective pore size). This is consistent with the results in the present nail hydration study (Fig. 2). The data also demonstrate reversibility and stability of the nail after iontophoresis over the course of the study.

Figure 4 is a plot of the iontophoretic permeability coefficients of MA and UR versus pH. The results at pH 7.4 in our previous study¹⁵ are included in the figure for comparison. When the pH of PBS was increased, the anodal permeability coefficients of both permeants increased while the cathodal permeability coefficients decreased. At around pH 5–6, anodal transport was essentially the same as cathodal transport for MA and UR; the permeability coefficients overlap at 5×10^{-8} and 5×10^{-7} cm/s for MA and UR, respectively. These values are consistent with the passive permeability coefficients of MA and UR.

To assess the contribution of electroosmosis, the Peclet numbers were determined using the enhancement factors of the permeants and Eq. (3), assuming that there was no electric field-induced alteration of the nail barrier. The Peclet number describes the contribution of convective transport such as electroosmosis²⁰ and allows a direct comparison of the electroosmosis data of MA and UR at different pH and ionic strengths. The Peclet numbers are presented in Figure 5. In anodal iontophoresis, MA transport was significantly enhanced at pH 9 and the Peclet number was 3.8 ($p < 0.05$). The Peclet number of UR under the same condition was 1.1 and did not show statistically significant enhancement over passive transport ($p > 0.05$). At pH 5, the Peclet numbers of MA and UR were close to zero, indicating minimal iontophoresis enhancement for both permeants. At pH 3 during anodal iontophoresis, the Peclet numbers for both permeants had negative values, suggesting that electroosmosis was from the cathode to anode, opposite to the direction of passive transport from the donor to receptor.

The results in cathodal iontophoresis were the opposite of those in anodal iontophoresis. The Peclet numbers of MA and UR at pH 3 were 2.5 and 1.1, respectively. Enhancement due to cathodal iontophoresis at pH 3 was statistically significant for MA ($p < 0.05$) but not for UR ($p > 0.05$). The MA and UR Peclet numbers at pH 3 were also comparable to those obtained in our previous anodal iontophoresis study at pH 7.4,¹⁵ suggesting that the magnitude of the electroosmotic solvent flow during cathodal iontophoresis at pH 3 was similar to that during anodal iontophoresis at pH 7.4, albeit that they were in opposite directions. At pH 5, the permeability coefficients were not statistically different from those in passive transport ($p > 0.05$) with little or no contribution of electroosmosis. Both permeants in cathodal iontophoresis at pH 9 had negative Peclet numbers, and the direction of electroosmotic solvent flow was from the anode to cathode.

Effect of Ionic Strength on Anodal Iontophoretic Transport

The passive permeability coefficients of MA and UR obtained in Protocol 2 are presented in Figure 3. Consistent with the results in the nail hydration study (Fig. 2), the effect of ionic strength upon the porosity of the nail plate and hence its passive permeability for the permeants is minimal (Fig. 3). The P1, P2, and P3 passive transport data also show general reversibility and stability of the nail after iontophoresis over the course of the ionic strength experiments in Protocol 2. Figure 6 presents the effect of solution ionic strength on the Peclet numbers in the anodal iontophoresis experiments. The results obtained in a previous study¹⁵ are also provided in the figure. When the ionic strength was 0.04 M during 0.1 mA anodal iontophoresis, the Peclet numbers of both MA and UR were higher than those at 0.16 M and 0.1 M. Following the same trend, the Peclet numbers of both permeants at 0.7 M ionic strength during 0.3 mA anodal iontophoresis were lower than those at 0.16 M and 0.3 M. These results indicate that

electroosmotic transport of the neutral permeants increased when the solution ionic strength decreased.

The mechanistic analysis of electroosmosis in constant direct current iontophoresis should take into account of the different voltage applied across the nail plate at different solution ionic strengths because electroosmotic transport is voltage dependent (see Eq. 5). To compare electroosmosis at the same applied voltage, the Peclet numbers normalized by the voltage across the nail plates are calculated. The normalized Peclet number increased approximately four times when the ionic strength decreased from 0.7 to 0.04 M. This trend is consistent with electrokinetic theory and will be discussed later. The normalized Peclet number of MA was about three times that of UR at all the solution ionic strengths studied. The higher normalized Peclet numbers of MA compared to those of UR were again attributed to the difference in their diffusion coefficients.

Electrical Resistance Measurements

Figure 7 shows the relationship between the logarithm of the electrical resistance of the nail plates and the logarithm of the ionic strength of the solution. Before the measurements at pH 9 and at the end of the resistance study, measurements were repeated at pH 7.4 and 0.15 and 0.04 M, respectively, to check for data reproducibility (the crosses in the plot). The electrical resistance of the nail depends on the concentrations of both the counterions to the surface of the pore wall and the bulk ions in the pores of the nail. In the present study, the ion concentration of the solution is approximately equal to the ionic strength of the solution, so these two terms can be used interchangeably. At low ionic strength such as below 0.07 M, the electrical resistance of the nail plate was relatively constant. It is believed that, at the low ionic strength, the solution resistance in the pores was dominated by the counterions of the fixed charges on the pore walls in the nail. Thus, the resistance of the nail plate was relatively independent of the ionic strength of the bulk solution in this region. When the ionic strength was increased to higher than 0.07 M, the resistance began to decrease, giving a slope of -1 in the log-log plot. The bulk ions in the pores became the dominant conducting ions above this ionic strength. The resistance of the nail plates at pH 5, 7.4, and 9 were not statistically different.

DISCUSSION

Electroosmosis

In general, the fixed charges on a pore wall in a charged porous membrane create a distribution of charges (ions) in the surrounding aqueous solution due to the requirement of electroneutrality.²³ This leads to the formation of an electrical double layer and an excess of counterions in the adjacent solution of the pore wall in the pores of the membrane. The counterions are distributed in the double layer extending a distance from the charged surface and is related to the Debye-Huckel thickness $1/\kappa$. When an electric field is applied across the membrane (parallel to the charged surface), forces are exerted on the ions in the double layer in the pores. The ions in the double layer move under the influence of the electric field, carrying the solvent with them by momentum. Electroosmotic flow is formed. The thickness of the electrical double layer in the pores is related to the ionic strength of the solution, and the hydrodynamics of the solvent associated with the double layer is related to the pore radius. Because the solvent flow velocity in convective transport is zero (in stationary) at the pore wall and increases to a maximum at the pore center, when the ionic strength increases and the $1/\kappa$ values decreases, the solvent flow velocity decreases. As a result, electroosmosis is a function of the pore charge, pore size, solution ionic strength in the pore, and the applied voltage.^{17, 24} In electroosmosis dominant transport, the flux of a permeant is directly related to the velocity of the electroosmotic flow and independent of the permeant diffusion coefficient. Therefore,

electroosmotic transport enhancement is a function of the molecular size of the permeant. Flux enhancement increases when the diffusion coefficient of the permeant decreases.²⁰

pH and Ionic Strength Effects on Electroosmotic Transport

The fully hydrated nail plate behaves like a hydrogel with aqueous solution filled pores. The keratins in the nail plate were reported to have pI around 4.9–5.4.⁵ Accordingly, the nail plate is expected to be essentially uncharged at pH 5 around its pI , net negatively charged above its pI at pH 7.4 and 9, and net positively charged at pH 3 below its pI . The findings in the present study (Figs. 4 and 5) are in agreement with the pI reported in a previous transungual iontophoresis study.¹³ When the nail plates were positively charged at pH 3, electroosmotic flow was from the cathode to anode, assisting cathodal iontophoresis. At pH 7.4 and 9, the nail plates were negatively charged and the convective solvent flow was from the anode to cathode, favoring anodal iontophoretic transport. Electroosmosis enhancement increased when pH was increased from 7.4 to 9 in anodal iontophoretic transport. No significant electroosmosis enhancement was observed at pH 5 when the pH approaches the pI of the keratins in the nail plates. Under this condition, the nail plates were essentially uncharged and no significant convective solvent flow was observed. Passive diffusion was the main transport mechanism of the neutral permeants during iontophoresis. In addition, the Peclet numbers of MA and UR also demonstrate that the contribution of electroosmosis was related to the molecular size of the permeant. Comparison of the MA and UR data under the same conditions during iontophoresis shows higher Peclet numbers of MA than those of UR in both anodal iontophoresis at pH 7.4 and 9 and cathodal iontophoresis at pH 3. This observation is consistent with the theory of electroosmotic transport²⁰ as the diffusion coefficient of UR is larger than that of MA.

In the ionic strength study, the nail plate is net negatively charged at pH 7.4. Assuming a constant surface charge density inside the “pores” of the nail plates and a pore radius sufficiently larger than the thickness of the double layer, the flow velocity is inversely proportional to the square root of the solution ionic strength in the nail (Eqs. 5 and 6). As a result, the solvent flow velocity as well as the Peclet number (Eq. 4) were expected to decrease by approximately a factor of five when the solution ionic strength increased from 0.04 to 0.7 M in the present study ($1/\kappa$ values decreased from around 2 to 0.4 nm). This is consistent with the experimental results: the Peclet numbers normalized by the voltage permeants obtained in the ionic strength experiments decreased about four times when the solution ionic strength increased from 0.04 to 0.7 M. An estimation of the surface charge density using the experimental data, hindered transport theory, and Eq. (5) suggests that the surface charge density of the pores in the nail plate at pH 7.4 is in the order of 10^{-4} C/m².

It should be noted that the increase in the Peclet number and electroosmosis contribution to iontophoretic transport is moderate even in the situation of low ionic strength such as 0.04 M ($Pe/V = 7.7$). The present nail electrical resistance data suggest the practical difficulties to further enhance electroosmotic flow by decreasing the ionic strength of the bulk solution equilibrating the nail. When the ionic strength decreased from 0.7 to around 0.07 M, the double-layer in the pores of the nail started to overlap. Under this condition, the pores were encompassed by the double-layer, and the ionic strength of the solution in the pores was mainly controlled by electroneutrality. A further decrease in the bulk solution ionic strength would not significantly affect the ionic strength in the pores. The number of ions in the pores would depend only on the charges on the pore surfaces, therefore, resulting in essentially constant ionic strength in the pores and electroosmosis. This relationship between the bulk ionic strength and the ionic strength of the solution in the pores was demonstrated in the present nail electrical resistance study (Fig. 7). The electrical resistance data of the nail show that this limiting value is around 0.07 M. Unexpectedly, this value is not significantly affected by solution pH, which

should affect the surface charges of the pores in the nail, possibly due to experimental variability such as nail-to-nail variability in the present study.

Transungual Iontophoretic Delivery

For transungual delivery of uncharged drugs, iontophoresis can moderately enhance the transport of neutral permeants with molecular size similar to MA via the mechanism of electroosmosis. The effects of pH on transungual transport of an uncharged drug compound have been previously examined.¹³ Electroosmotic transport can be optimized by adjusting the formulation factors like pH and ionic strength of the donor solution.^{17,25} In addition, the effect of electroosmosis is expected to be greater in the delivery of larger molecules or under higher electric current density.^{17,20} Unfortunately, it is difficult to significantly improve electroosmotic transport of neutral permeants by altering pH and ionic strength of a transungual delivery system as shown in the present study. In addition, transungual transport of large molecules is restricted by hindered diffusion across the small effective pore size of the nail.¹⁵ The use of higher electric current may also be difficult because of the limited surface area available on the nail. The small electrodes required to fit on the surface of the nail comfortably may not sustain the electrochemical reactions at high electric current in long transungual iontophoresis, and the resulting electric current density across the tissues in the nail bed is limited by skin/tissue tolerance.

For the delivery of ionic permeants with molecular size similar to MA such as ciclopirox, the effect of electroosmosis on iontophoretic transport under the conditions employed in the present study is relatively small compared to the theoretical 38-fold flux enhancement per voltage per charge due to electrophoresis according to the Nernst–Planck model.¹⁵ For example, at pH 7.4 and 3, the electroosmotic flow velocities are similar, but in opposite directions, with flux enhancement approximately three times over passive diffusion, which is less than 10% of the effect from electrophoresis (the direct-field effect) according to the Nernst–Planck theory.¹⁵ Increasing the pH from 7.4 to 9 enhanced electroosmosis by approximately 50%, which will still be relatively insignificant compared with the direct-field effect. A decrease in the ionic strength from 0.16 to 0.04 M at pH 7.4 would enhance electroosmosis by approximately two times, in which electroosmosis contribution could reach as high as 30%. Therefore, the contribution of electroosmosis is not significant to predominantly enhance or retard iontophoretic transport of small ionic permeants during iontophoresis. The results in the present study suggest that a transungual iontophoresis system should be tailored to the mechanism of electrophoresis in transungual delivery of small ionic compounds, irrespective of the electroosmotic effect at different pH and ionic strengths. For example, with a positively charged permeant of pK_a close to the pI of the nail, it would be effective to enhance iontophoretic transport by reducing the pH of the donor to sufficiently below the pK_a and utilize anodal iontophoresis despite the resulting unfavorable electroosmosis from the receptor to the donor. Overall, the utilization of electroosmosis to enhance transungual iontophoretic delivery is limited, and flux enhancement could be modified only to a small extent by changing the pH and ionic strength of the solution. It should also be pointed out that the present findings were obtained from fully hydrated nail and that electroosmosis can be affected by nail hydration. Thus, cautions must be excised if the data are to be extrapolated to the partially hydrated nail system.

CONCLUSION

The effects of pH and ionic strength on nail hydration, transungual electroosmotic transport, and nail electrical resistance were investigated. The solution pH and ionic strength in the present study had no significant effect on nail hydration and did not significantly alter passive transungual transport. During iontophoresis, transungual electroosmotic transport was pH

dependent, consistent with the existence of both proton donating and accepting functional groups in the nail plates and $pI \approx 5$. The net surface charge density of the nail plates increased when the solution pH moved away from the pI of the keratins in the nail plates. In addition to the effects of pH, the effects of solution ionic strength on transungual iontophoresis were also determined. This is the first study on the effects of ionic strength upon transungual electroosmosis. The results showed that transungual electroosmosis was affected by the ionic strength of the solution, and a decrease in solution ionic strength from 0.7 to 0.04 M enhanced electroosmotic transport. Under the pH and ionic strength conditions commonly encountered in pharmaceutical formulations, the effects of electroosmosis were generally small, providing less than 10 times flux enhancement over passive transport during 0.1 mA iontophoresis across 0.64 cm² nail surface, for example, at pH 9 and ionic strength of 0.04 M. The results in the present study also demonstrated that transungual transport enhancement of small neutral permeants increased with permeant molecular sizes, which is consistent with electroosmosis theory. Nail electrical resistance increased with decreasing the ionic strength of the equilibrating solution, but reached a plateau value when the ionic strength was less than approximately 0.07 M. This again is consistent with the electrokinetic theory. The apparent surface charge density of the transport pathway across the nail plate was estimated at pH 7.4. Because flux enhancement due to electroosmosis was generally small compared to that due to the direct-field effect in transungual iontophoretic transport, the mechanism of the direct-field effect should be the main focus in transungual iontophoresis formulation development of small to moderate size drug permeants.

ACKNOWLEDGMENTS

This research was supported in part by NIH grant GM063559. The authors thank Dr. Gerald B. Kasting for his help and discussion, Kelly A. Smith for performing some of the preliminary experiments, and Dr. William I. Higuchi and Dr. Kendall D. Peck for helpful discussion.

REFERENCES

1. Repka MA, O'Haver J, See CH, Gutta K, Munjal M. Nail morphology studies as assessments for onychomycosis treatment modalities. *Int J Pharm* 2002;245:25–36. [PubMed: 12270239]
2. Mertin D, Lippold BC. In-vitro permeability of the human nail and of a keratin membrane from bovine hooves: Penetration of chloramphenicol from lipophilic vehicles and a nail lacquer. *J Pharm Pharmacol* 1997;49:241–245. [PubMed: 9231338]
3. Mertin D, Lippold BC. In-vitro permeability of the human nail and of a keratin membrane from bovine hooves: Influence of the partition coefficient octanol/water and the water solubility of drugs on their permeability and maximum flux. *J Pharm Pharmacol* 1997;49:30–34. [PubMed: 9120766]
4. van Hoogdalem EJ, van den Hoven WE, Terpstra IJ, van Zijtveld J, Verschoor JSC, Visser JN. Nail penetration of the antifungal agent oxiconazole after repeated topical application in healthy volunteers, and the effect of acetylcysteine. *Eur J Pharm Sci* 1997;5:119–127.
5. Murdan S. Drug delivery to the nail following topical application. *Int J Pharm* 2002;236:1–26. [PubMed: 11891066]
6. Hui X, Chan TCK, Barbadillo S, Lee C, Maibach HI, Wester RC. Enhanced econazole penetration into human nail by 2-*n*-nonyl-1,3-dioxolane. *J Pharm Sci* 2003;92:142–148. [PubMed: 12486690]
7. Hui X, Wester RC, Barbadillo S, Lee C, Patel B, Wortzman M, Gans EH, Maibach HI. Ciclopirox delivery into the human nail plate. *J Pharm Sci* 2004;93:2545–2548. [PubMed: 15349963]
8. Kobayashi Y, Komatsu T, Sumi M, Numajiri S, Miyamoto M, Kobayashi D, Sugibayashi K, Morimoto Y. In vitro permeation of several drugs through the human nail plate: Relationship between physicochemical properties and nail permeability of drugs. *Eur J Pharm Sci* 2004;21:471–477. [PubMed: 14998577]
9. Repka MA, Mididoddi PK, Stodghill SP. Influence of human nail etching for the assessment of topical onychomycosis therapies. *Int J Pharm* 2004;282:95–106. [PubMed: 15336385]

10. Monti D, Saccomani L, Chetoni P, Burgalassi S, Saettone MF, Mailland F. In vitro transungual permeation of ciclopirox from a hydroxypropyl chitosan-based, water-soluble nail lacquer. *Drug Dev Ind Pharm* 2005;31:11–17. [PubMed: 15704853]
11. Kasting GB. Theoretical models for iontophoretic delivery. *Adv Drug Deliv Rev* 1992;9:177–199.
12. James MP, Graham RM, English J. Percutaneous iontophoresis of prednisolone—A pharmacokinetic study. *Clin Exp Dermatol* 1986;11:54–61. [PubMed: 3708894]
13. Murthy SN, Waddell DC, Shivakumar HN, Balaji A, Bowers CP. Iontophoretic permselective property of human nail. *J Derm Sci* 2007;46:150–152.
14. Murthy SN, Wiskirchen DE, Bowers CP. Iontophoretic drug delivery across human nail. *J Pharm Sci* 2007;96:305–311. [PubMed: 17080425]
15. Hao J, Li SK. Transungual iontophoretic transport of polar neutral and positively charged model permeants: Effects of electrophoresis and electroosmosis. *J Pharm Sci* 2008;97:893–905. [PubMed: 17683062]
16. Guy RH, Kalia YN, Delgado-Charro MB, Merino V, Lopez A, Marro D. Iontophoresis: Electropulsion and electroosmosis. *J Control Release* 2000;64:129–132. [PubMed: 10640651]
17. Pikal MJ. The role of electroosmotic flow in transdermal iontophoresis. *Adv Drug Deliv Rev* 2001;46:281–305. [PubMed: 11259844]
18. Li SK, Ghanem AH, Peck KD, Higuchi WI. Iontophoretic transport across a synthetic membrane and human epidermal membrane: A study of the effects of permeant charge. *J Pharm Sci* 1997;86:680–689. [PubMed: 9188050]
19. Zhu H, Peck KD, Li SK, Ghanem AH, Higuchi WI. Quantification of pore induction in human epidermal membrane during iontophoresis: The importance of background electrolyte selection. *J Pharm Sci* 2001;90:932–942. [PubMed: 11458340]
20. Peck KD, Srinivasan V, Li SK, Higuchi WI, Ghanem AH. Quantitative description of the effect of molecular size upon electroosmotic flux enhancement during iontophoresis for a synthetic membrane and human epidermal membrane. *J Pharm Sci* 1996;85:781–788. [PubMed: 8819006]
21. Peck KD, Hsu J, Li SK, Ghanem AH, Higuchi WI. Flux enhancement effects of ionic surfactants upon passive and electroosmotic transdermal transport. *J Pharm Sci* 1998;87:1161–1169. [PubMed: 9724571]
22. Sims SM, Higuchi WI, Srinivasan V. Interaction of electric field and electro-osmotic effects in determining iontophoretic enhancement of anions and cations. *Int J Pharm* 1991;77:107–118.
23. Hiemenz, PC.; Rajagopalan, R. Principles of colloid and surface chemistry. 3rd edition. Marcel Dekker; New York: 1997. p. 499-574.revised and expanded
24. Sims SM, Higuchi WI, Srinivasan V, Peck KD. Ionic partition coefficients and electroosmotic flow in cylindrical pores: Comparison of the predictions of the Poisson-Boltzmann equation with experiment. *J Colloid Interface Sci* 1993;155:210–220.
25. Marro D, Kalia YN, Delgado-Charro MB, Guy RH. Contributions of electromigration and electroosmosis to iontophoretic drug delivery. *Pharm Res* 2001;18:1701–1708. [PubMed: 11785689]

Figure 1. Schematic diagrams of the transport experimental procedure under (a) Protocol 1 and (b) Protocol 2.

Figure 2.

Percentage of water content in the nail clippings equilibrated with PBS of different pH and ionic strengths: 0.16 M of pH 3, 5, and 9; and pH 7.4 of 0.04, 0.16, and 0.7 M. Data represent the average and standard deviation of measurements with three nail samples. Data of 0.16 M pH 7.4 were taken from Hao and Li.¹⁵

Figure 3.

Passive permeability coefficients of MA (open bars) and UR (dotted bars) obtained from the transport experiments P2 (light background) at pH 3, 5, and 9 (0.16 M), ionic strength of 0.04 and 0.7 M (pH 7.4) and P3 (dark background) at 0.16 M, pH 7.4 under Protocols 1 and 2. The dotted and solid horizontal lines show the average passive permeability coefficients of MA and UR in experiments P1, respectively. Data represent the average and standard deviation of four nail samples.

Figure 4.

Effect of solution pH on permeability coefficients of mannitol (diamond symbols) and urea (square symbols) in 0.3 mA anodal (open symbols) and cathodal (closed symbols) iontophoretic transport experiments at ionic strength of 0.16 M for pH 3, 5, and 7.4 and 0.18 M for pH 9. Data represent the average and standard deviation of four nail samples. Data of pH 7.4 were taken from Hao and Li.¹⁵

Figure 5.

Peclet number (Pe) determined in 0.3 mA anodal and cathodal iontophoretic transport experiments at 0.16 M and pH 3 (open bars, light background), pH 5 (dotted bars, light background), pH 7.4 (open bars, dark background), and pH 9 (dotted bars, dark background) under Protocol 1. Data represent the average and standard deviation of four nail samples. Data of 0.16 M pH 7.4 were taken from Hao and Li.¹⁵

Figure 6.

Effect of solution ion strength on Peclet numbers of mannitol (open bars) and urea (dotted bars) in 0.1 mA (light background) and 0.3 mA (dark background) anodal iontophoretic transport experiments at pH 7.4. Data represent the average and standard deviation of four nail samples. Data of 0.16 M were taken from Hao and Li.¹⁵

Figure 7.

Electrical resistance of nail plates versus solution ionic strength at pH 5 (diamond symbols), pH 7.4 (square symbols), and pH 9 (triangle symbols) obtained in the resistance measurement study. The crosses represent the resistance values measured at the beginning and the end of the resistance studies. Data represent the average and standard deviation of the measurements with four nail samples.