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Ion-exchange membrane assisted transdermal iontophoretic delivery of salicylate and acyclovir

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ABSTRACT

The presence of endogenous competing counterions is a main reason for the generally low efficiency of transdermal iontophoretic drug delivery. The objective of the present study was to test the hypothesis that the incorporation of an ion-exchange membrane (Ionac) in an iontophoresis system to hinder transdermal transport of these counterions can enhance iontophoretic delivery. The properties of Ionac were characterized in passive and iontophoretic transport experiments. Iontophoretic transport across human epidermal membrane (HEM) and across HEM in series with Ionac was then studied. To assess the effect of HEM electrical resistance upon Ionac-assisted iontophoresis, HEM resistance was reduced in the iontophoresis experiments with alternating current (AC). Salicylate (SA) was the negatively charged permeant first tested in this study. Mannitol was the model permeant to examine the effects of electroosmosis. At the completion of the SA study, experiments were performed with acyclovir (ACV), an antiviral drug with limited water solubility. When Ionac was used to enhance SA transdermal fluxes, higher SA fluxes were observed with HEM of lower resistances in Ionac-assisted iontophoresis. Up to a four-fold flux enhancement was achieved when the electrical resistance of HEM was reduced using an AC iontophoresis method. For ACV, two-fold flux enhancement was observed in Ionac-assisted iontophoresis compared with the conventional iontophoresis baseline. In all experiments, the contribution of electroosmosis to drug transport was less than 10%. The present study has demonstrated the potential of a new approach using a positively charged ion-exchange membrane to enhance transdermal iontophoretic transport of negatively charged drugs.

1. Introduction

Transdermal iontophoresis is a method to enhance the delivery of a compound across the skin by an externally applied electric field (Banga, 1998). Iontophoresis has been shown to enhance transdermal permeation of ionic drugs (Kasting, 1992). A transdermal iontophoretic device consists of a donor and a receiver electrode. The donor electrode usually has the same polarity as the charge of the permeating drug. During transdermal iontophoresis, the permeant is delivered from the donor into systemic circulation. Endogenous ions, which have charge(s) opposite to that of the drug ion, migrate from the body fluids to the donor. The drug ion, any co-ions present in the drug formulations, and the counterions contribute to the total electric current applied across the skin. The efficiency of iontophoretic drug delivery is commonly assessed by the transference number (Phipps and Gyory, 1992), which is the ratio of the current carried by the ionized drug to the total current transported across the membrane (Kasting, 1992; Phipps and Gyory, 1992; Banga, 1998).

Because the endogenous counterions in body fluids (e.g. Na^+ or Cl^-) generally have higher electromobility than the drug ion, transdermal iontophoretic transport usually has low transport efficiency (low transference number). Particularly, in transdermal iontophoretic delivery of negatively charged compounds, typically less than 20% of the current is carried by the compounds (Kasting, 1992; Phipps and Gyory, 1992; Banga, 1998). It has been hypothesized that the incorporation of an ion-exchange membrane, having a charge opposite to that of the ionized drug, between the skin and the donor electrode would hinder the migration of the competing counterions, selectively enhance drug transport across skin, and thus can increase the efficiency of iontophoretic delivery. This enhancement allows the delivery of the same amount of drug at a

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lower applied electric current or shorter iontophoresis application times (Li et al., 2006).

Previous studies have shown that the use of ion-exchange membranes in series with the sclera provides two- to three-fold flux enhancement in transscleral iontophoresis with model permeants salicylate (SA) and tetraethylammonium (Li et al., 2006; Molokhia et al., 2008). In these studies, it was also found that the positively charged ion-exchange membrane suppressed transscleral electroosmosis. Although this technique has proven to be effective in enhancing transscleral iontophoresis, it is not clear whether the technique would be effective in transdermal delivery due to the significantly greater electrical resistance of the skin compared to the sclera. The resistive skin barrier may limit the ability of an ion-exchange membrane to enhance the efficiency of transdermal iontophoresis.

The assembly of two membranes such as an ion-exchange membrane and skin (Li et al., 2006) creates a multiple membrane system similar to those modeled in a recent study (Molokhia et al., 2008). It was shown in this study that the incorporation of an ion-exchange membrane between the iontophoretic device and a biological membrane would lead to a change in the ion concentration in these membranes. Permeant transport across the multiple membrane system was related to the passive permeability of each membrane in the system for the permeant. Accordingly, because of the correlation between the passive permeability coefficient of skin and its electrical resistance (Li et al., 1999), skin electrical resistance is expected to be a controlling factor in ion-exchange membrane enhanced transdermal iontophoresis. To fully utilize ion-exchange membrane enhanced transdermal iontophoresis, the relationship between skin electrical resistance and ion-exchange membrane enhanced transport should be investigated.

Acyclovir (ACV) is a potent and reliable antiviral agent used in the treatment of herpes simplex infection to relieve the painful sores associated with the infection. Local iontophoretic delivery of ACV for such indications is currently under development. ACV has two ionizable groups: pK_a of 2.4 for the basic group and 9.2 for the acidic group (Volpato et al., 1995; Abla et al., 2006). The low efficacy of dermatological formulation of ACV has been attributed to its poor percutaneous penetration partly due to the low aqueous solubility of ACV. Cathodal and anodal iontophoresis have been employed previously to enhance the flux of ACV across skin (Volpato et al., 1995, 1998; Stagni et al., 2004; Padula et al., 2005; Abla et al., 2006). However, iontophoretic transport of ACV with the assistance of an anion-exchange membrane has not been investigated in any systematic fashion.

The objectives of the present study were to (a) examine the use of an ion-exchange membrane to enhance the efficiency of transdermal iontophoresis of a model permeant SA, (b) determine the influence of skin resistance on flux enhancement in ion-exchange membrane enhanced iontophoretic transport, and (c) test the feasibility of this method with a low water solubility antiviral agent ACV. Direct current (DC) cathodal iontophoresis (cathode in the donor) was first conducted using an ion-exchange membrane (Ionac) and human epidermal membrane in a side-by-side diffusion cell *in vitro* with SA. Iontophoresis experiments without Ionac were the controls. SA (pK_a of 3) was the model permeant because of its high water solubility at physiological pH allowing the study of ion-exchange membrane enhanced iontophoresis and mechanistic interpretation under the symmetric condition with respect to the total ion concentrations and pH in the donor and receiver chambers. Mannitol (a neutral permeant of similar molecular size to SA) was used to assess the contribution of electroosmosis. Experiments with SA and mannitol were carried out using alternating current (AC) to reduce and control skin resistance and to assess the effect of skin electrical resistance upon flux enhancement in ion-exchange membrane assisted iontophoresis. It was hypothesized that effective iontophoresis enhancement using this technique would require the skin to have relatively low electric resistance. The feasibility of ion-exchange membrane to enhance transdermal iontophoretic delivery of ACV was then examined. Baseline control experiments were also conducted with ACV. The results of ACV not only demonstrate the feasibility of this technique on a drug with practical importance but provided a broader view in understanding the behavior of drugs in ion-exchange membrane assisted iontophoresis.

2. Materials and methods

2.1. Materials

^{14}C-SA and ^{3}H-mannitol at purity >97% were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). ACV was purchased from Lecto Meridian Pharmaceuticals, Inc. (Decatur, AL). Sodium salicylate (NaSA) was purchased from Acrōs Organics (Morris Plains, NJ). Acetonitrile, HPLC grade, was purchased from Fisher Scientific Co. (Fair lawn, NJ). Phosphate buffered saline (PBS, pH 7.4; 0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride) was prepared by dissolving PBS tablets purchased from Sigma–Aldrich, Co. (Saint Louis, MO) in distilled deionized water using the method specified by the supplier. Sodium azide (NaN_3) at 0.02% was used as a preservative in PBS solution. Other chemicals used in the present study are of analytical grade.

Side-by-side diffusion cells were custom-made and the area available for diffusion was approximately 0.8 cm^2. Ion-exchange (permselective) membrane Ionac MA 3475 was kindly supplied by Sybron Chemicals Inc. (Birmingham, NJ). NaSA solution of 0.15 M (pH 6–7) was prepared by dissolving an appropriate amount of NaSA in distilled deionized water (>99% SA was in the ionized form). The use of 0.15 M NaSA in the donor chamber was to maintain symmetric total ion concentrations in the donor and receiver chambers; otherwise, the constant electric field approximation would not be valid making data interpretation difficult (Kasting and Keister, 1989; Li et al., 2005). ACV solution of 0.01 M was prepared by dissolving ACV in deionized distilled water and subsequently adjusting the solution pH to 10 with concentrated sodium hydroxide. At pH 10, approximately 85% of ACV species was in its ionized form in the solution. Frozen split thickness cadaver skin was obtained from the New York Firefighters Skin Bank (New York, NY). The skin was from the posterior torso. The use of frozen cadaver skin was appropriate as it had been shown to have similar properties to freshly excised skin (Kasting and Bowman, 1990). Human epidermal membrane (HEM) was prepared by heat separation of the split-thickness human skin (Scheuplein, 1965; Sims et al., 1991; Peck et al., 1995).

2.2. Equations in the analysis of experimental data

The flux (J_i) and permeability coefficient (P, the flux normalized by donor concentration) for SA and ACV were calculated at steady state under sink conditions:

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

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

where C_D is the concentration of the permeant in the donor chamber, $\Delta Q/\Delta t$ is the slope of the plot of the cumulative amount of the permeant transported across the membrane into the receiver chamber versus time, and A_D is the area of the diffusional surface. Note that the term, permeability coefficient, is used not only to

describe passive transport but also for iontophoretic transport in the present study. In the plot of cumulative amount versus time, the straight line representing the steady-state region was extrapolated to the *x*-axis to determine the lag time. The flux of the permeant (J_i) was used to calculate the transference number (t_i):

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

where I_{total} is the total current, F is the Faraday constant, and z_i is the charge number. The transference number is the fraction of the current carried by the drug (permeant) to the total current carried by all ionic components in a system:

$$t_i = \frac{|z_i| J_i}{\sum_j |z_j| J_j} = \frac{I_i}{I_{\text{total}}} \tag{4}$$

where z_j is the charge number of ionic species j, J_j is the flux of all ionic species j in the system, and I_i is the current carried by the permeant. Ionic species j in the system represent both the negatively charged permeant as well as the positive charged counterions migrating in an opposite direction of the negatively charged permeant.

According to Nernst Planck theory, assuming a pore transport pathway model, steady-state flux of an ionized permeant molecule J_i during iontophoresis is expressed as:

$$J_i = \varepsilon_p \left[-D_p \frac{dC_p}{dx} - \mu_p C_p \frac{d\psi}{dx} \pm \upsilon_p C_p \right] \tag{5}$$

where ε_p is the porosity of the pore pathway, D_p is the diffusion coefficient, C_p is the permeant concentration, x is the position of the permeant in the membrane, μ_p is the electromobility of the permeant, υ_p is the convective solvent flow velocity, and ψ is the electrical potential. The first term in this equation represents diffusion, and the second term represents the movement of ionic species under the influence of an applied electric field (electrophoresis). The third term represents the movement of solvent along the charged pore surface in the membrane due to the momentum of the excess counterion in the solvent (electroosmosis). The effect of electroosmosis is generally small for small permeants from the anode to cathode at physiological pH in transdermal iontophoresis. However, ion-exchange membranes can alter electroosmosis in iontophoretic transport (Li et al., 2006).

2.3. Characterization and transport across Ionac

The ion-exchange membrane Ionac was characterized in permeant uptake and transport studies. In the uptake study, a similar procedure as described in a previous study (Li et al., 2006) was used to determine the amounts of ACV loaded in Ionac. Briefly, the membrane was cut to 1 cm × 1 cm pieces, weighed and pre-equilibrated in 0.01 M ACV solution at pH 10. Due to the low water solubility of ACV, each piece of 1 cm^2 Ionac membrane was pre-equilibrated in 20 ml of 0.01 M ACV aqueous solution shaken at 37 °C for 4 days. During pre-equilibration, the equilibrating solution was replaced with fresh solution every 12 h. At the end of pre-equilibration, the membranes were taken from the solutions and swabbed with Kimwipe to remove the solution on the membrane surface. The membranes were then equilibrated in 20 ml PBS adjusted to pH 10 with sodium hydroxide for 24 h at 37 °C with agitation for ACV extraction. After extraction, the membranes were withdrawn from the extracting solutions, and the amounts of ACV in the extracting solutions were determined. The extraction procedure was repeated for subsequent extraction/equilibration with fresh PBS at pH 10 (20 ml in vial) until the amounts of ACV extracted into PBS were less than 10% of that in the first extraction. In the ACV assay, samples were filtered using nylon membrane filters (Whatman, 0.45 μm). After filtration, 10-μl samples were diluted with 990 μl deionized water adjusted to pH 10 with sodium hydroxide. The samples were then assayed by high performance liquid chromatography (isocratic LC pump 20AT, Shimadzu) using a reverse-phase column (Microsorb-MV100-5, C18, 150 × 4.6 mm, Varian) and mobile phase composed of 98 parts of 0.1% acetic acid in water (v/v) and 2 parts of acetonitrile at flow rate of 1.0 ml/min and detector at 254 nm (UV–vis Spectrophotometer detector SPD-20A, Shimadzu). Calibration curves were prepared using ACV and deionized water at pH 10 and were found to be linear over the range of 10–500 μg/ml.

The loading capacity of SA in Ionac was determined in a previous study and this was not repeated here. In the transport study, a preliminary experiment with Ionac indicated a pre-equilibration step was appropriate; without pre-equilibration, the transport lag time of SA across Ionac was about 1 day (Li et al., 2006). Therefore, all experiments with SA using Ionac were conducted after the membrane had been pre-equilibrated in 0.15 M NaSA (the concentration of NaSA in the donor chamber). In the experiment, Ionac was cut to an area of about 1 cm^2. Each piece of 1 cm^2 Ionac was then placed in 10 ml of 0.15 M NaSA and left to equilibrate for 1–2 days. After pre-equilibration, the membrane was removed from the 0.15 M NaSA solution and placed in 2 ml of fresh donor solution spiked with radiolabeled ^{14}C-SA (at approximately 1 μCi/ml) and left to equilibrate for another day. Similarly, all experiments of ACV with the ion-exchange membrane were carried out after the pre-equilibration of membrane in 0.01 M ACV solution at pH 10. The same ACV equilibration procedure described above in the uptake study was used.

Experiments of passive and iontophoretic transport of SA and mannitol across Ionac were carried out to characterize the membrane in the present study and to compare the present results with those in a previous study. In the passive transport experiments of SA, the pre-equilibrated Ionac membranes were mounted in a side-by-side diffusion cell. The donor chamber was filled with 2 ml of the pre-equilibrating radiolabeled SA solution (0.15 M NaSA with ^{14}C-SA) and the receiver chamber was filled with 2 ml of PBS. An appropriate amount of ^{3}H-mannitol was then added to the donor chamber as the neutral model permeant. Both solutions were well stirred during the experiment. The passive transport experiment was carried out at 37 °C for 6 h. At predetermined time intervals, samples of 10 μl from the donor solution and 2 ml from the receiver solution were taken and then mixed with 10 ml of scintillation cocktail (Ultima Gold, Boston, MA). The samples were analyzed using a liquid scintillation counter (Beckman Coulter LS 6500 Multipurpose Scintillation Counter, Fullerton, CA). The amount of permeant transported across Ionac was calculated from the radioactivity and the specific activity of the radiolabeled permeant in the donor solution. The specific activity of the donor solution was calculated by dividing the radioactivity of the permeant by the total concentration of the radiolabeled and non-radiolabeled permeant in the donor solution. The permeability coefficient was then calculated from the steady-state flux region in the amount transported versus time plots assuming sink conditions, Eq. (2). This assumption is reasonable as the concentration in the receiver compartment was always less than 10% of that in the donor chamber. Iontophoretic transport of SA across one Ionac membrane and across an assembly of five membranes was carried out in a side-by-side diffusion cell at 0.5 mA DC. The experimental setup in these experiments was the same as the passive transport experiments described above. Phoresor II Auto, Model PM 850 (Iomed, Inc., Salt Lake City, UT) was the iontophoresis power supply, and Ag and Ag/AgCl electrodes were placed in the receiver and donor chambers, respectively. The anode was in the receiver and cathode was in the donor. The duration of the experiment was 1 h as the transport lag times were short

and the permeabilities were quite high under the 0.5 mA condition.

The same experimental protocols of passive and cathodal iontophoretic transport across one Ionac membrane as those of SA were used in the ACV experiments except that 0.01 M ACV at pH 10 was the donor solution. In these ACV experiments, each donor sample of 10 μl was diluted with 990 μl deionized water adjusted to pH 10 with sodium hydroxide. Then, the donor and receiver samples were filtered and assayed using the HPLC method described above.

In a separate experiment to assess the influence of the loading capacity of the membrane in the case of SA, the donor solution was completely replaced with fresh PBS 1 h after the start of the iontophoresis experiment. Particularly, this step was to address the question of how long the ion-exchange membrane could maintain quasi-steady-state transport during iontophoresis before the membrane SA was depleted (replaced by Cl ions).

2.4. HEM DC baseline experiments

HEM was mounted in a side-by-side diffusion cell with the stratum corneum facing the donor chamber and was supported by a 0.22 μm Millipore filter (Peck et al., 1993) on each side. Each half-cell had a volume of 2 ml, and the two half-cells were sealed at the connection with two rubber gaskets and Parafilm. A low electrical potential of ≤100 mV (waveform generator, Agilent 33220A, Santa Clara, CA) was applied across the system consisting of HEM in the diffusion cell and a fixed resistor in series. The voltage drop across the fixed resistor and that across HEM were measured and used to determine the initial resistance of HEM. Only HEM samples of initial resistance ≥10 kΩ were used in the present study. The mounted HEM was then allowed to equilibrate in PBS for 12–24 h at 37 °C in a circulating waterbath. The electrical resistance of HEM was checked again after this equilibration period. This equilibrating step was necessary to achieve a stable equilibrated HEM state. The integrity of HEM after this preparation was studied previously, and HEM was found to be stable without any significant changes in electrical resistance and permeability for several days (Peck et al., 1993). This condition also mimicked the conditions of fully hydrated skin in transdermal delivery systems such as long device and patch applications in practice. DC iontophoresis experiments of SA and ACV were conducted similar to that described in Section 2.3. Constant current DC of 0.1, 0.5, 1, or 2 mA was applied across HEM in the well-stirred diffusion cells using the phoresor. The voltage drop between the Ag/AgCl and Ag electrodes was measured, and the electrical resistance of the HEM system was calculated using Ohm's law. The duration of the experiments was 3–6 h, and samples were withdrawn from the donor and receiver chambers at predetermined time intervals (every 30–60 min). During sampling, the entire receiver chamber was withdrawn and replaced with 2 ml fresh PBS. Ten microliters of the donor solution was also withdrawn. The entire donor solution was replaced at predetermined times with fresh donor solution to prevent any changes in the pH or in the chemical composition of the donor solution as a result of ion transport and electrochemical reactions at the electrode surface (Li et al., 2006). The pH of the removed donor solution was checked and was found to be constant in all the experiments. The donor and receiver samples were then assayed as described in Section 2.3. The permeability coefficient and transference number were calculated from the steady-state flux region in the plot assuming sink conditions with Eqs. (2) and (3).

In order to obtain an accurate electrical resistance measurement of HEM, it was necessary to determine the electrical resistance contribution of the solution in the diffusion cell without HEM but only the Millipore filter support. In these control experiments, DC of 0.5 mA was applied across two Millipore filters mounted in the diffusion cells. The voltage drop without HEM was measured and the electrical resistance was calculated using Ohm's law. The electrical resistance of the solution and the Millipore filters (the system without HEM) were then subtracted from the resistance of the whole system (HEM, Millipore filters, and the solution) to obtain the resistance of HEM in the transport experiments. A four-electrode system (Li et al., 1999) was also used to provide occasional checks of the electrical resistance measurements of HEM. No significant difference was found between the results of the four-electrode system and the present system under the experimental conditions in the present study.

In a separate set of experiments, control experiments of SA transport across a three-electrode system was conducted using 0.1 mA DC. In this set of experiments, the three-electrode system described in Section 2.5 was used to determine the electrical resistance of HEM. These experiments were carried out to determine (a) whether the three-electrode system configuration might have an influence on the transference number of SA across HEM, and (b) if there might be a difference in the resistance measurements between the two (used in Section 2.4) and three-electrode systems (used in Section 2.5).

2.5. HEM in series with Ionac

Ionac membrane was pre-equilibrated in 0.15 M NaSA or 0.01 M ACV donor solutions as described in Section 2.3. In this HEM in series with Ionac study, HEM, Millipore filters, and the Ionac membrane were mounted together in the diffusion cell with the viable epidermis of HEM facing the receiver and Ionac facing the donor. The Ionac membrane was separated from HEM by the Millipore filter and an Ag/AgCl electrode was inserted between the Ionac and Millipore. The Ag/AgCl electrode permitted the direct determination of the voltage drop across HEM in this three-electrode system. Two rubber gaskets were placed on both sides of the "Millipore-HEM-Millipore-electrode-Millipore-Ionac" composite between the diffusion half-cells and the membrane composite and sealed with Parafilm to prevent potential leakage in the system. The initial resistance of HEM was measured using a method similar to that described in Section 2.4. A minor modification was made in the side-by-side diffusion cell setup in the later experiments with ACV, where the Millipore filter on the stratum corneum side in the assembly was further separated from HEM by a rubber gasket: this was found to improve the likelihood of success in the HEM integrity screening using the initial electrical resistance of HEM. The addition of the gasket could prevent/minimize current leakage between the stratum corneum and Millipore. After the HEM was allowed to equilibrate 12 h at 37 °C in PBS, cathodal iontophoresis of 0.1, 0.5, 1, and 2 mA DC was carried out for 3–6 h. Sampling followed the same protocol described in Section 2.4. The total electrical resistance of the HEM assembly was determined by the voltage drop and electric current across the assembly. The electrical resistance of HEM was calculated by subtracting the resistance of the solution and that of the Millipore filters from the total resistance.

2.6. Alternating current iontophoresis with Ionac

Previous studies have shown that HEM electrical resistance can be controlled by an AC electric field (Li et al., 1999; Song et al., 2002). The purpose of AC iontophoresis in the present study was therefore to reduce the electrical resistance of HEM and to maintain it at a constant level (0.5 or 2 kΩ) in Ionac-assisted iontophoresis. Permeabilization of HEM and controlling its resistance at a relatively low value with AC was hypothesized to be a critical condition for the ion-exchange membrane to dominate SA and ACV transport

Table 1
Transference numbers and permeability coefficients of SA and ACV and ratios of mannitol to SA permeability coefficients in passive and iontophoretic transport across an assembly of one and five Ionac membranes[a].

DC current (mA)	System	Permeant	Permeability coefficient (cm/s)	Transference number	*P* of mannitol divided by *P* of SA
0	1-Ionac	SA	$7.6 \pm 0.9 \times 10^{-6}$	–[b]	0.05 ± 0.02
0	1-Ionac	ACV	$6.0 \pm 1.2 \times 10^{-5}$	–[b]	–[c]
0.5	1-Ionac	SA	$3 \pm 1 \times 10^{-5}$	0.7 ± 0.2	0.03 ± 0.01
0.5	1-Ionac	ACV	$2.6 \pm 0.2 \times 10^{-4}$	0.54 ± 0.03	–[c]
0.5	5-Ionac	SA	$3.1 \pm 0.2 \times 10^{-5}$	0.69 ± 0.05	0.02 ± 0.01

[a] Mean ± SD ($n \geq 4$).
[b] Not applicable.
[c] Not determined.

across the system. The AC iontophoresis study was divided into three parts: Protocol A (the control), HEM alone at 0.5 kΩ maintained using AC with DC offset of 0.5 mA; Protocol B, HEM in series with one Ionac membrane with the resistance of HEM maintained at 0.5 kΩ using AC with DC offset of 0.5 mA; Protocol C, HEM alone at 2 kΩ maintained using AC with DC offset of 0.5 mA. These protocols allow the study of iontophoretic transport using SA and direct comparison with previous transscleral iontophoresis data (Li et al., 2006). For ACV, Protocol A was not conducted.

Iontophoretic transport experiments of HEM were carried out in the experimental setups described in Section 2.4 for Protocols A and C and Section 2.5 for Protocol B. In addition, a fixed resistor of known resistance was placed in series with HEM mounted in the diffusion cells in the electric circuit. A 1 kHz-AC field was applied across the system using a function generator (BK Precision Function Generator 4017A, Yorba Linda, CA) connected to a custom-made amplifier (Salt Lake City, UT) to reduce and maintain the resistance of HEM at 0.5 or 2 kΩ, and a cathodal DC offset current of 0.5 mA superpositioned with the AC was applied. Voltmeters were connected across HEM as well as the fixed resistor to measure the DC offset voltage across the HEM sample and that across the fixed resistor. The DC offset current applied across the system was monitored and calculated by the DC offset voltage across the fixed resistor using Ohm's law. The electrical resistance of the HEM membrane composite in the diffusion cell was calculated by the DC offset voltage across the composite and the DC offset current (Ohm's law). The electrical resistance of HEM was determined by subtracting the resistances of the solution and the Millipore filters from those of the HEM membrane composites of the two-electrode system for Protocols A and C and of the three-electrode system for Protocol B, respectively.

2.7. Statistical analyses

The data in the present study are presented as means and standard deviations (SD) with the n values representing the number of replicates. One-way ANOVA with Tukey post test was performed using GraphPad InStat (GraphPad Software, San Diego, CA) to compare the HEM transference number data of SA under all the iontophoresis conditions in the present study. The HEM transference numbers of ACV were compared separately using the same method. Unpaired *t*-tests were used to compare the data in Section 3.1.

3. Results

3.1. Characterization of Ionac

Using the method described previously (Li et al., 2006), the concentration of ACV in Ionac was determined to be approximately 1.6 M. This concentration is significantly higher than that of the equilibrating solution (0.01 M), consistent with the high surface charge density of the membrane. On the other hand, the concentration of ACV in the Ionac was lower than the 3 M of SA (Li et al., 2006). Given the size exclusion property of Ionac, this is consistent with the larger molecular size of ACV (molecular weight = 225 g/mol) compared to that of SA (molecular weight = 138 g/mol).

The results of the SA passive transport experiments with one Ionac membrane are consistent with those in a previous study (Li et al., 2006). Higher apparent permeability than the steady-state value was observed in the first 2 h of the experiment, likely due to SA unloading from Ionac before the steady-state SA concentration profile was established in the membrane. The permeability coefficient of mannitol was also determined for one Ionac membrane and found to be $3.0 \pm 0.1 \times 10^{-7}$ cm/s. The results of these experiments suggest that the Ionac membrane in the present study had the same characteristics as those in the previous study (Li et al., 2006).

Table 1 summarizes the transference numbers of SA across one Ionac membrane and across an assembly of five Ionac membranes in the iontophoresis experiments. The transference numbers of SA were 0.7 ± 0.2 and 0.69 ± 0.05 for one Ionac membrane and an assembly of five Ionac membranes, respectively. There was no significant difference observed between the one and five membrane results (*t*-test, $p = 0.96$), consistent with the electrotransport theory that fluxes across an assembly of membranes of the same pore size and charge are independent of the number of membranes in the assembly when iontophoresis is carried out at constant current and electrotransport is the dominant transport mechanism. Similar to the passive transport study, there were high initial fluxes during iontophoresis in both systems (one and five Ionac) before steady state was attained. This was again attributed to a higher initial release of SA similar to that seen under the passive transport condition. It was also observed that the time required to reach steady-state transport was longer in the five membrane system than a one membrane system. The transport of mannitol was used to assess the contribution of electroosmotic transport across the membrane systems. The last column in Table 1 shows the ratios between the permeability coefficients of mannitol and SA (calculated by dividing the permeability coefficients of mannitol to that of SA at steady state). These ratios suggest that the contribution of electroosmosis was not significant during iontophoresis.

Based upon the observation of no significant difference between the SA permeability coefficients in 1-Ionac and 5-Ionac systems, only the 1-Ionac system was employed in the ACV transport study. The permeability coefficient of ACV in passive transport was $6.0 \pm 1.2 \times 10^{-5}$ cm/s. The higher permeability coefficient of ACV than that of SA was primarily due to the lower concentration of ACV in the donor in the ACV experiment than that in the SA experiment, but having comparable SA and ACV concentrations in the membrane. The ACV permeability coefficient of DC iontophoretic transport was $2.6 \pm 0.2 \times 10^{-4}$ cm/s. This corresponds to around 4 times increase in ACV transport during iontophoresis from that of passive transport, and is consistent with the result in the SA study above. The transference of ACV across Ionac was 0.54, lower than the value of 0.7 of SA, which is possibly related to the larger molecular size (lower effective diffusivity and electromobility) of ACV than that of SA.

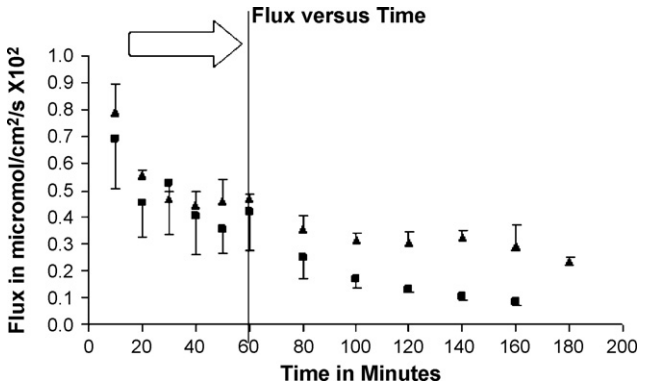

Fig. 1. Iontophoretic transport of SA across the ion-exchange membrane systems (one and five Ionac membranes). The flux $\times 10^2$ in micromol/cm^2/s is plotted against time in min. *Symbols*: 1-Ionac system, squares; 5-Ionac system, triangles. The arrow indicates the time when the donor solution was replaced with PBS.

To illustrate the loading capacity of SA in Ionac and its effects upon SA transport, the donor solution was replaced at 60 min in the iontophoresis transport study. When the donor solution was replaced with PBS (indicated by an arrow in Fig. 1), the flux across the 1-Ionac system dropped to about 25% of the steady-state flux value in 40 min. In the 5-Ionac system, the flux dropped to about 70% in 40 min. The five membrane system, in general, maintained a higher SA flux than the one membrane system over a significant length of time after the removal of the donor solution. In these transport experiments, the ratios of mannitol permeability to that of the permeability of SA were <0.05, indicating relatively negligible electroosmosis contribution to iontophoretic transport.

3.2. Ionac-assisted DC iontophoresis across HEM

Tables 2 and 3 present the transference numbers of SA and ACV across HEM during constant current iontophoresis of 0.1, 0.5, 1.0, and 2.0 mA without Ionac. The electric current levels did not significantly affect the transference numbers of SA and ACV, respectively, and the average transference numbers of SA was approximately 0.1 and that of ACV was approximately 0.009 under different current levels from 0.1 to 2 mA without Ionac. The relatively low transference numbers are consistent with the reported values in previous transdermal iontophoresis studies of similar anionic drugs (Bellantone et al., 1986; Phipps et al., 1988). For example, the transference number of ACV calculated from the data of a previous study (Volpato et al., 1995) was about 0.002 (compared to the transference number of 0.009 in the present HEM study). Given the different experimental condition of this previous study, e.g., buffer system and pH, the difference in transference numbers between the previous and present studies is reasonable.

Table 2
Summary of the transference numbers for SA transport across HEM and HEM in series with Ionac during 0.1, 0.5, 1.0, and 2.0 mA constant current DC iontophoresis.

DC current (mA)	Range of HEM resistance (kΩ)[a]	System	Transference number[b]
0.1	1.7–5	HEM	0.10 ± 0.04
0.1	0.2–2.2	HEM + Ionac	0.13 ± 0.05
0.1[c]	1.6–2.7	HEM	0.08 ± 0.02
0.5	2.5–5	HEM	0.12 ± 0.02
0.5	2.5–5	HEM + Ionac	0.15 ± 0.03
1.0	0.6–1.3	HEM	0.10 ± 0.02
1.0	0.7–2	HEM + Ionac	0.19 ± 0.04
2.0	1.0–1.2	HEM	0.08 ± 0.01
2.0	0.7–1.2	HEM + Ionac	0.15 ± 0.01

[a] Electrical resistance of HEM in diffusion cells of diffusional area approximately 0.8 cm^2.
[b] Mean ± SD ($n \geq 4$).
[c] Represents the experiments where a three-electrode setup (the same electrode setup as in the "HEM + Ionac" experiments) was used.

Table 3
Summary of the transference numbers of ACV transport across HEM and HEM in series with Ionac during 0.1, 0.5, 1.0, and 2.0 mA constant current DC iontophoresis.

DC current (mA)	Range of HEM resistance (kΩ)[a]	System	Transference number[b]
0.1	6–20	HEM	0.009 ± 0.003
0.1	2.8–5	HEM + Ionac	0.014 ± 0.008
0.5	2.3–5	HEM	0.009 ± 0.006
0.5	1–2	HEM + Ionac	0.008 ± 0.001
1.0	2.7–4	HEM	0.008 ± 0.003
1.0	0.7–1.8	HEM + Ionac	0.006 ± 0.002
2.0	1.0–2.2	HEM	0.010 ± 0.005
2.0	0.3–0.9	HEM + Ionac	0.009 ± 0.005

[a] Electrical resistance of HEM in diffusion cells of diffusional area approximately 0.8 cm^2.
[b] Mean ± SD ($n \geq 4$).

Tables 2 and 3 also present the SA and ACV 0.1, 0.5, 1.0, and 2.0 mA iontophoresis data when HEM was assembled with Ionac. Although there was a trend of higher average transference of SA with Ionac than that without (Table 2), no significant increase in the transference number of SA between DC iontophoresis and Ionac-assisted DC iontophoresis was observed (ANOVA, $p > 0.05$). In the case of ACV, there is no statistical difference between the transference numbers of ACV with and without Ionac (ANOVA, $p > 0.05$); the transference numbers fluctuated between 0.006 and 0.014 in the same current range (Table 3).

To examine the trend of higher SA transference numbers in Ionac-assisted DC iontophoresis, Fig. 2 shows a plot of the transference numbers of SA across HEM alone and HEM in series with Ionac at applied electric current of 0.1, 0.5 and 1, and 2 mA versus HEM electrical resistance during iontophoresis. It can be seen that the resistance of HEM tends to be lower at a higher applied current. Of more significance, HEM of lower resistance seems to show a higher degree of enhancement (determined by the ratio of the transference number in the presence of Ionac to that in the absence of Ionac); the enhancement tends to decrease as the resistance of HEM increases. The data in Fig. 2 suggest that higher permeant transference can be

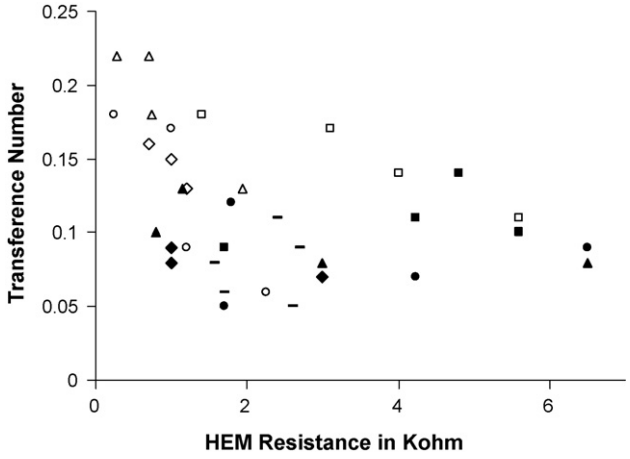

Fig. 2. Transference numbers of SA versus HEM resistance in constant current DC iontophoresis experiments with HEM alone at 0.1 mA (solid circles), HEM alone at 0.1 mA using a three-electrode system (dashes), HEM alone at 0.5 mA (solid squares), 1 mA (solid triangles), and 2 mA (solid diamonds) and with HEM in series with one Ionac at 0.1 mA (open circles), 0.5 mA (open squares), 1 mA (open triangles), and 2 mA (open diamonds).

Table 4
Transference numbers of SA and ACV and ratios of mannitol to SA permeability coefficients in 0.5 kΩ constant skin resistance AC iontophoretic transport of HEM and HEM in series with Ionac.

DC offset current (mA)[a]	HEM resistance (kΩ)[b]	System/permeant	Transference number[c]	*P* of mannitol divided by *P* of SA
0.5[a]	0.5	HEM, SA	0.13 ± 0.03	<0.05
0.5[a]	2	HEM, SA	0.11 ± 0.01	<0.1
0.5[d]	2	HEM, ACV	0.002 ± 0.001	–[e]
0.5[a]	0.5	HEM + Ionac, SA	0.32 ± 0.12[f]	<0.02
0.5[d]	0.5	HEM + Ionac, ACV	0.022 ± 0.010[f]	–[e]

[a] AC peak-to-peak voltage was 5–20 V.
[b] Electrical resistance of HEM in diffusion cells of diffusional area approximately 0.8 cm^2.
[c] Mean ± SD ($n \geq 4$).
[d] AC peak-to-peak voltage was 20–80 V.
[e] Not determined.
[f] Represents a significantly higher transference number in the presence of Ionac at 95% confidence using ANOVA.

obtained in Ionac-assisted iontophoresis as the resistance of HEM decreases. It was also observed that the resistance of HEM in series with Ionac, in some cases, was lower than that of HEM under the same experimental conditions.

The results of the three-electrode control experiments with SA at 0.1 mA DC are also presented in Table 2 to examine if the three-electrode configuration may affect iontophoretic transport across HEM and HEM electrical resistance. No significant differences of the transference number and HEM electrical resistance results were observed between the experiments conducted with the three-electrode and two-electrode setups under the studied condition (*t*-test, $p > 0.3$). There was no significant effect of the electrode setups upon the electrical resistance and the transference number.

3.3. Effect of Ionac on AC exposed HEM at 0.5 and 2 kΩ

To test the hypothesis that a reduction of HEM resistance would improve ion-exchange membrane enhanced iontophoretic transport, the resistance of HEM was lowered (relative to that of HEM during constant DC iontophoresis) and maintained at 0.5 or 2 kΩ by the application of AC. Experiments without Ionac at 0.5 or 2 kΩ were the control. In these experiments, AC voltages ranging from 5 to 80 V were applied to reduce the electrical resistance of HEM to the target value of 0.5 or 2 kΩ. A DC offset of 0.5 mA was applied across the system to provide the main driving force. The fluxes of SA and ACV transported across HEM in the AC iontophoresis experiments with Ionac as well as those transported across HEM under the same AC iontophoresis condition without Ionac were determined. For ACV, it was observed that the application of AC could not lower and maintain HEM electrical resistance in the HEM alone experiments constant at 0.5 kΩ. This is possibly due to the low concentration of ACV in donor solution in the experiments. Therefore, ACV data of 0.5 kΩ AC iontophoresis without Ionac are not available.

Table 4 summarizes the transference numbers of iontophoretic transport across HEM in series with Ionac during constant skin resistance AC iontophoresis of 0.5 and 2 kΩ. In these AC permeabilization experiments, the transference of SA was approximately 0.13 without the ion-exchange membrane. When one Ionac was used in conjunction with 0.5 kΩ AC iontophoresis and 0.5 mA DC offset, the average transference number increased to 0.32 (ranging from 0.23 to 0.55 due to skin-to-skin variability). An average three-fold increase of SA transference was demonstrated in the presence of Ionac (ANOVA, $p < 0.05$) relative to the control without Ionac when HEM electrical resistance was maintained at a relatively low level (0.5 kΩ). The average transference number of ACV was also significantly enhanced in the presence of Ionac with AC lowering the HEM resistance to 0.5 kΩ (ANOVA, $p < 0.05$). The average ACV transference under the "0.5 kΩ AC + 0.5 mA DC" condition in the presence of Ionac increased to 0.022 (ranging from 0.014 to 0.031), greater than two-fold enhancement compared to the HEM 0.5 mA DC baseline of 0.009. This is consistent with the SA results in the present study. Possible reasons of the smaller transference enhancement achieved for ACV than that for SA was (a) the larger molecular size of ACV than SA, (b) the different pK_a values of ACV and SA, and (c) the lower concentration of ACV compared to that of SA in Ionac.

It should be noted that the skin-to-skin variability (based on the coefficient of variation) observed in the presence of Ionac under the AC conditions was not significantly different from those observed in the DC experiments with and without Ionac. It was originally thought that the incorporation of an ion-exchange membrane can reduce skin-to-skin flux variability during iontophoresis when the iontophoretic flux becomes more controlled by the ion-exchange membrane. However, the ion-exchange membrane did not improve skin-to-skin variability, probably due to the dependence of the transference number on the HEM barrier and the variability in controlling the HEM barrier using its electrical resistance.

Another noteworthy observation was the relatively high sensitivity of the transference enhancement to the electrical resistance of HEM for SA and ACV with the AC Ionac technique. No significant Ionac-enhancement was found when the electrical resistance of HEM was not lowered to the 0.5 kΩ level. For example, in preliminary AC iontophoresis experiments with Ionac when HEM resistance was approximately 0.7 kΩ for ACV and 2 kΩ for SA, lower transference numbers were observed and the transference numbers approached that of the DC baseline (data not shown).

4. Discussion

4.1. Utility of Ionac-enhanced transdermal iontophoresis

Transdermal iontophoresis typically has low efficiency due to the relatively high concentration of endogenous ions having a charge opposite to that of the permeating drug (e.g. Na^+ and Cl^-). The low efficiency is also attributed to the high electromobility of these endogenous ions in comparison to the drug ion. Ion-exchange resins, membranes, or fibers have been employed previously to modify and improve iontophoretic drug delivery, but these systems do not operate under the same mechanism as that in the present study. For example, Conaghey et al. (1998) studied iontophoretically assisted delivery of nicotine, bound to ion-exchange resin, from a hydrogel vehicle. The utility of the ion-exchange resin in the drug depot was to increase its drug loading capacity. Yu et al. (2006) incorporated an anion-exchange fiber between the iontophoretic donor electrode and the drug (ketoprofen) reservoir to reduce "fluctuation of the release" of drug during iontophoresis. In other studies, cationic exchange membranes were incorporated

between the anode electrode and the drug loaded ion-exchange fiber to control iontophoretic delivery of tacrine (Kankkunen et al., 2002; Vuorio et al., 2004). Different from these previous studies, the present study investigated the use of an ion-exchange membrane to enhance iontophoretic transport of model drugs by the incorporation of an ion-exchange membrane between the tissue and the donor compartment. In this method, the flux enhancing mechanism is the hindrance of the competing current carrying counterions from the receiver across the skin into the donor. It has been previously shown in transscleral iontophoresis that the incorporation of an ion-exchange membrane this way can enhance permeant transference (Li et al., 2006). Other studies had proposed the use of ion-exchange membranes to enhance iontophoretic transport in implants (Schwendeman et al., 1994) and in transdermal delivery (Untereker et al., 1995). However, the conditions for effective ion-exchange membrane enhanced transdermal iontophoresis and the relationships between HEM electrical resistance and iontophoresis enhancement had not been extensively studied.

The ion-exchange membrane Ionac MA 3475 is permselective to negatively charged permeants such as SA and ACV. The transference number of SA across Ionac during iontophoresis was determined to be approximately 0.7, consistent with the results in a previous study (Li et al., 2006), and similar transference of ACV (transference number $\approx$0.5) was observed. Other properties of Ionac have also been investigated in the previous study. Ionac has high membrane charge density and small effective pore size. The concentration of SA and ACV in the membrane (in equilibrium with 0.15 M NaSA and 0.01 M ACV) was determined to be around 3 and 1.6 M, respectively. The effective pore size of Ionac was estimated to be 0.8 nm. While the small effective pore size could limit the use of Ionac to enhance iontophoresis of small molecules, this was not a concern for the purpose of the present study. With the high permselectivity to anionic permeants and the high membrane capacity for these anions, when Ionac was placed in series with a low resistance tissue in constant current iontophoresis, significant iontophoretic transport enhancement has been observed (Li et al., 2006; Molokhia et al., 2008). In addition, the high SA capacity in Ionac could sustain SA iontophoretic transport even in the absence of SA in the donor chamber as shown in the present study (Fig. 1). These characteristics made Ionac a good model membrane for the evaluation of ion-exchange membrane assisted iontophoresis in the present study. The high loading capacity of Ionac also suggests the utility of this membrane as the donor matrix that can be placed directly on HEM without an additional donor chamber in the iontophoresis device in ion-exchange membrane enhanced iontophoretic drug delivery.

4.2. Ionac-assisted transdermal iontophoresis

The present study shows no significant effect of electric current (within the range of 0.1–2 mA) upon permeant transference in HEM without Ionac. This result is consistent with the hypothesis that the properties of HEM transport pathway such as the effective pore size are invariant during constant current iontophoresis. Under constant current iontophoresis, when the electrical resistance of HEM decreases, the electrical potential applied across the membrane will decrease to maintain the same total current passage through HEM. If the iontophoresis electric current does not affect the properties of the transport pathway in HEM, transdermal flux will remain essentially constant. In the presence of Ionac with higher electric current during iontophoresis, the resistance of HEM decreased, and a trend of increasing transference was observed (i.e. greater influence of the ion-exchange membrane upon the iontophoretic transport) for SA and no effect was observed for ACV. Thus, the studied DC iontophoresis conditions provided only limited utility in ion-exchange membrane enhanced transdermal transport because these conditions were unable to sufficiently permeabilize HEM. To this end, AC iontophoresis that can reduce and control HEM electrical resistance was examined to exploit the potential of ion-exchange membrane enhanced iontophoresis.

Constant skin resistance AC iontophoresis is a convenient and robust method to reduce and control HEM electrical resistance (Li et al., 2003; Yan et al., 2004, 2005). Without Ionac, essentially the same permeant transferences were observed during 0.5 and 2 kΩ constant skin resistance AC iontophoresis of 0.5 mA DC offset for SA. In the presence of Ionac and when HEM was permeabilized to 0.5 kΩ during AC iontophoresis, an average of two- to three-fold enhancement was attained for SA and ACV. Transference numbers of up to 0.55 and 0.031 were achieved with this technique for SA and ACV, respectively, in the present study. Such enhancement is consistent with the hypothesis that transdermal iontophoretic transport utilizing an ion-exchange membrane can be improved by controlling HEM electrical resistance at the low value. Further flux enhancement may be possible as demonstrated in a previous transscleral iontophoresis study (Li et al., 2006). In this previous study, transference numbers as high as 0.6 was achieved with SA because the sclera membrane has higher SA passive permeability coefficient than that of 0.5 kΩ HEM. Future studies will attempt to optimize ion-exchange membrane enhanced iontophoresis.

Two studies were also conducted in parallel to the present study that provided insights into ion-exchange membrane assisted transdermal iontophoresis. First, neat ethanol was used to chemically perturb HEM before iontophoresis to examine the effects of lowering HEM resistance by ethanol upon Ionac assisted iontophoretic transport of SA. No significant flux enhancement was observed in iontophoretic transport across ethanol-perturbed HEM in the presence of Ionac compared to that of unperturbed HEM (unpublished results), and the inability of ethanol to lower HEM resistance sufficiently might explain the failure of this method to enhance iontophoretic transport in the presence of the ion-exchange membrane. Second, iontophoretic transport of a number of drug compounds including nicotine, lidocaine and TEA across HEM with and without an ion-exchange membrane was also investigated (unpublished results). Different from the results obtained in the ACV and SA studies, no significant enhancement in transport efficiency was achieved (compared to the HEM DC baseline) using the AC Ionac technique with these positively charged drugs and a cation-exchange membrane. This might be related to the high transference of the positively charged drug molecules in the DC baseline experiments (transference numbers were ~0.2–0.5) and the positive ion permselectivity of skin. Further investigation on pore size, pore charge and charge density of HEM and other factors such as the electrical resistance controlled by AC is needed.

5. Conclusions

The present study evaluated the ability of an ion-exchange membrane to enhance transdermal iontophoretic transport of a model permeant SA and an antiviral agent ACV. It was hypothesized that the high electrical resistance of HEM and its general barrier nature during iontophoresis significantly limited the utility of the ion-exchange membrane to enhance transdermal delivery compared to iontophoretic enhancement of more permeable tissues such as the sclera. To overcome this problem, AC iontophoresis was employed to reduce the electrical resistance of HEM to improve transdermal transport enhancement by Ionac. The results show that constant skin resistance AC iontophoresis, by maintaining HEM resistance at 0.5 kΩ, was effective in enhancing

transdermal iontophoretic transport of SA and ACV. Although it is uncertain whether the studied techniques may be applied to human subjects, the present study has demonstrated ion-exchange membrane enhanced transdermal iontophoresis of negatively charged drugs, and proposed a new approach to enhance transdermal iontophoretic transport.

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References

Abla, N., Naik, A., Guy, R.H., Kalia, Y.N., 2006. Topical iontophoresis of valaciclovir hydrochloride improves cutaneous aciclovir delivery. Pharm. Res. 23, 1842–1849.

Banga, A.K., 1998. Electrically Assisted Transdermal and Topical Drug Delivery. Taylor & Francis, London, Bristol, PA.

Bellantone, N.H., Rim, S., Francoeur, M.L., Rasadi, B., 1986. Enhanced percutaneous-absorption via iontophoresis. 1. Evaluation of an in vitro system and transport of model compounds. Int. J. Pharm. 30, 63–72.

Conaghey, O.M., Corish, J., Corrigan, O.I., 1998. Iontophoretically assisted in vitro membrane transport of nicotine from a hydrogel containing ion exchange resins. Int. J. Pharm. 170, 225–237.

Kankkunen, T., Sulkava, R., Vuorio, M., Kontturi, K., Hirvonen, J., 2002. Transdermal iontophoresis of tacrine in vivo. Pharm. Res. 19, 705–708.

Kasting, G.B., 1992. Theoretical-models for iontophoretic delivery. Adv. Drug Deliv. Rev. 9, 177–199.

Kasting, G.B., Bowman, L.A., 1990. Electrical analysis of fresh, excised human skin: a comparison with frozen skin. Pharm. Res. 7, 1141–1146.

Kasting, G.B., Keister, J.C., 1989. Application of electro-diffusion theory for a homogeneous membrane to iontophoretic transport through skin. J. Control. Release 8, 195–210.

Li, S.K., Ghanem, A.H., Peck, K.D., Higuchi, W.I., 1999. Pore induction in human epidermal membrane during low to moderate voltage iontophoresis: a study using AC iontophoresis. J. Pharm. Sci. 88, 419–427.

Li, S.K., Higuchi, W.I., Zhu, H.G., Kern, S.E., Miller, D.J., Hastings, M.S., 2003. In vitro and in vivo comparisons of constant resistance AC iontophoresis and DC iontophoresis. J. Control. Release 91, 327–343.

Li, S.K., Zhang, Y., Zhu, H., Higuchi, W.I., White, H.S., 2005. Influence of asymmetric donor-receiver ion concentration upon transscleral iontophoretic transport. J. Pharm. Sci. 94, 847–860.

Li, S.K., Zhu, H., Higuchi, W.I., 2006. Enhanced transscleral iontophoretic transport with ion-exchange membrane. Pharm. Res. 23, 1857–1867.

Molokhia, S.A., Zhang, Y., Higuchi, W.I., Li, S.K., 2008. Iontophoretic transport across a multiple membrane system. J. Pharm. Sci. 97, 490–505.

Padula, C., Sartori, F., Marra, F., Santi, P., 2005. The influence of iontophoresis on acyclovir transport and accumulation in rabbit ear skin. Pharm. Res. 22, 1519–1524.

Peck, K.D., Ghanem, A.H., Higuchi, W.I., 1995. The effect of temperature upon the permeation of polar and ionic solutes through human epidermal membrane. J. Pharm. Sci. 84, 975–982.

Peck, K.D., Ghanem, A.H., Higuchi, W.I., Srinivasan, V., 1993. Improved stability of the human epidermal membrane during successive permeability experiments. Int. J. Pharm. 98, 141–147.

Phipps, J.B., Gyory, J.R., 1992. Transdermal ion migration. Adv. Drug Deliv. Rev. 9, 137–176.

Phipps, J.B., Padmanabhan, R.V., Lattin, G.A., 1988. Transport of ionic species through skin. Solid State Ionics 28, 1778–1783.

Scheuplein, R.J., 1965. Mechanism of percutaneous adsorption. I. Routes of penetration and the influence of solubility. J. Invest. Dermatol. 45, 334–346.

Schwendeman, S.P., Amidon, G.L., Labhasetwar, V., Levy, R.J., 1994. 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. 83, 1482–1494.

Sims, S.M., Higuchi, W.I., Srinivasan, V., 1991. Skin alteration and convective solvent flow effects during iontophoresis. 1. Neutral solute transport across human skin. Int. J. Pharm. 69, 109–121.

Song, Y., Li, S.K., Peck, K.D., Zhu, H.G., Ghanem, A.H., Higuchi, W.I., 2002. Human epidermal membrane constant conductance iontophoresis: alternating current to obtain reproducible enhanced permeation and reduced lag times of a nonionic polar permeant. Int. J. Pharm. 232, 45–57.

Stagni, G., Ali, M.E., Weng, D., 2004. Pharmacokinetics of acyclovir in rabbit skin after i.v.-bolus, ointment, and iontophoretic administrations. Int. J. Pharm. 274, 201–211.

Untereker, D.F., Phipps, J.B., Cahalan, P.T., Brennen, K.R., 1995. Iontophoresis electrode, US Patent 5,395,310.

Volpato, N.M., Nicoli, S., Laureri, C., Colombo, P., Santi, P., 1998. In vitro acyclovir distribution in human skin layers after transdermal iontophoresis. J. Control. Release 50, 291–296.

Volpato, N.M., Santi, P., Colombo, P., 1995. Iontophoresis enhances the transport of acyclovir through nude mouse skin by electrorepulsion and electroosmosis. Pharm. Res. 12, 1623–1627.

Vuorio, M., Murtomaki, L., Hirvonen, J., Kontturi, K., 2004. Ion-exchange fibers and drugs: a novel device for the screening of iontophoretic systems. J. Control. Release 97, 485–492.

Yan, G., Higuchi, W.I., Szabo, A., Li, S.K., 2004. Correlation of transdermal iontophoretic phenylalanine and mannitol transport: test of the internal standard concept under DC iontophoresis and constant resistance AC iontophoresis conditions. J. Control. Release 98, 127–138.

Yan, G., Li, S.K., Higuchi, W.I., 2005. Evaluation of constant current alternating current iontophoresis for transdermal drug delivery. J. Control. Release 110, 141–150.

Yu, L.M., Li, S.M., Yuan, Y., Dai, Y., Liu, H.Z., 2006. The delivery of ketoprofen from a system containing ion-exchange fibers. Int. J. Pharm. 319, 107–113.