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Human epidermal membrane constant conductance iontophoresis: alternating current to obtain reproducible enhanced permeation and reduced lag times of a nonionic polar permeant

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Abstract

An experimental protocol, using an initial 1 min direct current (DC) applied potential of 4 V followed by alternating current (AC), was established to: (a) increase conductance and permeability and decrease lag time for human epidermal membrane (HEM) relative to unaltered HEM and; (b) maintain constant conductance and permeability during flux studies. The protocol allowed specific permeation parameters of the membrane to be characterized under electrically enhanced, constant flux conditions. The permeability, lag time, and effective membrane thickness were determined using a nonionic polar permeant, urea, while the enhanced conductance was maintained at a constant level with AC. A tortuous pore pathway model was employed to analyze the data. The AC protocol increased membrane permeability, and decreased lag time and effective membrane thickness relative to similar parameters obtained in previous studies from unaltered HEM. Lag times ranged from 32.0 to 105.5 min, and permeability coefficients calculated from steady state fluxes ranged from 1.68 to 6.03×10^{-7} cm/s for HEM samples with electrical resistance values during transport of 2.3–8.0 kΩ cm^2. Effective membrane thicknesses were calculated to range from 0.34 to 0.61 cm during AC iontophoresis. Significant additional results were obtained when the protocol was applied for two consecutive runs using the same HEM sample, with time for the HEM sample to recover between runs. During the second run, the applied potential was adjusted to reproduce the conductance obtained on the first run. Under these conditions, the consecutive runs yielded essentially the same lag time, permeability and effective membrane thickness values. These results suggest that constant fluxes can be achieved by keeping HEM electrical conductance constant during AC iontophoresis.

Keywords: Transdermal; Iontophoresis; Constant conductance; Permeation; Human epidermal membrane; Lag time

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1. Introduction

It has been well documented that transdermal drug delivery has many potential advantages relative to other routes of administration. Due to the remarkable barrier properties of the stratum corneum in skin, however, only a few drugs can be delivered transdermally at therapeutic levels by passive diffusion. For most therapeutic agents, the permeability values are too low, the lag times for permeation are too long and there is significant intra and inter subject variability. Iontophoresis has been studied as a means to achieve effective levels of transdermal drug flux in a reasonable time period by applying an electrical field across the skin to increase drug permeation and lower the lag time (Hinsberg, et al., 1995; Geest et al., 1997). Some products have been developed utilizing transdermal iontophoresis technology including the E-TRANS (fentanyl) electrotransport transdermal system (ALZA Corporation, Palo Alto, CA) (Gupta et al., 1998) and GlucoWatch automatic glucose biographer (Cygnus Inc, Redwood City, CA) (Tamada et al., 1999). Although adequate skin permeation enhancement has been achieved, long lag times and flux variability remain key issues. Transdermal delivery of fentanyl to relieve postoperative and other acute pain and the measurement of systemic glucose levels to guide the administration of insulin or oral hypoglycemic agents require, or would benefit from, shortened lag times and minimal flux variability. Despite the importance of lag time characterization, lag times determined in direct current (DC) iontophoresis are affected by the variable changes in the human epidermal membrane (HEM) barrier as a result of pore induction during iontophoresis. Iontophoretic transdermal drug delivery systems have been developed based upon DC as the driving force with the expectation that this method would yield constant permeant flux. Current information indicates that significant inter and intra subject variability is an issue that must be addressed. In short, the development of practical iontophoretic drug delivery devices would be greatly enhanced by the characterization of transport lag time and the ability to decrease flux variability.

The primary enhancement in permeant flux associated with iontophoresis is typically attributed to the direct interaction between the permeant and the electric field (electrophoresis) and/or the solvent flow in the pores (electroosmosis). Additional studies have shown that alterations in the barrier properties of HEM (pore induction or electroporation) that occur under an electric field are effective means to lower lag times and enhance transport flux (Hinsberg et al., 1995; Prausnitz, 1996; Li et al., 1998a, 1999; Chang et al., 2000). In addition to the electroporation caused by high voltage pulses (Prausnitz et al., 1994; Edwards et al., 1995; Chang et al., 2000), iontophoresis with low to moderate voltages also results in the induction of pores (Li et al., 1998a, 1999). A recent study showed that increases in conductance caused by alternating current (AC) iontophoresis were accompanied by proportional increases in permeability (Li et al., 1999). These data indicate that AC constant skin (or HEM) conductance iontophoresis may provide an alternative method to reduce the lag time and increase the permeability, while obtaining a more constant flux for transdermal drug delivery. The rationale for this expectation is the strong correlation that exists between permeant flux and HEM conductance (Flynn et al., 1974; Li et al., 1998b). Based on this correlation, it is reasonable to expect that if conductance could be maintained at a constant level, flux would also be constant.

In the present study, an AC technique was developed with the objectives of (a) increasing permeation and decreasing lag time of HEM transport (relative to untreated HEM); and (b) maintaining constant electrical conductance during iontophoresis. This technique was applied to characterize the membrane barrier properties of HEM during AC iontophoresis. A fundamental difference between these AC studies and similar DC experiments is the tendency for continuous pore induction during DC iontophoresis accompanied by continuous changes in membrane resistance. The AC studies rapidly achieved a constant level of membrane resistance, indicating constant membrane barrier properties, making it possible to characterize the transport lag time and permeability as well as the effective membrane thickness

for the HEM under conditions induced by AC iontophoresis.

2. Experimental section

2.1. Materials

Radio labeled [^{14}C]urea was purchased from Moravek Biochemicals and Radiochemicals (Brea, CA). HEM, which includes the stratum corneum and viable epidermis, was obtained by heat separation from back, abdomen or thigh split-thickness skin. Briefly, after the split-thickness skin was received from skin banks, HEM was removed from the dermis by immersing the skin in 60 °C water for 1 min and by careful peeling. HEM was then stored at −5 °C for later use. HEM electrical resistance was measured with a four-electrode potentiostat system (JAS Instrumental System, Inc, Salt Lake City, UT) (Li et al., 1997). Only HEM with electrical resistance of 15–110 kΩ cm^2 after more than 15 h equilibrating in 37 °C phosphate-buffered saline (PBS) was used in this study. Millipore membrane, used as a support membrane for HEM, was purchased from Millipore Corp (Bedford, MA). Polycarbonate Nucleopore membrane with a pore radius of 75 Å and porosity of 0.001 was obtained from Nuclepore Corp (Pleasanton, CA). PBS with ionic strength of 0.1 M and pH 7.5 was prepared from reagent grade chemicals and distilled deionized water. Sodium azide (0.02%) was added as a bacteriostatic agent.

2.2. Nuclepore membrane transport studies

The side-by-side diffusion cells used in these studies had diffusion areas of 0.75 cm^2, half-cell volumes of approximately 2.5 ml, and were maintained at 37 °C by a circulating water bath. A stack of 50 Nuclepore membranes was presoaked and sonicated in PBS before being mounted between two half-cells of a diffusion cell. The purpose of the around 30 min sonication was to help remove any air bubble trapped in the Nuclepore membrane. The junction between donor and receiver cells was clamped together and sealed with Parafilm. Two milliliter PBS was added to the well-stirred donor and receiver chambers. The Nuclepore experiments were divided into three stages. Stage I was a [^{14}C]urea passive diffusion experiment. Stage II was also a [^{14}C]urea diffusion experiment; however, immediately prior to the experiment, the membrane was pretreated by 1 min of 4 V DC, and during the transport experiment the applied field was changed to, 3 V, 12.5 Hz square-wave (symmetric) AC applied by a four-electrode potentiostat system (JAS Instrumental System, Inc) and a waveform programmer (Model JJ 1276, JAS Instrumental System) (Li et al., 1999). This electrical field protocol was selected because of our previous experience with AC; the frequency must be low enough for the measurement of membrane electrical resistance with the oscilloscope using the electrode system, and high enough to avoid AC flux enhancement. In these experiments, the electrical current across the Nuclepore membrane was monitored by an oscilloscope (Model 2211, Tektronix Inc, Beaverton, OR). Both the DC and AC electric fields were maintained by Ag/AgCl electrodes in both diffusion chambers. Calomel electrodes were the reference electrodes. Nuclepore electrical conductance was always found to be constant with electrical conductance being calculated by the ratio of electrical current to the applied voltage across the membrane. Stage III was a repeat of stage II.

The permeation studies were conducted during each stage by filling the receiver chamber with PBS and the donor chamber with PBS containing tracer-levels of [^{14}C]urea (in stage II and III, [^{14}C]urea was added into the donor chamber immediately after the 1 min of 4 V DC). At timed intervals, 1 ml samples were withdrawn from the receiver chamber and replaced with 1 ml of fresh PBS. The donor concentration was monitored by removing 10 μl samples. The donor and receiver samples were mixed with 10 ml of scintillation cocktail (Ultima Gold™, Packard Instrument Co., Meriden, CT) and assayed by liquid scintillation counting (1900TR, Liquid Scintillation Analyzer, Packard TriCarb Model). The permeation fluxes, J, and permeability coefficients, P, were calculated by:

$$J = \frac{\Delta Q}{A \Delta t} \quad \text{and} \quad P = \frac{J}{C} \tag{1}$$

where Q is the cumulative amount of solute transported across the membrane, t is time, C is the permeant concentration in the donor chamber, and A is the diffusion surface area of membrane. Between stages I and II, the diffusion cells were thoroughly washed with PBS and the membrane was equilibrated in PBS. After equilibrating for at least 12 h, the cells were again rinsed several times with fresh PBS prior to starting stages II and III. Residual permeant in the membrane after stage I could shorten the lag time of stage II and the same for stage III after stage II; therefore, the donor concentration for stages II and III was successively increased by a factor of four relative to the preceding stage in order to minimize any error caused by urea remaining in the membrane.

2.3. HEM transport studies

The HEM transport studies were conducted using the four electrode potentiostat system described in the Nuclepore section. The HEM sample was supported between the two half-cells of the diffusion cell with a Millipore membrane on the viable epidermis side facing the receiver chamber. Following cell setup, the HEM was allowed to hydrate in PBS at 37 °C for 12–24 h before the transport study. Following hydration, the initial resistance was calculated from Ohm's law by applying 100 mV DC across HEM and measuring the resulting current. Electrical conductance during the iontophoresis experiment was determined from the applied voltage and electrical current measured by the oscilloscope. During AC iontophoresis, the voltage and sampling protocol followed that used in stage II of the Nuclepore studies. The HEM transport studies were divided into two parts.

2.3.1. Part I

In these 1-day experiments, HEM was pretreated by 4 V DC for 1 min to rapidly induce pores, then the potential was switched to 3 V, 12.5 Hz square-wave (symmetric) AC and the electrical current across HEM was monitored until it reached a stable level. It took 1–1.5 h for HEM electrical conductance to reach a constant state. Once a constant conductance state was reached, tracer-level [^{14}C]urea was added to the donor chamber and the applied AC voltage was adjusted as necessary throughout the flux experiment (5–6 h) to maintain constant conductance (i.e. if the conductance decreased, the voltage was manually increased and if the conductance increased, the voltage was manually decreased). One milliliter samples were withdrawn from the receiver chamber every 50–70 min and replaced with 1 ml fresh PBS solution. Ten microliter samples were withdrawn from the donor chamber at each sample time. These samples were assayed as described for the Nuclepore membrane transport studies.

2.3.2. Part II

Following analysis of the HEM results from Part I, a new protocol was developed to allow two consecutive trials using the same HEM sample. These 2-day experiments included two stages. Stage I of the protocol was identical to the protocol described in Section 2.3.1. Following stage I, the cells were thoroughly rinsed with PBS and allowed to recover for at least 20 h before starting stage II. One hour prior to stage II, the cells were again rinsed several times with PBS. Similar to that in the Nuclepore membrane transport study, the donor concentration for stage II was increased by a factor of four relative to stage I in order to minimize any error caused by urea remaining in the membrane. During stage II, the applied voltage sequence followed the same protocol as stage I with the exception that following the 1 min of 4 V DC, the AC voltage was adjusted to achieve the same constant conductance level that was observed during stage I. AC voltage adjustments were made throughout the flux portion of stage II so that conductance levels were maintained as close as possible to stage I conductance levels measured during the previous flux experiment.

2.4. Theory

Permeability coefficients were determined directly from the steady state region of cumulative permeant transported into the receiver chamber

versus time plots using Eq. (1). Lag time, T_{lag}, was determined by extrapolating the linear region of the receiver permeant versus time plot (steady state flux data) to the abscissa (Martin and Bustamante, 1993). Steady state data, used to determine the permeability coefficient, were collected at times greater than 1.5 T_{lag}. Errors in lag times and permeability coefficients caused by extrapolation from assumed steady state flux at times greater than 1.5 T_{lag} (data from 1.5 to 6 T_{lag}) to the abscissa were estimated by simulating diffusion data using Scientist® software (MicroMath Scientific Software Inc, Salt Lake City, UT) for a membrane with an effective thickness of 0.41 cm and a porosity of 0.017. These simulations indicate that assuming steady state is reached within 1.5 T_{lag} leads to a T_{lag} value that differs from the theoretical T_{lag} by less than 4% and a permeability coefficient that differs from the theoretical value by less than 1%.

Due to the aqueous nature of the urea permeation pathway through HEM, lag time data are useful in studying permeation parameters such as porosity and tortuosity of the membrane (Peck et al., 1994, 1995; Li et al., 1998b). In a previous study, lag time data were successfully utilized to characterize the pore pathway in HEM before and after ethanol or chloroform–methanol treatment (Li et al., 1998b). Effective membrane thickness ($h\tau$) and porosity, ε, were calculated using the tortuous pore pathway model represented by Eqs. (2) and (3) (e.g. Flynn et al., 1974; Li et al., 1998b):

$$T_{lag} = \frac{(h\tau)^2}{6DH} \quad (2)$$

$$P = \frac{\varepsilon DH}{h\tau} \quad (3)$$

where h is the thickness of the HEM, τ is the tortuosity, D is the aqueous diffusion coefficient of the permeant, and H is the permeant hindrance factor for diffusion in the membrane. The pores during AC iontophoresis were modeled as cylindrical channels with constant cross section area. The membrane pore radius was assumed to be similar to the radius of the preexisting pore ($r \approx$ 20 Å) (Peck et al., 1994), giving a hindrance factor of 0.55 for urea diffusing in a 20 Å pore. The aqueous diffusion coefficient is 1.8×10^{-5} cm^2/s for urea (Peck et al., 1994).

3. Results and discussion

3.1. Nuclepore transport experiment

The Nuclepore experiments were conducted to establish the capability of the instrumentation in performing the proposed protocol before proceeding with HEM. Similar studies with Nuclepore membrane and 12.5 Hz square-wave AC iontophoresis at lower applied voltage have been conducted with mannitol as the model permeant (Li et al., 1999). These earlier studies showed that the permeability of Nuclepore membrane was the same during AC iontophoresis and passive diffusion (Li et al., 1999). The transport data obtained from the Nuclepore studies are shown in Fig. 1. As expected, the present study showed that the electrical resistance and urea permeability of Nuclepore during AC iontophoresis were essentially the same as those values measured during the passive stage. Moreover, as shown in Fig. 1, T_{lag} was the same for passive diffusion (stage I), 3 V square wave AC iontophoresis (stage II) and stage

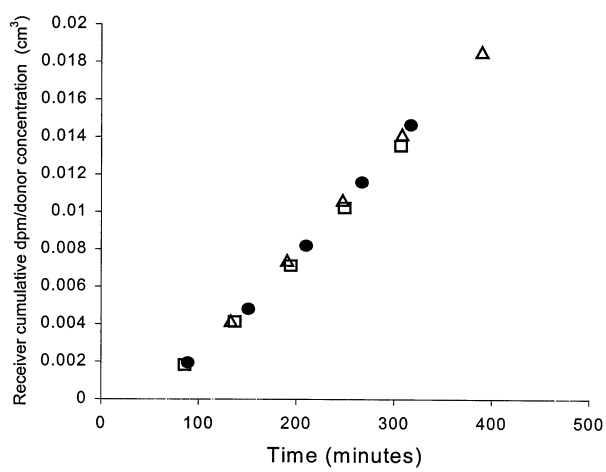

Fig. 1. Representative flux data of passive and 3 V, 12.5 Hz square wave AC iontophoretic permeation studies in Nuclepore membranes. Symbols: passive permeation (□), Stage II (●) and Stage III (△).

Table 1
Summary of Part I HEM 3 V, 12.5 Hz iontophoretic permeation study data

Sample	$R_{initial}$ (kΩ cm^2)	R_{AC} (kΩ cm^2)	P ($\times 10^{-7}$ cm/s)	T_{lag} (min)	$h\tau$ (cm)
1	83	8.0	1.7	105	0.61
2	44	5.6	2.7	69	0.50
3	20	2.6	4.5	41	0.38
4	15	4.5	3.6	58	0.46

Permeability coefficients (P) and lag times (T_{lag}) were determined from steady state flux data, electrical resistance (R) was directly measured, and effective thickness ($h\tau$) of HEM was calculated from the tortuous pore pathway model assuming a pore size of 20 Å.

III (repeat of stage II). These results support the expectation that AC iontophoresis, in the absence of membrane alteration, does not affect permeant flux and lag time (i.e. negligible effects from electroosmosis) and therefore provide a baseline for the HEM transport studies.

3.2. HEM experiment part I

Table 1 summarizes the data generated from four HEM samples for Part I of the HEM studies. The initial resistance ($R_{initial}$) of each HEM sample prior to the 4 V DC applied potential is shown and these observed values indicate representative intact HEM samples based upon electrical resistance criteria suggested by Kasting and Bowman (1990). R_{AC} represents the average resistance of the HEM following the application of the 4 V DC potential when the HEM reached a stable conductance level during 3 V, 12.5 Hz square-wave AC iontophoresis. The applied voltage regimen led to approximately an order of magnitude reduction in electrical resistance relative to $R_{initial}$. While the $R_{initial}$ values differ by a factor of 5.6 (ranging from 15 to 83 kΩ cm^2), the R_{AC} values differ by only a factor of 3.1 (ranging from 2.6 to 8 kΩ cm^2, which is in the same order of magnitude of the resistance observed during conventional constant current DC iontophoresis). As expected, based upon a porous permeation pathway, the permeability is inversely proportional to the electrical resistance measured during the flux studies, and there is no significant contribution of electroosmosis to flux as suggested in a previous study (Li et al., 1999). Urea permeability coefficient values in the range of 1.7 to 4.5×10^{-7} cm/s, T_{lag} values in the range of 41–106 min, and effective membrane thicknesses in the range of 0.38–0.61 cm indicate a general increase in permeability, decrease in lag time and a decrease in effective membrane thickness relative to unaltered HEM values. Values measured in a previous passive permeation study of unaltered HEM gave permeability coefficients ranging from 9.1×10^{-9} to 1.1×10^{-7} cm/s, lag times ranging from 64 to 733 min and effective membrane thicknesses ranging from 0.47 to 1.6 cm (Li et al., 1998b). The electrical resistance values measured in the previous study ranged from 16 to 81 kΩ cm^2 (Li et al., 1998b). These changes are attributed to membrane alterations such as pore induction caused by the combination of the initial 1 min 4 V DC potential pulse followed by the continuous 3 V AC electrical potential. Despite the significant inter-sample variability that is observed in the measured parameters, these results demonstrate that it is possible to generate an applied voltage regimen that initially alters the membrane by the induction of new pores and then maintains the membrane in a relatively constant flux state by applying AC. This observation could have significant implications in the development of practical iontophoretic devices where flux variations must be tightly controlled. These findings coupled with the correlation between permeability and electrical conductance could be a powerful means to obtain a constant target drug flux during iontophoresis.

The initial observations obtained from the Part I HEM studies led to Part II experiments that were designed to test the hypotheses that, (a) as the electrical conductance and permeation are directly proportional between HEM samples, if a

second trial was performed with the same HEM sample at the same conductance level as the first trial, the same permeability coefficient should be obtained; and (b) as conductance should be an indicator of membrane barrier parameters, a consecutive flux experiment using the same HEM sample at the same conductance level should yield the same T_{lag} value.

3.3. HEM experiment part II

Fig. 2a–d show the raw voltage and conductance data for the four HEM samples used in the Part II HEM experiments. Stage I data are identical to the description given above for Part I data. A large increase in conductance was observed due to 1 min of 4 V DC followed by a significant decrease in conductance, relative to the peak conductance, when the potential was changed to 3 V AC 12.5 Hz square-wave. Once the conductance became relatively constant, usually between 40 and 80 min, tracer-level urea was added to the donor chamber and the urea flux was measured. During the flux experiment, conductance was maintained constant by manually adjusting the output voltage. The amount of voltage adjustment necessary to maintain a constant conductance level varied from sample to sample. Stage II, performed after the HEM sample had recovered for at least 20 h after Stage I, followed the same protocol with the exception that the objective in adjusting the AC potential throughout the flux experiment was to obtain the same conductance that was observed during Stage I. Although it was not possible to exactly duplicate the Stage I conductance level during Stage II by manual AC potential adjustments, the Stage I and Stage II R_{AC} levels matched quite closely (Table 2).

Table 2 summarizes data obtained from Stage I and II of the Part II HEM studies. In each case, the HEM samples showed significant recovery following Stage I (compare $R_{initial}$ values of column 2 and 3). On average, the Stage II $R_{initial}$ values were 56% of the Stage I values while the Stage I R_{AC} values were 14% of the Stage I $R_{initial}$ values. Changes in the $R_{initial}$ values from Stage I to II indicate a degree of irreversible membrane alterations, at least in the time frame allowed for recovery, caused by the applied voltage. The primary motivation for conducting Stage II was to be able to compare permeability coefficients, lag times and effective membrane thicknesses from two consecutive trials when conductance was maintained constant. Fig. 3 shows the flux data for HEM sample b normalized by the donor concentration. As may be expected based upon the changes in $R_{initial}$, there is a trend of increasing permeability from Stage I to II. From the data in Table 2, on average, the Stage II P-values are within 25% of the Stage I P-values. Considering the variability in the $R_{initial}$ values between the two stages, the permeability coefficients are relatively constant. The greatest deviation in P-values was observed for sample c, where the permeability increased by 40%. For this sample, the Stage I conductance remained constant and required almost no adjustment of applied potential. During the Stage II flux study, it became difficult to maintain a constant conductance by voltage adjustments as indicated in Fig. 2c. During much of the Stage II flux study, the HEM resistance was lower than the Stage I resistance as attempts were

Fig. 2. Experimental applied voltage and HEM conductance data for 3 V, 12.5 Hz square wave AC iontophoresis transport study for, (a) HEM sample a, (b) HEM sample b, (c) HEM sample c, and (d) HEM sample d. The HEM electrical conductance was kept constant during the HEM AC iontophoretic permeation experiment by adjusting the applied voltage as necessary. Symbols: HEM electrical conductance (●) and applied voltage (△).

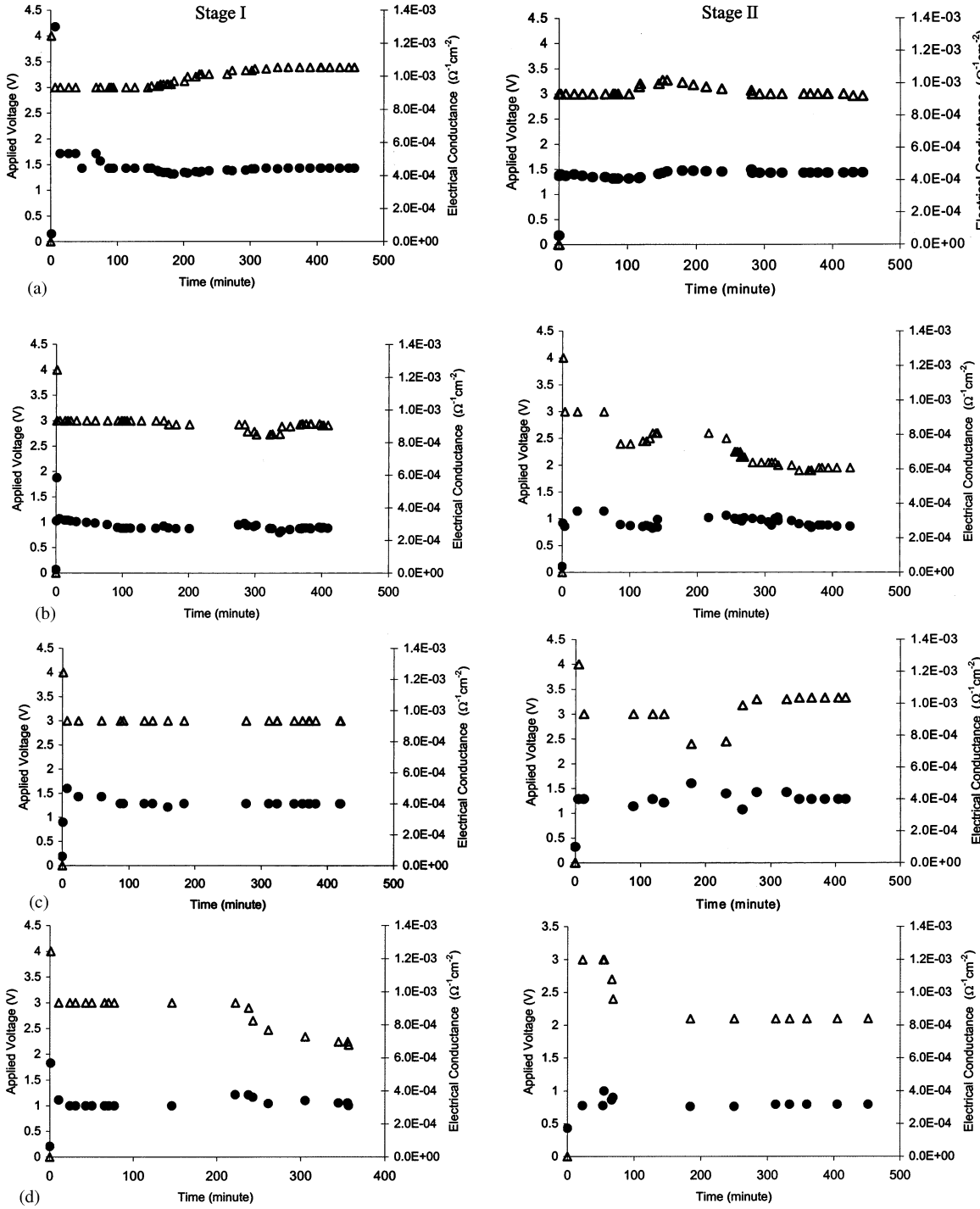
Stage I
Stage II
Applied Voltage (V)
Electrical Conductance (Ω⁻¹cm⁻²)
Time (minute)
(a)
(b)
(c)
(d)

Fig. 2.

Table 2
Summary of Part II HEM 3 V, 12.5 Hz iontophoretic permeation study data

Sample	$R_{initial}$ (kΩ cm^2)		R_{AC} (kΩ cm^2)		P ($\times 10^{-7}$ cm/s)		T_{lag} (min)		$h\tau$ (cm)	
Stage	I	II	I	II	I	II	I	II	I	II
a	21	13	2.3	2.3	5.2	6.0	40	32	0.38	0.34
b	48	30	3.8	3.8	3.3	4.1	54	63	0.44	0.47
c	17	10	2.5	2.3	4.3	6.0	47	41	0.41	0.38
d	16	5.8	3.3	3.2	4.6	5.5	58	57	0.45	0.45

Permeability coefficients (P) and lag times (T_{lag}) were determined from steady state flux data for each stage of the protocol, electrical resistance (R) was directly measured, and effective thickness ($h\tau$) of each HEM was calculated from the tortuous pore pathway model assuming a pore size of 20 Å.

made to make voltage corrections. The changes indicated significant membrane damage or breakdown during this experiment. As further evidence that HEM sample c did not remain completely intact, this sample did not show any resistance recovery following the Stage II flux study (data not shown).

The lag times and effective membrane thicknesses for the four HEM samples were essentially unchanged from Stage I to II. These constant parameters indicate essentially constant effective membrane barrier properties when constant conductance is maintained. To our knowledge, this is the first time a study has attempted to maintain a constant state of HEM conductance or resistance during iontophoresis. Despite significant differences in the initial resistance of the HEM samples between Stages I and II, returning the HEM to the same conductance seems to reestablish essentially the same barrier properties of the HEM. The only uncontrollable variable is the occasional significant breakdown of the HEM sample.

To gain insights into the actual enhancing effect of the applied potential upon urea permeation, the data obtained from HEM sample b with an initial electrical resistance of 48 kΩ cm^2 can be compared with the permeation data obtained from two HEM samples with electrical resistance of 46 and 49 kΩ cm^2 measured in a previous study (Li et al., 1998b). This comparison is valid in the present application because of the strong correlation that exists between urea permeation and electrical resistance of HEM (Peck et al., 1995; Li et al., 1998a,b). In the previous study, the HEM samples had P-values of 3.5×10^{-8} cm/s, lag times of greater than 300 min and effective membrane thicknesses of approximately 1 cm. During Stage I of the AC enhanced flux study, sample b yielded a P-value of 3.3×10^{-7} cm/s, a lag time of 54 min and an effective membrane thickness of 0.44 cm. The 10-fold enhancement in permeability, coupled with marked decreases in lag time and the effective length of the pore pathways in the membrane, strongly indicate the induction of new pores due to the applied voltage regimen used in this study (Li et al., 1998b). As the nature of the structural changes that occur during iontophoresis is still not clear (Hinsberg et al., 1995; Chizmadzhev et al., 1998; Banga et al., 1999), further studies are needed in this area. As further evidence that the permeant is following a porous pathway through the membrane and that passive diffusion is the transport mechanism (i.e. negligible electroosmosis contribution), the permeability coefficients and resistance during the permeation studies were plotted, along with data obtained from a previous study (Li et al., 1999), on a log–log plot that shows a slope of -1.0 (see Fig. 4). Despite differences in experimental conditions, urea permeability versus resistance data generated from a number of studies always seems to fall on the same line as shown in Fig. 4. The slope of -1.0 of the permeability and conductance data in Fig. 4 also is strong evidence that the increase in conductance is due to AC field induced pathway formation rather than changes in the ionic content of the existing pathways; for the latter, there would be no apparent a priori basis. It should be

considered a very surprising coincidence if significant electroosmosis during AC (which is unlikely according to the results in the Nuclepore membrane transport study) and any significant changes in the ionic content of the existing pathways would not result in significant deviation of the data from the line in Fig. 4.

3.4. Constant skin conductance iontophoresis

The outcomes of the present study have significant experimental and practical importance in the development of iontophoretic transdermal drug delivery devices. The applied electrical potential regimen succeeded in accomplishing several practical objectives. The membrane permeability and conductance was enhanced by an order of magnitude relative to baseline HEM properties by a short pretreatment with a moderate voltage DC pulse followed by the application of a sustained AC potential. Although maintaining an order of magnitude enhancement in permeability relative to baseline HEM properties is significant but not entirely surprising, it is possible that by adjusting the electrical potential parameters such as DC magnitude and pulse length, and AC potential and frequency that the enhancement in permeability and conductance could be further increased. Along with the permeation enhancement that was observed, the applied AC potential that followed the 1-min DC pulse effectively maintained the HEM in a state of enhanced permeability for urea. DC iontophoresis suffers from continuous membrane alterations. Although current is constant, changes in membrane parameters such as pore size can lead to significant changes in permeant flux (i.e. the transference number for a permeant is a function of both the permeant properties and the membrane properties). Continuous changes in the membrane barrier properties during the application of a DC field may account for some of the iontophoretic flux variability commonly observed in the literature in in-vivo (Gupta et al., 1998; Stagni et al., 2000) and in-vitro (Green et al., 1991; Delgado-Charro and Guy, 1994; Singh et al., 1995) studies. Larger flux vari-

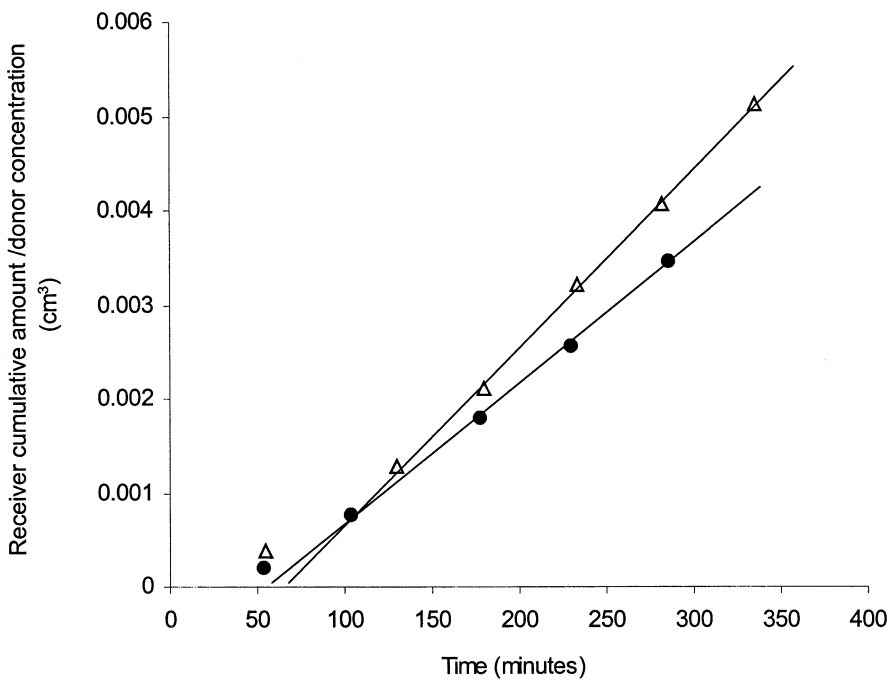

Fig. 3. Representative flux data of a 3 V, 12.5 Hz square wave AC iontophoretic permeation study of HEM (sample b). The HEM electrical conductance was maintained constant for each stage. Symbols: Stage I (●) and Stage II (△).

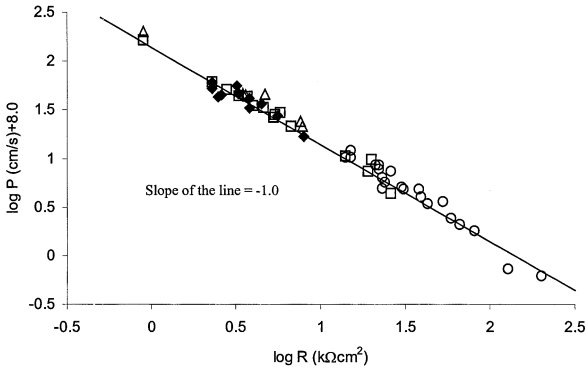

Fig. 4. Correlation between HEM electrical resistance and urea permeability showing data from the present study (solid diamonds) and data from a previous study (Li et al., 1999) (open marks). Symbols from the previous studies: passive before iontophoresis, circles; 12.5 Hz square-wave AC iontophoresis, squares; passive permeation after iontophoresis, triangles. Symbols from the present studies: 12.5 Hz square wave AC iontophoresis with constant electrical conductance, solid diamonds.

ability is generally observed for neutral permeants than that for small ionic permeants. A decrease in flux variability would be a major advancement in the practical applications of iontophoresis such as glucose monitoring and transdermal drug delivery (Tamada et al., 1995; Garg, et al., 1999). In the present study, there was an equilibration period between the DC pulse and the point that AC HEM conductance became stable. However, the magnitude of AC can be accordingly adjusted to eliminate or at least shorten this equilibration period. Once the HEM conductance stabilizes, a constant conductance state of the membrane can be maintained with only minor AC voltage adjustments. This is the first study to demonstrate the ability to maintain constant HEM conductance during iontophoresis, leading to an enhanced constant flux of a neutral permeant by electrically maintaining the membrane in a constant state of enhanced permeability. Future studies will investigate the practicality of using an AC potential to maintain the enhanced conductance of the HEM in parallel with a low DC potential to achieve electrophoretic and electroosmotic transport of charged and neutral permeants.

Part II of the HEM studies demonstrated that returning the HEM to a previous conductance state made it possible to duplicate the permeability value of a neutral permeant during a repeat experiment. It is reasonable to expect that obtaining a specific desired flux could be accomplished by obtaining a specific membrane conductance. A recent study by Zhu et al. (2001) demonstrated that increases in electrical conductance due to an applied electric field quantitatively predict flux enhancement due to the induction of pores when the background electrolyte ion size matches the permeant ion size. The correlation between HEM permeability and its conductance in the present study benefits from the relatively close match between urea and the background electrolyte size. It is not entirely clear at this point whether this correlation will continue to hold when the permeant molecular or ion size is significantly different from that of the background electrolyte ion size. Although preliminary data from our laboratory show such a correlation with another neutral permeant mannitol during constant skin conductance square-wave AC iontophoresis (unpublished data), future studies are required to establish how much general predictive capability lies in the correlation between electrical conductance and permeant flux. The findings of the present study and the results of Zhu et al. imply that it may be possible to obtain a desired permeability during iontophoresis simply by obtaining a particular membrane conductance through an appropriate AC voltage regimen. The ability to reduce variability and use electrical conductance to predict/control flux levels would be major milestones in practical transdermal iontophoresis development.

4. Conclusions

The present study demonstrated that the application of AC iontophoresis could maintain a constant state of enhanced electrical conductance of HEM. The enhanced conductance was attributed to the induction of pores caused by the application of the electric field. This method was applied to maintain a constant electrical conductance during the transport of urea across HEM. The constant conductance state made it possible to measure urea permeability, and lag time, as well

as the effective membrane thickness of the HEM during transport without the complications associated with the continual membrane alteration that is typically associated with DC iontophoresis. The application of the electric field increased urea permeability and decreased lag time and effective membrane thickness relative to untreated HEM. Consecutive transport studies with an individual HEM sample demonstrated that when identical conductance levels are maintained in consecutive trials, similar permeabilities and lag times are also observed. The outcomes of this study have important implications relative to reducing variability and controlling flux by maintaining a constant state of enhanced skin electrical conductance in practical iontophoretic devices.

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