Published in final edited form as:

J Pharm Sci. 2008 January ; 97(1): 490–505. doi:10.1002/jps.21231.

Iontophoretic Transport Across a Multiple Membrane System

SARAH A. MOLOKHIA¹, YANHUI ZHANG^{1,2}, WILLIAM I. HIGUCHI¹, and S. KEVIN LI^{1,3}

¹ Department of Pharmaceutics & Pharmaceutical Chemistry, University of Utah, Salt Lake City, Utah 84112

² Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

³ Division of Pharmaceutical Sciences, College of Pharmacy, University of Cincinnati, Cincinnati, Ohio 45267

Abstract

The objective of the present study was to investigate the iontophoretic transport behavior across multiple membranes of different barrier properties. Spectra/Por® (SP) and Ionac membranes were the synthetic membranes and sclera was the biomembrane in this model study. The barrier properties of SP membranes were determined individually in passive and iontophoresis transport experiments with tetraethylammonium ion (TEA), chloride ion (Cl), and mannitol as the model permeants. Passive and iontophoretic transport experiments were then conducted with an assembly of SP membranes. The contribution of electroosmosis to iontophoresis was assessed using the mannitol data. Model analysis was performed to study the contribution of diffusion and electromigration to electrotransport across the multiple membrane system. The effects of membrane barrier thickness upon ion-exchange membrane-enhanced iontophoresis were examined with Ionac, SP, and sclera. The present study shows that iontophoretic transport of TEA across the membrane system was related to the thicknesses and permeability coefficients of the membranes and the electromobilities of the permeant across the individual membranes in the assembly. Model analysis suggests significant contribution of diffusion within the membranes across the membrane system, and this mechanism is relatively independent of the current density applied across the system in iontophoresis dominant transport.

Keywords

iontophoresis; membrane; transport; transference number; electrophoresis; diffusion; mathematical model; drug delivery

INTRODUCTION

Iontophoresis is a method to deliver a compound across a membrane with the assistance of an electric field. Examples of electric field assisted drug delivery are transdermal iontophoresis,¹ ocular iontophoresis,² and electroporation delivery.³ In constant current iontophoretic delivery, the efficiency of iontophoretic transport is commonly assessed by the transference number, which is the ratio of the current carried by the permeant to the total electric current applied across the membrane.^{4–6} The flux and transference number of a permeant across a membrane (or a homogenous membrane system) at constant current are independent of the porosity and thickness of the membrane but depend on the pore size and charge of the membrane transport pathways. For example, when the effective thickness of the membrane increases or when membrane porosity decreases, the electric field across the membrane increases to maintain a constant level of electric current across the system. Therefore, the

iontophoretic fluxes across an assembly of two membranes of the same pore size and pore charge will be the same as those of a single membrane during constant current iontophoresis. On the other hand, when two membranes of different barrier properties, that is, different transference numbers to a permeant, are stacked together to form a membrane system, the transport behavior of permeant across the membrane system will be different from those of the two individual membrane types. The mechanism and transport behavior of iontophoretic transport across such a membrane system is not well defined.

Electrically driven transport across multiple membrane or multiple pathway systems is commonly encountered in pharmaceutical devices. For example, synthetic membranes are often used in devices for transdermal iontophoretic drug delivery; the result here is a two-membrane system involving the synthetic membrane and skin.⁷ Other investigators control drug release from implants with heterogeneous cation-exchange membranes⁸ or enhance transdermal and ocular iontophoretic delivery with ion-exchange membranes.^{9,10} These applications are also multiple membrane systems involving a synthetic membrane and a biological tissue. Multiple pathway systems are also encountered in chemical separations such as electrically driven microfluidic channel separation,¹¹ which employs complex systems involving multiple channels of different mixing, reacting, and separating zones. These analytical separation processes usually involves nonsteady state transient transport different from the steady-state transport normally encountered in iontophoretic drug delivery and are more complicated.

The purposes of the present study were (a) to investigate the interplay of electrophoresis, diffusion, and the resulting ion concentration profiles in the membranes during iontophoresis when membranes of different barrier properties (or permeant transference) are assembled together in series to form a barrier and (b) to provide a mechanistic understanding of iontophoretic transport across such multiple membrane systems so this information can be applied to data interpretation in an ongoing study of ion-exchange membrane-enhanced iontophoresis. The effects of the thicknesses and permeability coefficients of the individual membranes in the assembly upon the total iontophoretic fluxes across the system were to be studied under the constant current iontophoresis conditions. Spectra/Por[®] (SP) membranes were the synthetic model membranes and tetraethylammonium ion (TEA) was the model permeant ion. Mannitol was the neutral model permeant to assess the contribution of electroosmosis. Ion-exchange membrane enhanced iontophoretic transport and the effects of the tissue barrier resistance upon transport were to be examined with Ionac, SP, and sclera. Model simulations were to be performed to study the flux enhancing mechanisms in the iontophoresis system.

EXPERIMENTAL

Materials

³H-mannitol and ¹⁴C-tetraethyl ammonium (TEA) at >98% purity were purchased from Perkin-Elmer Life and Analytical Sciences (Boston, MA). 36-chloride (³⁶Cl, NaCl in aqueous solution) was purchased from GE Healthcare Bio-Sciences Corp. (Piscataway, NJ). The physical properties of the model permeants are provided in Table 1. Tetraethylammonium chloride (TEACl) was purchased from Sigma-Aldrich Co. (St. Louis, MO). The TEACl crystals had approximately 10–12% water content as reported from the manufacturer and were further checked by Karl Fischer titration. TEACl solution of approximately 0.13 M was prepared in deionized water. Phosphate buffered saline (PBS), pH 7.4, composed of 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride, was prepared by PBS tablets purchased from Sigma-Aldrich Co. and deionized distilled water. Millipore membranes, 0.22 μm pore diameter, Type GV, were purchased from VWR International (West Chester, PA). SP dialysis membranes of molecular weight cutoff 500 and 1000 (SP500 and

SP1000, respectively) were purchased from Spectrum Laboratories, Inc. (Rancho Dominguez, CA). Ion-exchange membrane Ionac (cation, MA-3470) was obtained from Sybron Chemicals (Birmingham, NJ). Before used in the transport experiments, Ionac membranes were preequilibrated in 0.13 M TEACl solution (approximately 10 mL solution per membrane) in screw-capped vials and shaken at room temperature for at least 24 h and then subsequently preequilibrated for another 24 h in a solution of (0.13 M TEACl with trace levels of ^{14}C -TEA) as that to be used as the donor solution in the transport experiments.

The sclera tissues were obtained from both the superior and inferior temporal sections of the globe about 0.5 cm away from the limbus, after the eye was separated from the New Zealand rabbit (Western Oregon Rabbit Co., Philomath, OR) and freed from adhering extraocular debris such as the conjunctiva and muscles. The rabbits were euthanized in other studies at the University of Utah Animal Resource Center under the approval of the Institutional Animal Care and Use Committee at the University of Utah.

Transport Experiments

Passive and iontophoresis experiments were carried out in a well-stirred two-chamber side-by-side diffusion cell system with effective diffusion area around 0.2 cm^2 similar to those described previously.¹⁴ Passive and iontophoresis experiments were carried out with dual permeants ^{14}C -TEA/ ^3H -mannitol pair and ^{36}Cl . Passive experiments were also carried out immediately after iontophoresis to check for membrane reversibility and stability. An assembly of membranes was sandwiched between the two diffusion half-cells with the edge of the membrane sealed with parafilm. The diffusion cell was placed in a circulating water bath at $36 \pm 1^\circ\text{C}$. SP500, SP1000, Ionac, and sclera were the membranes to construct the membrane systems in Table 2 in the present study. Millipore membranes were used to examine possible effects of aqueous unstirred boundary layer (ABL) in the diffusion cell setup. Unless otherwise stated 2 mL of PBS and 2 mL donor solution were then pipetted into the receiver and donor chambers, respectively. The donor solution was prepared by mixing an appropriate amount of the radiolabeled permeant with 0.13 M TEACl in deionized distilled water. The final concentration of the radiolabeled permeant in the solution was around 4×10^4 – 4×10^5 dpm/mL. Typically, 20 μL aliquots were taken from the donor chamber, and 1 mL samples were withdrawn from the receiver chamber at predetermined time intervals (20–30 min). Fresh PBS solution was then added back to the receiver chamber to maintain a constant volume in the chamber. The donor and receiver samples were mixed with 10 mL of scintillation cocktail (Ultima Gold, Packard Instrument, Meriden, CT) and analyzed by a liquid scintillation counter (Packard TriCarb Model 1900TR Liquid Scintillation Analyzer). The duration of the experiments was approximately 1.5 h unless otherwise stated.

In the iontophoresis experiments, a DC current was applied with a constant current iontophoretic device (Phoresor II Auto, Model PM 850, Iomed, Inc., Salt Lake City, UT) using Ag/AgCl (cathode in the receiver) and Ag (anode in the donor) as the driving electrodes for TEA experiments and an opposite configuration (cathodal iontophoresis) for ^{36}Cl experiments. An electric current of 1 or 2 mA was applied across the membrane assembly. Iontophoresis transport of 2 mA was not conducted with SP500 due to irreversible changes observed after iontophoresis with the membrane. The pH of the donor and receiver solution was checked and was found to be essentially constant in the present study. The applied voltages across the SP and Ionac were measured with a multi-meter (Fluke, Model 75) during TEA transport iontophoresis.

The amount of permeant transported across the membrane was determined by the radioactivity (in dpm) transported across the membrane and the specific activity (dpm/mol) of the radiolabeled permeant in the donor solution. The flux (J) and permeability coefficient (P) were calculated at steady-state under sink conditions:

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

$$P = \frac{1}{C_D A_D} \frac{\Delta Q}{\Delta t} \quad (2)$$

where C_D is the concentration of the permeant in the donor chamber, A_D is the diffusional surface area, and $\Delta Q/\Delta t$ is the slope of the cumulative amount of the permeant transported across the membrane into the receiver chamber versus time plot.

In the iontophoresis experiments with ^{14}C -TEA and ^{36}Cl , the flux of the permeant (J_i) is related to the current by

$$J_i = \frac{t_i I_{\text{total}}}{A_D F |z_i|} \quad (3)$$

where F is the Faraday constant, I_{total} is the total current, z_i is the charge of the permeant, and t_i is the apparent transference number of the permeant. The apparent transference number equals the fraction of the current carried by an ionic species of interest (i.e., the permeant ion). It is the ratio of the current carried by the permeant (I_i) to the total current carried by all ionic species (I_{total}) in the system:

$$t_i = \frac{I_i}{I_{\text{total}}} \quad (4)$$

and

$$t_i = \frac{|z_i| J_i}{\sum_j |z_j| J_j} \quad (5)$$

where J_i is the flux of species i (the permeant), J_j is the flux of ionic species j in the system, I_i is the current carried by the permeant, and z_j is the charge of ionic species j . The ionic species j represents both the ions migrating into the receiver from the donor including ionic species i and the oppositely charged counterions migrating into the donor from the receiver. It should be noted that transference number is traditionally defined as the fraction of the galvanic current carried by the ion in a pure conduction process. In the present study, an apparent transference number is defined, that is the fraction of current transported by the ion in the membrane system with no restriction except the conditions of steady-state and iontophoresis-dominated transport (i.e., the apparent transference number also accounts for the contribution of electric current from ion diffusion and convection).^{15,16} This definition allows the integral characterization of the fraction of current transported in the membranes as a whole and is preferred due to the concentration gradients developed in the membranes with defined boundary conditions.

To assess the effects of electroosmosis, iontophoresis experiments of mannitol were conducted to determine the effects of electroosmosis across the membrane system. Passive transport experiments without the application of an electric field were to serve as the baseline. The contribution of electroosmosis to total electrotransport (i.e., the influence of the convective solvent flow) was assessed by the enhancement factor of mannitol during iontophoresis. The enhancement factor is

$$E = \frac{J_{\text{iont}}}{J_{\text{passive}}} \quad (6)$$

where J_{iont} and J_{passive} are the iontophoresis and passive fluxes across the same membrane, respectively.

To examine possible effects of the background electrolyte in the asymmetric condition of 0.13 M TEACl solution in donor and PBS in receiver upon iontophoretic transport across SP500 and SP1000 in the present study, iontophoresis transport experiments of TEA were also performed under the symmetric condition (of 0.13 M TEACl in both chambers) at 1 mA constant current. The results obtained under the symmetric conditions were compared with those under the asymmetric conditions.

Numerical Model Simulation

According to the Nernst–Planck theory and assuming a pore transport pathway model, the flux of an ionic species j across a membrane during iontophoresis can be expressed as

$$J_j = \varepsilon \left(-D_j \frac{dC_j}{dx} - z_j u_j C_j \frac{d\psi}{dx} \pm v_j C_j \right) \quad (7)$$

where the first term $D_j(dC_j/dx)$ is the diffusion component, the second term $z_j u_j C_j (d\psi/dx)$ is related to electrophoresis, $v_j C_j$ describes electroosmotic transport, and ε is the combined porosity and tortuosity factor of the membrane.^{17–20} ψ is the electric potential, v_j is the average effective velocity due to convection resulting from electroosmosis, u_j is the effective electromobility, D_j is the effective diffusion coefficient, and z_j is the charge number of the ion. The effective diffusion coefficient D_j and effective electromobility u_j are related to the hindrance factor and the free diffusion coefficient of the ionic species as previously described.¹⁴

Finite-element simulations of electrotransport in a single pathway across the membrane system were performed with the computer software Comsol (FEMLAB v3.0a, Comsol, Inc., Burlington, MA) as described previously.¹⁴ Briefly, the space dimension was set to 1D (1 dimension), and the Physics Model was the Nernst–Planck equation at steady-state. The input information included the thickness of membranes. The thicknesses of 2 layers of SP500, 2 layers of SP1000 and an Ionac membrane were 0.11, 0.11, and 0.41 mm, respectively. The boundary conditions for the two end points of the line (depending on thickness of membrane) were set to the constant experimental donor and receptor concentrations. Concentration of TEA and Cl at donor boundary was 0.15M (150 mol/m³) and at receiver boundary was zero and 150 mol/m³, respectively. The boundary current density was set at 50 and 100 A/m² for the 1 and 2 mA experiments, respectively. The current densities were calculated from the current and the total cross-sectional area of the pores estimated by the total membrane surface area and the porosity of the membrane. The electromobility and diffusivity were set in the subdomain settings. The effective electromobility and diffusivity used in the model simulations were determined as follows. The effective electromobility and diffusivity are related to the passive permeability coefficient (P_{passive}) and the thickness of the membrane according to the following relationships:

$$P_{\text{passive}} = \frac{\varepsilon D_{\text{aq}} H}{h} \quad (8)$$

$$D_{\text{eff}} = \varepsilon D_{\text{aq}} H = P_{\text{passive}} h \quad (9)$$

$$\mu_{\text{eff}} = \frac{D_{\text{eff}}}{RT} = \frac{P_{\text{passive}} h}{RT} \quad (10)$$

where ε is the combined porosity and tortuosity factor, D_{eff} is the effective diffusivity, D_{aq} is the aqueous diffusion coefficient, H is the hindrance factor, h is the thickness, R is the gas constant, T is the temperature, and μ_{eff} is the effective mobility. Due to the asymmetric condition of TEACl in the donor and PBS in the receiver across the membranes, the effective diffusivities of the permeants in the membranes cannot be determined directly from passive transport experiments. Effects such as Donnan equilibrium and diffusion potential were important for passive permeation across SP500 and Ionac. In passive transport experiments under this condition, TEA, Na, and Cl do not move independently according to their diffusion coefficients in the membranes but interact with each other to maintain electroneutrality such that, for example, permeation of Na ion would enhance the permeation of TEA. As a result, passive permeability values were calculated from the iontophoretic enhancement factor and the voltage drop ($\Delta\psi$) measured across the membrane in the iontophoresis study rather than directly measured in passive transport study:

$$P_{\text{passive}} = \frac{P_{\text{iont}}}{E} = \frac{P_{\text{iont}}(1 - \exp(-K - Pe))}{K + Pe} \quad (11)$$

where

$$K = \frac{z_j F(\Delta\psi)}{RT} \quad (12)$$

and P_{iont} is the permeability coefficient during iontophoresis and Pe is the Peclet number. The average hypothetical passive permeability coefficients of SP500, SP1000, and Ionac for TEA and Cl under 1 mA and asymmetric (donor/receiver: TEACl/PBS) conditions were determined using the single 2 SP500, 2 SP1000, and 1 Ionac systems, respectively, and Eqs. (11) and (12). The effective electromobilities and diffusion coefficients of the permeants in the SP membranes were then determined from the passive permeability values according to Eqs. (9) and (10). It should be pointed out that the model simulations in the present study did not account for changes in electromobility at high solute concentration. Charge neutrality is assumed in the system, and aqueous unstirred boundary effects are assumed to be negligible. The boundary mesh parameters had maximum element size of 1×10^{-9} . As for the membrane assembly model simulation, current and flux continuity was chosen at the interior boundary. The stationary nonlinear solver was chosen. The simulation took about 1 min in a Dell Inspiron 1100 computer. The apparent transference numbers of TEA in all model simulations were calculated using:

$$t_{\text{TEA}} = \frac{J_{\text{TEA}}}{J_{\text{TEA}} + J_{\text{Cl}}} \quad (13)$$

where J_{TEA} and J_{Cl} are the fluxes of TEA and Cl, respectively.

RESULTS

Transport Across the Millipore Membranes and ABL

Possible effects from the ABL were assessed in the transport experiments with Millipore membranes in the diffusion cell setup. Millipore membranes were chosen (over SP membranes) because of the high permeability of Millipore which permitted better assessment of the ABL. Passive transport experiments were carried out with dual permeants TEA and mannitol and membrane systems of an assembly of 1, 2, and 5 Millipore membranes. Permeability coefficients in the experiments (P_{total}) were then calculated using Eq. (2) and the data were analyzed using:

$$\frac{1}{P_{\text{total}}} = \frac{1}{P_{\text{ABL}}} + \frac{n}{P_{\text{millipore}}} \quad (14)$$

where P_{ABL} and $P_{millipore}$ are the permeability coefficients of the ABL and a single Millipore membrane, and n is the number of Millipore membranes. In the plots of $1/P_{total}$ versus number of Millipore membranes, the y-intercepts ($1/P_{ABL}$) were close to the origin, and there was no statistical difference between the $1/P_{ABL}$ values and zero. This suggests that the effect of ABL is minimal (data not shown).

Transport Across the SP1000 Membranes

Table 3 presents the passive and iontophoresis permeability coefficients of mannitol across the SP1000 membrane systems. Stage 1 was passive permeation before 1 mA iontophoresis (Stage 2), and Stage 3 was passive transport after iontophoresis with the same membranes. The enhancement factor of iontophoretic transport for mannitol was approximately 1.3. These results suggest that the pores in SP1000 are slightly negatively charged but can be assumed as neutral for the purpose of electroosmosis in this study. Table 3 also shows insignificant differences between the passive permeability of SP1000 for mannitol in Stage I and Stage III. There was no significant irreversible membrane alteration as a result of the application of the electric field.

Table 4 shows the apparent transference numbers of TEA across the SP1000 membrane systems. The data show that the flux and transference of TEA across a homogenous membrane system were independent of the thickness of the membrane assembly under constant current iontophoresis. In addition, the transference numbers of TEA were relatively independent of the applied electric current density (1 and 2 mA). The average transference number of TEA for SP1000 is 0.29, which is consistent to the aqueous electromobilities of TEA and Cl.

To verify that Cl and TEA ions are the main carriers of the electric current across the membrane and to examine the contribution of the other ion species such as phosphate and sodium ions to the total current during iontophoresis, iontophoresis transport of ^{36}Cl was studied under constant current of 1 mA. The permeability coefficients of Cl ion in this study were $2.8 \times 10^{-4} \text{ cm/s} \pm 0.3 \times 10^{-4} \text{ cm/s}$ ($n=2$) and $3.1 \times 10^{-4} \text{ cm/s} \pm 0.1 \times 10^{-4} \text{ cm/s}$ ($n=3$), corresponding to transference numbers (t_i) of 0.72 and 0.80 for the 4 SP1000 and the 2 SP1000 systems, respectively. The sum of the t_i of TEA and that of Cl is close to unity. This result suggests that TEA and Cl are the main ion species that carry the electric current across the membranes.

Transport Across the SP500 Membranes

Table 3 also presents the passive and iontophoresis permeability coefficient of mannitol across the SP500 membrane systems. The enhancement due to iontophoresis was 1.2, suggesting minimal electroosmosis effect. Similar to the SP1000 results, the mannitol data in Stage 1 and Stage 3 also suggest no significant irreversible membrane alteration due to the application of the electric field under the iontophoresis condition of 1 mA. The data also show that the TEA transference number for SP500 is independent of the thickness of the SP500 membrane assembly and is approximately 0.005. This value is significantly smaller than that found with SP1000 due to the much greater size exclusion effect with TEA than that with Cl.

Iontophoresis transport experiments of Cl under asymmetric condition were also performed as in the SP1000 study using 2 SP500. The permeability coefficient value is $3.6 \times 10^{-4} \text{ cm/s} \pm 0.1 \times 10^{-4} \text{ cm/s}$ ($n \geq 3$), corresponding to t_i of 0.93. The sum of the transference number of TEA and that of Cl is close to unity.

Iontophoretic Transport Experiments Under the Symmetric Condition of TEACl in Donor and Receiver

The results showed that the iontophoretic permeability coefficients of TEA under the symmetric TEACl/TEACl (donor/receiver) condition within data scatter were essentially the

same as those under the asymmetric TEACl/PBS condition for both SP500 and SP1000 (data not shown). This result is consistent with TEA in the donor chamber and Cl in the receiver chamber being the main carriers of the electric current across the membrane under both TEACl/TEACl and TEACl/PBS conditions. The transference number of TEA was not affected by the Na background electrolyte and phosphate buffer in the receiver during iontophoresis. Na ion transport was in the opposite direction of and against the electric current applied across the membrane. The transference numbers of the phosphate buffer ions were small probably due to the relatively low concentrations of the phosphate buffer compared to those of TEA and Cl in the system.¹⁴

Iontophoresis transport of ¹⁴C-TEA and ³⁶Cl was also studied at current levels of 0.3, 0.5, and 1 mA under symmetric conditions of 0.13 M TEACl in both chambers with 2 SP500. Voltage across the membrane during iontophoresis was measured at different current intensities and the hypothetical passive permeability was calculated using Eqs. (11) and (12). The hypothetical passive permeability coefficients of 2 SP500 for TEA and Cl were relatively constant from 0.3 to 1 mA with the average values of 1.9×10^{-8} cm/s $\pm$ 0.7×10^{-8} cm/s and 2.6×10^{-6} cm/s $\pm$ 0.7×10^{-6} cm/s for TEA and Cl, respectively, at 0.3, 0.5, and 1 mA. The relatively constant passive permeability value for TEA and Cl from 0.3 to 1 mA suggests good stability of the 2 SP500 membrane system under these applied current conditions. These results support the use of the hypothetical passive permeability values later in the model simulations in the present study.

Transport Across the Assembly of SP500 and SP1000

Table 4 summarizes the transport results of the assembly of SP500 and SP1000 membranes. In the SP500 and SP1000 experimental setup, SP500 was the model barrier and SP1000 was the flux enhancing membrane. The effects of the thickness and barrier resistance of SP500 and SP1000 upon the transference number of TEA across the assembly were examined by varying the number of SP500 and SP1000 membranes in the assembly during iontophoresis. From the data, iontophoretic transport of TEA across the SP500 membranes was enhanced when SP1000 (which have higher TEA transference number) were placed in series with the SP500. The increase in the number of SP1000 (the total thickness of SP1000) further enhances TEA iontophoretic transport. Table 4 shows an increase in the flux enhancement of almost 2.5 times when the thickness of SP1000 increases from “2 SP1000 + 2 SP500” to “16 SP1000 + 2 SP500”. On the contrary, when the number of SP500 membranes in the membrane assembly increases (from two to four membranes) the flux and transference number of TEA decreases approximately by a factor of 2.

Transport Across the Assembly of Ionac and SP1000

In the membrane system of Ionac and SP1000, the model barrier was the SP1000, and Ionac having a higher TEA transference number than SP1000 was the flux enhancing membrane. Table 5 summarizes the transference number of TEA across the Ionac and SP1000 membrane systems. The transference number of TEA across Ionac was independent of the number of Ionac membrane (one or three membranes) with an average value of 0.97. When the thickness of SP1000 in the assembly increased, total TEA iontophoretic transport decreased. From “1 Ionac” to “1 Ionac + 8 SP1000”, the transference number decreases almost two times. When SP1000 was replaced with SP500 in the assembly, the transference number of TEA decreased to less than one-twentieth of the “Ionac + 2 SP1000” transference value due to the higher resistance barrier of SP500 for TEA than that of SP1000.

Transport Across the Assembly of Ionac and Sclera

Table 5 also shows the transference numbers of TEA iontophoretic transport across Ionac, sclera, and the assembly of Ionac and sclera. Ionac membrane was facing donor chamber and

sclera facing the receiver chamber to mimic the practical situation of biological tissue as the barrier membrane. The average transference number of TEA iontophoretic transport across sclera was 0.38. With Ionac, the Ionac + sclera membrane assembly provided an iontophoresis enhancement of approximately twofold from 0.38 to 0.83. When the order of Ionac and sclera assembly was reversed (i.e., Ionac facing the receiver and sclera facing the donor), there was no effect upon TEA fluxes and hence no significant difference between the transference numbers was observed under these two configurations.

Model Simulation: Membrane Concentration Profile and Electrical Potential Gradient

Model analysis was performed to study the contribution of diffusion and electromigration (or electrophoresis) in the membranes across the membrane systems. The phosphate buffer ions in the system were not considered in the model calculation due to their relatively low concentrations and transference numbers in the system¹⁴ demonstrated by the Cl ion data in the present study. The model simulation also did not account for electroosmosis because no significant electroosmosis contribution was observed in the iontophoresis experiments of SP500 and of SP1000.

Figure 1a shows the steady-state concentration of TEA across two SP500 membranes during 1 mA iontophoresis. As shown in the figure, the boundary condition at the membrane/receiver interface dictates that only diffusion occurs for the permeant at the interface when Na, Cl and the permeant TEA are present; there is no electro-migration for TEA electrotransport at this membrane/receiver interface. This result is consistent with previous findings.¹⁴ Figure 1b–d shows the steady-state concentration of TEA across the membrane assemblies under 1 mA. Not shown in the figures are the concentration profiles of Cl. In the assemblies of SP500 and SP1000, the concentration profiles of Cl and TEA overlap in most parts of the SP membranes (except near the membrane boundaries at the receiver) to maintain charge neutrality in the membranes. During iontophoresis, high TEA concentrations were established at the interface of SP500 and SP1000 to maintain a constant total steady-state flux across membrane assembly with TEA concentration in the membranes higher than those in the donor and receiver chambers. Diffusion of TEA due to the high concentration of TEA at the membrane–membrane interface enhanced the transport of TEA across the SP500 and hindered the transport across SP1000. The concentration gradients of TEA in the 2 SP500 membranes increase with increasing the thickness of SP1000. When the number (or thickness) of SP1000 increased, the concentration at the membrane–membrane interface increased. The simulation data show that the contribution of diffusion to the total flux in the membranes is significant and comparable to that of electromigration during iontophoresis. This is different from a homogenous membrane system, in which the contribution of diffusion to total flux is significant only near the membrane/receiver interface.

Figure 2 presents the steady-state electrical potential gradient (electric field) in the membrane assemblies under 1 mA. The negative electrical potential gradients are due to with the electrical polarities in the donor and receiver and direction of electric current flow from the donor to receiver across the membranes. The magnitude of the potential gradient is related to the driving force for electromigration. The constant electrical potential gradient in the two-SP500 membrane system in Figure 1a is consistent with a homogenous membrane system with relatively constant total concentration of ions across the membrane. In the assemblies of SP500 and SP1000 (Fig. 2b–d), the absolute value of the electrical field was higher in the SP500 than that in SP1000. This is consistent with the lower permeability (higher resistance) of SP500 than that of SP1000. As expected from the concentration profiles of TEA and Cl in the membranes, the absolute value of the electric field decreases from the donor to the SP1000/SP500 membrane interface within the SP1000 membranes and increases from the interface across the SP500 membranes to the receiver. The nonlinear electrical potential gradients in the

membrane assembly imply that the Nernst-Planck model with Goldman approximation would not be appropriate for predicting iontophoretic transport across heterogeneous systems of multiple membranes similar to those in the present study.

Model Simulation: Contribution of Diffusion and Electromigration in the Membrane

The simulation data of the SP500 and SP1000 multiple membrane systems show linear concentration gradients of TEA in the membranes and the fluxes due to diffusion and electromigration are relatively constant in each membrane type in the membrane assembly. For example, the contribution of diffusion or electromigration at different positions in the SP500 membranes is essentially constant except near the boundaries of the SP500/SP1000 interface and the SP500/receiver interface. Figure 3 summarizes the passive (diffusive) and electrophoresis (electromigrative) flux contributions within SP500 (away from the interface regions) in the different SP membrane assemblies. Note that the total flux across the SP500 is the same as that across SP1000 in the membrane assembly under steady-state and is equivalent to the total flux across the whole membrane assembly of SP1000 in series with SP500. As the thickness of SP1000 increases, the flux due to diffusion from the SP500/SP1000 interface to the donor (or the concentration gradient of TEA) in SP1000 decreases, the SP500/SP1000 interface concentration increases, the flux due to diffusion from the SP500/SP1000 interface to the receiver increases, and the contribution of diffusion to total transport increases. In the case of “2 SP1000 + 2 SP500,” the diffusion flux increased eight times from approximately 4.0×10^{-7} to 3.1×10^{-6} mol/m²/s when the number of SP1000 increased from 2 to 16 in “16 SP1000 + 2 SP500,” but the migration flux increased less than two times from 4.0×10^{-6} to 6.6×10^{-6} mol/m²/s.

Model Simulation: Effect of Electric Current

Figure 4 shows the concentration profiles of TEA across the same membrane assemblies as in Figure 1 but under constant current of 3 mA. Figure 4 suggests that the mechanism of TEA transport across the membranes is relatively independent of the applied electric current levels when electrotransport is the dominant transport mechanism. When the electric current increased, the concentration of TEA at the interface between SP1000 and SP500 increased. The increase in the slopes of TEA (concentration gradient of TEA) in the membranes was roughly proportional to the electric current increase. The plots of computer simulated TEA fluxes against the current intensities are presented in Figure 5. The data in the figure show good linearity of TEA flux with electric current in the multiple membrane systems. Although diffusion is a major component in electrotransport of the ions in the membranes across the multiple membrane systems, the apparent transference numbers of ions remain relatively constant and TEA flux is proportional to the electric current. The percent contributions of diffusion and electromigration to total TEA transport was also observed to be relatively independent of the electric current applied across the membrane assemblies under these conditions.

Transference Number Prediction and Comparison of the Model Simulation and Experimental Transport Data

Figure 6 shows the model simulation results of the relationships between the transference numbers of permeant transport across membrane assemblies of two types of membranes and the passive permeability coefficient ratios of the membranes for the permeant. The two types of membranes are one with higher transference number (flux enhancing membrane) and other with lower transference number (barrier membrane) with the lower transference number membrane facing the receiver. The donor contained the permeant and its counterion, and the receiver contained the counterion and coion at the same concentration as those of the donor. Permeability coefficient ratio equals the passive permeability coefficient of the lower

transference number membrane divided by that of the higher transference number membrane. At small passive permeability coefficient ratios (i.e., permeability coefficient of the higher transference number membrane $\gg$ permeability coefficient of the lower transference number membrane), permeant transference across the membrane assembly approaches the transference number of the membrane with the lower transference number. When the permeability coefficient of the lower transference number membrane for the permeant is much higher than that of the higher transference membrane, the transference number of the membrane assembly approaches the value of the membrane with the higher transference number. The model simulation results in this figure allow the prediction of permeant transference across a membrane assembly of two membrane types (e.g., a flux-enhancing membrane and a biomembrane barrier) when their individual transference numbers and passive permeability coefficients for the permeant are known.

The apparent transference numbers of the computer model simulation are compared with the experimental data (last columns in Tables 4 and 5). Within the scattering of data, the computer model simulation results follow the same trend as those of the experimental data with the single and combined membrane systems. It should be pointed out that the model simulation values are expected to correlate with the experimental values for the single membrane system of 2 SP500 and of 2 SP1000 since the parameters used for the model simulation was calculated directly from the experiment results in these single membrane systems (2 SP500, 2 SP1000 or Ionac) as explained in Numerical Model Simulation Section.

DISCUSSION

Transport Mechanisms

The general mechanisms of electrotransport during iontophoresis of low to moderate electric field for the synthetic membranes are: electrophoresis (facilitation of the movement of ionic species by the applied electric field), electroosmosis (assisted transport of both neutral and charged species by electric field induced convective solvent flow), and to a small extent diffusion. Electroporation (barrier alterations that increase the intrinsic permeability of the membrane) is not expected to occur. The effects of electroosmosis are negligible in the present SP study as shown in the mannitol experiments (Tab. 3). Thus, it was originally expected that electrophoresis is the dominant transport mechanism of iontophoretic transport.

The present model simulation has shown significant contribution of diffusion to the total permeant flux in the membranes in addition to electrophoresis as the primary mechanisms of iontophoretic transport across the assembly of SP membranes under the electric current conditions studied in the present study (Fig. 3). According to the model simulation, the observed iontophoretic transport enhancement in the SP1000 + SP500 system is a consequence of the higher electrotransport of TEA in the SP1000 than that in the SP500, resulting in high TEA concentration at the interface of the SP1000 and SP500 membranes (Fig. 1). Diffusion of TEA in the membrane due to the high concentration at the interface enhances the transport of TEA across the SP500 and retards TEA transport in SP1000 (the large and opposite TEA concentration gradients in the membranes). This provides the same total flux (electrophoresis and diffusion) of TEA across the SP500 and SP1000 membranes during iontophoresis at steady state. For the counterion Cl, the apparent transference of Cl in the SP500 is higher than that in the SP1000. As a result, the diffusion of Cl due to the high concentration at the interface retards Cl transport across SP500 and enhances Cl transport across SP1000 in the SP1000 + SP500 system. The same is observed with Ionac and SP1000 where Ionac is the flux enhancing membrane and SP1000 is the barrier membrane.

Effects of Membrane Permeability and Thickness upon Iontophoretic Transport

As discussed Introduction Section, iontophoretic transport across a homogenous membrane system is independent of the porosity and thickness of the barrier during constant current iontophoresis. Under the constant current iontophoresis approach, the fluxes and apparent transference numbers of TEA across two, four and eight membranes (a homogenous membrane system) were essentially the same (Tab. 4). This result is consistent with this general view.

Different from a homogenous membrane system, iontophoretic fluxes were observed to be related to the electrical resistance and thickness of both SP500 and SP1000 membranes in the multiple membrane system because diffusion is a major component in iontophoretic transport across the system (Tab. 4). According to the transport model, when the thickness of SP1000 (flux enhancing membrane) decreases, the concentration gradient in the membrane increases. Hence, the flux due to diffusion (from the interface to the donor) in the flux enhancing membrane increases, and this effectively reduces the concentration of the permeant at the interface, diffusion from the interface to the receiver, and the total flux across the system (Figs 1 and 3). When the thickness of the flux enhancing membrane increases, the opposite effect is expected: diffusion from the interface to the donor decreases, concentration at the interface increases, and total flux across the assembly increases. This relationship is believed to hold as long as the concentration of the permeant in the membrane system is not limited by the solubility of the permeant salt. The effects of membrane porosity upon iontophoretic flux are the opposite of the membrane thickness effect. This hypothesis is supported by the experimental data and model simulations in the present study.

Drug Delivery

Under constant electric current iontophoretic drug delivery, the efficiency of drug transport is commonly assessed by the ratio of the current carried by the drug to that of the total electric current applied across the membrane. The ions migrating from the body fluids into the donor electrode are endogenous ions having a charge opposite to the polarity of the drug. These ions compete with the drug as carrier of the electric current across the tissue membrane and reduce the efficiency of drug transport. If the transport of the current-carrying counterions from the body fluids to the donor electrode is hindered, the efficiency of iontophoretic delivery will be enhanced at the same applied electric current. Particularly, iontophoretic transport can be enhanced by incorporating an ion-exchange membrane between the body surface and the donor electrode to exclude the transport of current-carrying counterions from the body fluids. A previous study has examined the use of a positively charged ion-exchange membrane to enhance transscleral iontophoresis of a model anion salicylate *in vitro*.¹⁰ In that study, the ion-exchange membrane eliminated the flux-retarding effect of electroosmosis and enhanced salicylate electromigration across the sclera, resulting in a threefold flux enhancement. The results in the present study demonstrate a twofold flux enhancement with a different permeant and membrane system (a negatively charged ion-exchange membrane and a positively charged model permeant).

An objective of the present study was to study the relationship between the resistance of a tissue barrier and its effect upon ion-exchange membrane enhanced iontophoresis. The present results demonstrate the importance of the barrier resistance of the tissue and the resulting concentration at the membrane interface. This finding supports the previous hypothesis that the technique to enhance iontophoretic transport by ion-exchange membrane is particularly suited for ocular iontophoresis because of the relative permeable transscleral barrier. Unpublished data of ion-exchange membrane enhanced iontophoresis with skin that has electrical resistances higher than that of the sclera show moderate flux enhancement using this technique. This is consistent with the model hypothesis (Fig. 6). For high resistance

biomembranes, a means for permeabilizing the local tissue is a means for effective ion-exchange membrane enhanced iontophoretic drug delivery.

Acknowledgements

This research was supported by NIH Grant EY15181. The authors thank Dr. Henry S. White for helpful discussion and providing assistance in the computer simulations and Dr. Rajan P. Kochambilli for his help in the experiments.

References

1. Banga AK, Chien YW. Iontophoretic delivery of drugs: Fundamentals, developments, and biomedical applications. *J Controlled Rel* 1988;7:1–24.
2. Hughes L, Maurice DM. A fresh look at iontophoresis. *Arch Ophthalmol* 1984;102:1825–1829. [PubMed: 6508622]
3. Prausnitz MR, Bose VG, Langer R, Weaver JC. Electroporation of mammalian skin: A mechanism to enhance transdermal drug delivery. *Proc Natl Acad Sci USA* 1993;90:10504–10508. [PubMed: 8248137]
4. Phipps JB, Gyory JR. Transdermal ion migration. *Adv Drug Deliv Rev* 1992;9:137–176.
5. Sage, BH. Iontophoresis. In: Smith, EW.; Maibach, HI., editors. *Percutaneous penetration enhancers*. Chapter 15.1. Boca Raton: CRC Press; 1995.
6. Delgado-Charro, MB.; Guy, RH. Iontophoresis of peptides. In: Berner, B.; Dinh, SM., editors. *Electronically controlled drug delivery*. Chapter 7. Boca Raton: CRC Press; 1998.
7. Stamatialis DF, Rolevink HH, Koops GH. Delivery of timolol through artificial membranes and pig stratum corneum. *J Pharm Sci* 2003;92:1037–1039. [PubMed: 12712423]
8. Schwendeman SP, Amidon GL, Labhasetwar V, Levy RJ. Modulated drug release using iontophoresis through heterogeneous cation-exchange membranes. 2. Influence of cation-exchanger content on membrane resistance and characteristic times. *J Pharm Sci* 1994;83:1482–1494. [PubMed: 7884674]
9. Untereker, DF.; Phipps, JB.; Cahalan, PT.; Brennen, KR. Iontophoresis electrode. US Patent 5,395,310. Mar 7. 1995
10. Li SK, Zhu H, Higuchi WI. Enhanced trans-scleral iontophoretic transport with ion-exchange membrane. *Pharm Res* 2006;23:1857–1867. [PubMed: 16841198]
11. Bilitewski U, Genrich M, Kadow S, Mersal G. Biochemical analysis with microfluidic systems. *Anal Bioanal Chem* 2003;377:556–569. [PubMed: 14504677]
12. 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]
13. Lide, DR. *CRC handbook of chemistry and physics*. 84. New York: CRC Press; 2004.
14. Li SK, Zhang Y, Zhu H, Higuchi WI, White HS. Influence of asymmetric donor–receiver ion concentration upon transscleral iontophoretic transport. *J Pharm Sci* 2005;94:847–860. [PubMed: 15736190]
15. Manzanares, JA.; Kontturi, K. Transport numbers of ions in charged membrane systems. In: Sørensen, TS., editor. *Surface chemistry and electrochemistry of membranes*. Chapter 11. New York: Marcel Dekker; 1999.
16. Lebedev KA, Nikonenko VV, Zabolotskii VI. Selectivity of ion-exchange membranes. Theoretical analysis of ionic transport numbers in membrane systems. *Sov Electrochem* 1987;23:459–465.
17. 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]
18. Kasting GB. Theoretical models for iontophoretic delivery. *Adv Drug Deliv Rev* 1992;9:177–199.
19. Li SK, Ghanem AH, Peck KD, Higuchi WI. Pore induction in human epidermal membrane during low to moderate voltage iontophoresis: A study using AC iontophoresis. *J Pharm Sci* 1999;88:419–427. [PubMed: 10187752]

20. Chizmadzhev YA, Indenbom AV, Kuzmin PI, Galichenko SV, Weaver JC, Potts RO. Electrical properties of skin at moderate voltages: Contribution of appendageal macropores. *Biophys J* 1998;74:843–856. [PubMed: 9533696]

Figure 1.

Steady-state concentration profiles of TEA in membrane assemblies of (a) 2 SP500, (b) 2 SP1000 + 2 SP500, (c) 8 SP1000 + 2 SP500, and (d) 16 SP1000 + 2 SP500 during constant current iontophoresis of 1 mA (50 A/m^2). The donor/SP1000 and SP500/receiver interfacial regions of (b) are enlarged in (bI) and (bII), respectively. The concentration, C_j , in the membrane is plotted against the position, x , in the membrane assembly from the donor. In (b)–(d), the interfaces between SP500 and SP1000 are located at approximately 1.1×10^{-4} , 4.4×10^{-4} , and 8.8×10^{-4} m from the donor which correspond to the thickness of 2, 8, and 16 SP1000 membranes, respectively. Increasing the thickness of SP1000 increases the accumulation of TEA at the SP1000/SP500 interface of the membrane assembly, and the slopes of TEA (concentration gradient of TEA) in the membranes increase. The increase of the slope of TEA in the rate limiting barrier (2 SP500) is also observed to be greater than that in the SP1000 membranes with an increase in the thickness of SP1000 from (b) to (d).

Figure 2.

Steady-state electrical potential gradient in membrane assemblies of (a) 2 SP500, (b) 2 SP1000 + 2 SP500, (c) 8 SP1000 + 2 SP500, and (d) 16 SP1000 + 2 SP500 during constant current iontophoresis of 1 mA (50 A/m^2). The potential gradient, $\Delta\psi/\Delta x$, in the membrane is plotted against the position, x , in the membrane assembly from the donor.

Figure 3.

Diffusive (passive) and migrative (electrophoresis) contributions of steady-state TEA transport in the rate limiting SP500 membranes during constant current iontophoresis of 1 mA in the systems of 2 SP1000 + 2 SP500, 8 SP1000 + 2 SP500, and 16 SP1000 + 2 SP500 and total flux across the membrane assembly. The data exclude the regions near the SP500/SP1000 and SP500/receiver interfaces, in which contributions of diffusion and migration are significantly affected by the boundary conditions.

Figure 4.

Steady-state concentration profiles of TEA in the membrane assemblies of (a) 2 SP1000 + 2 SP500, (b) 8 SP1000 + 2 SP500, and (c) 16 SP1000 + 2 SP500 during constant current iontophoresis of 3 mA (150 A/m²). The concentration, C_j , in the membrane is plotted against the position, x , in the membrane assembly from the donor. The donor/SP1000 and SP500/receiver interfacial regions in (a) are enlarged in (aI) and (aII), respectively. With an increase in the electric current density, a proportional increase of the slopes of TEA (concentration gradient of TEA) in the membranes is observed.

Figure 5.

Relationship between steady-state TEA fluxes and current intensities from computer model simulation of iontophoretic transport across the membrane systems of 2 SP500 + 2 SP1000, 8 SP1000 + 2 SP500, and 16 SP1000 + 2 SP500. The current densities of 25, 50, 100, and 150 A/m² correspond to current intensities of 0.5, 1, 2, and 3 mA in the experiments, respectively.

Figure 6.

The apparent transference numbers across membrane assemblies of two membrane types: two membranes of different permeability coefficients and transference numbers with the lower transference number membrane facing the receiver. The total transference number is plotted against the ratio of the passive permeability coefficient ratio. Permeability coefficient ratio equals the passive permeability coefficient of the lower transference number membrane divided by that of the higher transference number membrane. Symbols and transference numbers of the membrane types (the first number is the transference number of the membrane facing the receiver, the second number is the transference number of the membrane facing the donor): solid line with crosses, 0.0044, 0.24; solid line with open circles, 0.0069, 0.28; solid line with open squares, 0.0069, 0.55; solid line with closed diamonds, 0.0069, 0.80; dotted line with open triangles, 0.13, 0.28; dotted line with open diamonds, 0.13, 0.55; dotted line with closed triangles, 0.127, 0.80; dash-dot line with closed squares, 0.33, 0.80.

Table 1

Molecular Characteristics of TEA, Mannitol, Urea, Sodium Ion, and Chloride Ion

Permeant	Molecular Weight	Charge	Free Aqueous Diffusion Coefficient (10^{-5} cm ² /s) ^{12,13}
TEA	130	+1	1.1
Mannitol	182	0	0.9
Na ⁺	23	+1	1.8
Cl ⁻	35	-1	2.7

Table 2

Various Membrane Systems Examined

Single Membrane System Testing	Combined Membrane System Testing ^a	Ionac Enhanced Iontophoresis Testing ^b	Enhanced Transscleral Iontophoresis ^c
2 SP1000	2 SP1000 + 2 SP500	1 Ionac + 1 SP1000	Sclera
4 SP1000	8 SP1000 + 2 SP500	1 Ionac + 2 SP1000	1 Ionac + Sclera
8 SP1000	16 SP1000 + 2 SP500	1 Ionac + 8 SP1000	Sclera + 1 Ionac
2 SP500	16 SP1000 + 4 SP500	1 Ionac + 2 SP500	
4 SP500			
1 Ionac			
3 Ionac			

In the Combined Membrane Systems, the First Membrane Listed Faced the Donor Chamber and Second Faced the Receiver Chamber.

^aSP1000 can be viewed as the flux enhancing membrane with SP500 as the biomembrane.

^bSP1000 or SP500 can be viewed as the biomembrane and Ionac as the flux enhancing membrane.

^cIonac as the flux enhancing membrane and sclera is the biomembrane.

Table 3

Passive and Iontophoresis (1 mA) Permeability Coefficients for Mannitol*

System ^a	P Passive Mannitol (10 ⁻⁵ cm/s) (Stage 1)	P Iontophoresis Mannitol (10 ⁻⁵ cm/s) (Stage 2)	P Passive Mannitol (10 ⁻⁵ cm/s) (Stage 3)	(P, Stage 3)/(P, Stage 1)
(2) SP1000	2.9 ± 0.5	3.8 ± 0.1	3.0 ± 0.5	1.0
(4) SP1000	1.5 ± 0.3	2.1 ± 0.2	1.6 ± 0.3	1.1
(8) SP1000	0.7 ± 0.1	0.8 ± 0.2	0.6 ± 0.1	0.9
(2) SP500	0.051 ± 0.004	0.062 ± 0.004	0.045 ± 0.005	0.9
(4) SP500	0.019 ± 0.001	0.020 ± 0.004	0.018 ± 0.001	0.9

* Mean ± SD, $n \geq 3$.^a Number of membranes in parentheses.

Table 4
Apparent Transference Numbers of TEA Iontophoretic Transport Across the Membrane Systems*

System ^a , Electric Current	Transference Number (t_i)	
	Experiment ^b	Model Simulation ^c
(2) SP1000, 2 mA	0.30 ± 0.02	0.28
(4) SP1000, 2 mA	0.28 ± 0.01	0.28
(2) SP1000, 1 mA	0.30 ± 0.03	0.28
(4) SP1000, 1 mA	0.30 ± 0.03	0.28
(8) SP1000, 1 mA	0.28 ± 0.03	0.28
(2) SP500, 1 mA	0.004 ± 0.002	0.0069
(4) SP500, 1 mA	0.005 ± 0.002	0.0069
(2) SP1000 + (2) SP500, 1 mA	0.005 ± 0.001	0.0085
(8) SP1000 + (2) SP500, 1 mA	0.006 ± 0.002	0.013
(16) SP1000 + (2) SP500, 1 mA	0.012 ± 0.004	0.019
(16) SP1000 + (4) SP500, 1 mA	0.007 ± 0.002	0.013

* Mean $\pm$ SD, $n \geq 3$.

^a Number of membranes in parentheses.

^b Calculated using Eq. (3).

^c Calculated using Eq. (13).

Table 5

Apparent Transference Numbers of TEA Iontophoretic Transport Across Membrane Systems of Ionac and Sclera at 1 mA *

System ^a	Transference Number (<i>t_i</i>)	
	Experiment	Model Simulation
(1) Ionac	1.02 ± 0.18	0.91
(3) Ionac	0.93 ± 0.10	0.91
Sclera	0.38 ± 0.03	— ^b
(1) Ionac + (1) SP1000	0.77 ± 0.08	0.86
(1) Ionac + (2) SP1000	0.70 ± 0.08	0.81
(1) Ionac + (8) SP1000	0.50 ± 0.03	0.65
(1) Ionac + (2) SP500	0.03 ± 0.01	0.04
(1) Ionac + Sclera	0.83 ± 0.08	— ^b
Sclera + (1) Ionac	0.85 ± 0.20	— ^b

* Mean ± SD, *n* ≥ 3.

^a Number of membranes in parentheses.

^b Not determined.