Factors Affecting Sulfisoxazole Transport through Excised Rat Skin during Iontophoresis

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Permeation of sulfisoxazole (SIX) across the excised rat skin were studied using two chamber cell with four electrodes, under three successive experimental conditions: without current for 3 h (treatment I), with current for 4 h (treatment II) and without current for 3 h (treatment III). Transport of SIX was significantly increased by iontophoresis. The enhancement ratio of SIX flux were reasonably predicted by Goldman's equation. There was no significant difference ($p<0.05$) between the flux in treatment I and treatment III. On the basis of the flux reversibility, it was concluded that skin alteration did not occur when the applied electric potential was below 5.025 V. Although a prominent current-induced volume flow (from the anodal side to the cathodal side) was observed during current exposure, SIX flux was not influenced by the volume flow. The flux enhancement of SIX was mainly dependent on transdermal potential difference.

Keywords iontophoresis; skin permeation; sulfisoxazole; ionized drug; transdermal drug delivery; electro-osmosis; skin alteration; current-induced volume flow

Introduction

Iontophoresis is a process which causes an increased penetration of solute molecules into tissues by use of an applied electric current through the tissue.[1] Three factors have been shown to affect the enhancement of drug transport by iontophoresis, *i.e.* (1) the increased driving force of ionized solute molecules when an electrical potential gradient is applied[1] (electric factor), (2) the current-induced volume flow[2] (electro-osmotic factor), and (3) the alteration of skin properties induced by an electric current[3] (skin alteration).

Without current exposure, the permeation of ionized drug across the skin is known to follow Fick's law of diffusion (diffusion factor). The iontophoretic flux enhancement is considered to be caused by both electric and electro-osmotic factors in addition to the diffusion factor. If the properties of the skin were changed during iontophoresis, the diffusional characteristics, *i.e.* diffusion coefficients in the skin or partition coefficients to the skin would also be changed. Increased penetration of a nonionized or ionized drug may occur due to electro-osmosis or skin alteration during iontophoresis.[2b]

The purposes of the present study were to determine the effect of the three aforementioned factors, and to clarify the mechanism of iontophoretic enhancement of drug penetration using excised rat skin. Sulfisoxazole (SIX, $pK_{a1}=1.55$, $pK_{a2}=5.10$) was used as a model ionized drug. The iontophoretic flux enhancement ratios (flux with current/flux without current), transdermal potential difference (*Er*), the flux of SIX before and after exposure to the electrical current and the current-induced volume flow (*Vf*) on SIX transport were investigated.

Experimental

Preparation of Solution Unless otherwise stated, all chemicals were used as received and all solutions were made using ion exchanged water. Most transport studies were carried out using McIlvaine buffer solution: 0.181 M Na_2HPO_4, 9.08 mM citric acid, 8.35 mM NaCl, ionic strength 0.5 M (pH was brought to 7.4 at 32 °C by the addition of less than 2 ml of 1 N HCl per liter of buffer solution). The resulting osmolarity of the buffer was about 300 mOsm as determined by the osmometer (electric semi-micro osmometer, Type M, Knauer, Berlin, West Germany). Sulfisoxazole (JP XI, Yamanouchi, Tokyo, Japan) was dissolved in the buffer solution (5 mg/ml) and [^{3}H]H_2O (NEN Research Products, Boston, MA) was diluted with the buffer solution (5 μCi/ml).

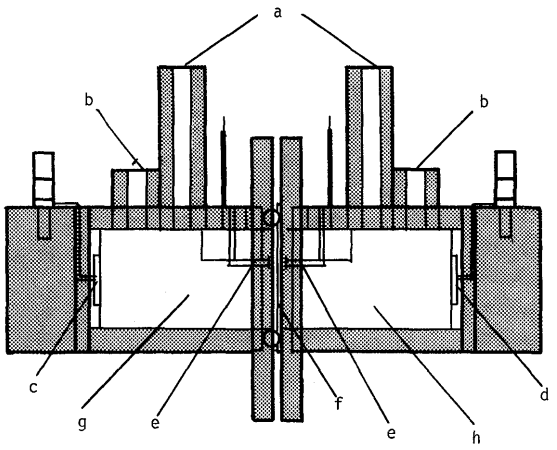

Fig. 1. Two-Chamber Diffusion Cell System with Four Electrodes

a, stirring port; b, sampling port; c, anode; d, cathode; e, reference electrode; f, skin; g, epidermal side; h, dermal side. The volume of the half cell is 15.6 ml.

Diffusion Studies Diffusion cells with four electrodes (Fig. 1) were made by us and used in all transport studies. A 5.6 cm^2 area of tissue membrane was exposed to the donor and receptor compartments of each diffusion cell. The reservoirs (15.6 ml each) were stirred mechanically by external electric motors (Oriental Motor Co., Ltd., Tokyo, Japan). Diffusion cells were immersed in a water bath maintained at 32±0.5 °C to simulate the condition in which a transdermal therapeutic system is used. A set of electrodes consisted of an anode, a cathode, and two reference electrodes. Each electrode was made of Pt plate (99.9% purity). Unless otherwise stated, the anode and cathode were each positioned 4 cm from the side of the tissue membrane; the cathode was placed on the epidermal side, whereas the anode was placed on the dermal side. The reference electrodes were attached carefully to the tissue membrane so as not to damage it. Constant electric potential was supplied by a programmable constant electric potential source (Seto Elec. Co., Toyama, Japan). The pH of reservoirs was monitored by a pH meter (Horiba F-7 type, Kyoto, Japan) during iontophoresis and was maintained within 7.4±0.1.

Skin Transport Studies All transport studies were carried out by using freshly excised abdominal skin of male albino rats (Wistar strain, Shizuoka Laboratory Animal Center, Hamamatsu, Japan) weighing 300 to 350 g. Transport studies were performed by mounting the excised tissue in the diffusion cell, placing plain buffer solution in the chamber adjacent to one side of the tissue, adding buffer solution containing SIX or [^{3}H]H_2O to the other chamber and turning on the external electric motor. The starting time was defined as the time when the buffer solution containing drug or radiotracer was added to the chamber. The duration of each treatment was chosen as a compromise so as to provide enough time to achieve a steady state. Unless otherwise stated, skin transport studies involved the following three steps of treatment. Transport of SIX or volume flow was

monitored for 3 h without current (treatment I), for 4 h with current (treatment II) and for 3 h without current (treatment III). In treatment II, each of applied electric potentials were 0 V (data not shown), 1.675 V (data for volume flow not shown), 3.35 and 5.025 V. It has been shown that the current density at these electric potentials did not cause perceptible physical discomfort in humans.[4] The sample solutions (0.5 ml each) were withdrawn from the chamber at predetermined time intervals. The volume of cell fluid was kept constant by addition of 0.5 ml of buffer solution. The concentration of SIX was determined by colorimetry after diazotization (wave length = 560 nm, Shimadzu UV-240, Kyoto, Japan). The $[^3H]H_2O$ samples were mixed with 4 ml of Atomlight (NEN Research Products, Boston, MA) and counted on a liquid scintillation counter (type 300c, Packard Instrument Company, Downers Grove, IL).

The potential difference between two reference electrodes, the transdermal potential difference (*Er*), was measured by a digital potentiometer (Sanwa MD-150C, Tokyo, Japan) at predetermined intervals. Details of the experimental conditions are shown in Table I.

TABLE I. *In Vitro* Experimental Conditions

Exp. No.	Epidermal side	Dermal side	Appl. volt. (V)
1	A	B[a,b]	1.625, 3.35, 5.025
2	A[b]	B[a]	3.35, 5.025
3	C	B[a,b]	3.35, 5.025
4	B[a]	C[b]	3.35, 5.025
5	C[b]	B[a]	3.35, 5.025
6	B[a,b]	C	3.35, 5.025

A is SIX (5 mg/ml) in pH 7.4 McIlvaine buffer solution, B is the pH 7.4 McIlvaine buffer solution and C tritiated water (5 μCi/ml) in pH 7.4 McIlvaine buffer solution. *a*) Sampling side. *b*) Anodal side.

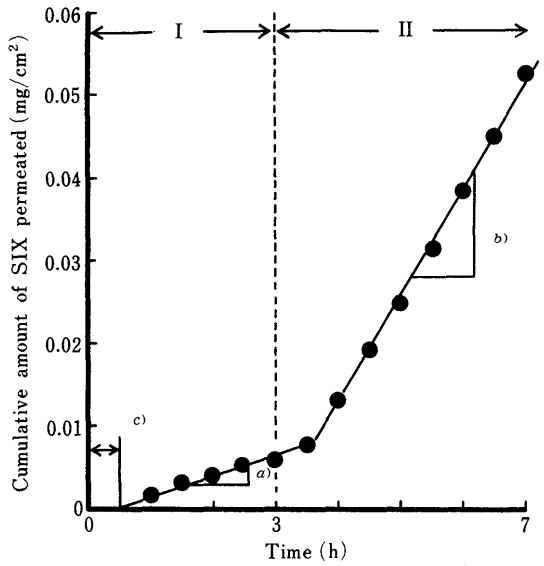

Fig. 2. Schematic Representation of Steady-State Permeation Rate (Q_{ss}) in Treatments I and II, and Lag Time

a—*c*) are steady-state flux in treatments I and II and lag time, respectively.

Determination of Diffusion Coefficient in the Skin and Partition Coefficient to the Skin (Exp. No. 1) Cumulative quantities of the SIX appearing in the receiver compartment were plotted as a function of time. The flux value at the steady-state without current (Q_{ss} in treatments I and III) and with current (Q_{ss} in treatment II) were calculated from the slope of the linear portions of the plot. Diffusion coefficient (*D*) and partition coefficient (*K*) were calculated from Eqs. 1 and 2.[5]

$$D=\frac{L^2}{6\ \text{lag time}} \tag{1}$$

$$K=\frac{Q_{ss}(\mathrm{I})L}{DC_0} \tag{2}$$

where L is the thickness of skin, and C_0 is the concentration of drug solution. $Q_{ss}(\mathrm{I})$ represents the Q_{ss} in treatment I. The lag time was determined from the intercepts on the time axis by extrapolating from the steady-state permeation profile. These procedures are shown schematically in Fig. 2.

Determination of Volume Flow (Exp. No. 3—6) The *i*-th apparent volume flow ($F_{e\to d}$ (*i*), $F_{d\to e}$ (*i*): subscript e→d indicates the flow from epidermis to dermis) was calculated from the permeation data of $[^3H]H_2O$ using Eqs. 3 and 4:

$$F_{e\to d}(i)=\frac{\mathrm{cumDPM}_{e\to d}(i)-\mathrm{cumDPM}_{e\to d}(i-1)}{t\,\mathrm{DPM}_e} \tag{3}$$

$$F_{d\to e}(i)=\frac{\mathrm{cumDPM}_{d\to e}(i)-\mathrm{cumDPM}_{d\to e}(i-1)}{t\,\mathrm{DPM}_d} \tag{4}$$

where $\mathrm{cumDPM}_{e\to d}(i)$ and $\mathrm{cumDPM}_{d\to e}(i)$ represent the cumulative amount of tritiated water per cross sectional area of diffusion cell (expressed in DPM/cm²) appearing at the *i*-th sampling time on the dermal side and epidermal side, respectively. DPM_e and DPM_d are the concentration (expressed in DPM/ml) of radiotracer solution on the epidermal and the dermal side, respectively, t is the interval between the *i*-th and $i-1$-th samplings, and i, k and n are sampling number, initial sampling number and final sampling number, respectively. $F_{e\to d}$ value consists of diffusion and volume flow terms, and $F_{d\to e}$ value consists of only the diffusion term, if the volume flow occurs in the direction of the dermal side. Assuming that these diffusion terms have equal value, total volume flow in the direction of dermal side ($Vf_{e\to d}$) was calculated by the use of Eq. 5.

$$Vf_{e\to d}=\frac{\sum_{i=k}^{n}(F_{e\to d}(i)-F_{d\to e}(i))}{n-k+1} \tag{5}$$

Statistics The statistical significance was evaluated by the two-tailed Student's *t* test. The 0.05 level of probability was taken as the level of significance.

Results and Discussion

SIX Permeation across the Skin (Exp. No. 1) Figure 3 shows the cumulative amount of drug on the dermal side as

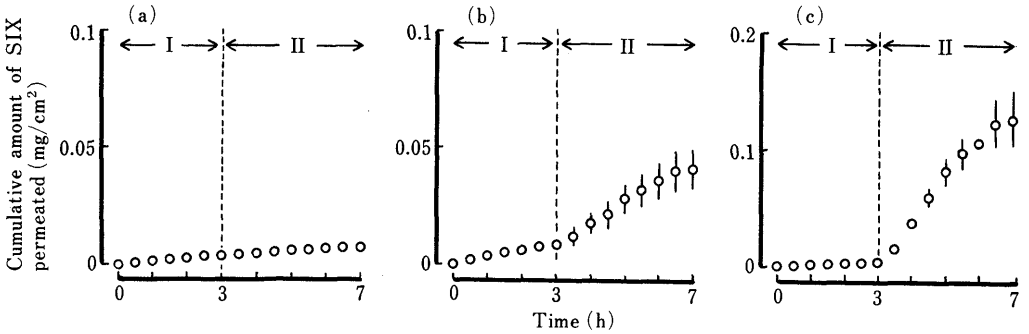

Fig. 3. Time Course of Cumulative Amount of SIX before Current Exposure (Treatment I) and during Iontophoresis (Treatment II) in Exp. No. 1

The applied electrical potential (E_a) was (a) 1.675 V, (b) 3.35 V, and (c) 5.025 V. (I) and (II) refer to treatments I and II. Results are expressed as the mean ± S.D. of 3—5 experiments.

a function of time during treatment I and treatment II (exp. No. 1). In this case, the applied potential was 3.35 or 5.025 V. Each drug flux was significantly increased and achieved a steady-state value (Q_{ss} in treatment II) thereafter.

Applied Electric Potential Difference (E_a) and Transdermal Potential Difference (Er) (Exp. No. 1) Figure 4 shows the relationship between E_a and Er which was determined 1 h after the initiation of the current exposure. The relationship between Er and E_a was not linear but Er increased abruptly above an E_a level of 3.0 V. This result may be explained by a potential drop caused by the electric double layer existing in the solution on the surface of both cathode and anode.[6] That is, the applied potential does not directly affect the potential gradient in solution.

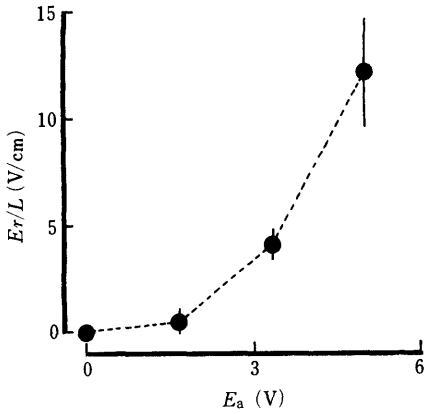

Fig. 4. Relationship between Applied Potential (E_a) and Potential Gradient in the Skin (Er/L)

Each point and bar represent the mean ± S.D. of 3—5 experiments.

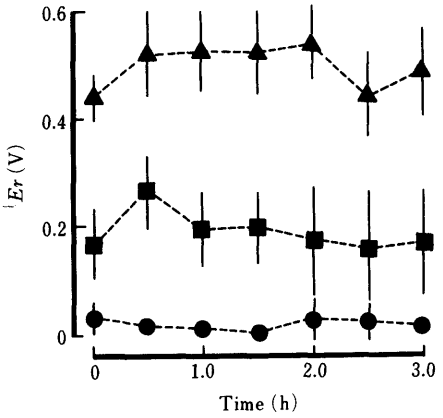

Fig. 5. Time Course of Transdermal Potential Difference (Er)

The applied electrical potential (E_a) was 1.675 V (●), 3.35 V (■) and 5.025 V (▲). Results are expressed as the mean ± S.D. of 3—5 experiments.

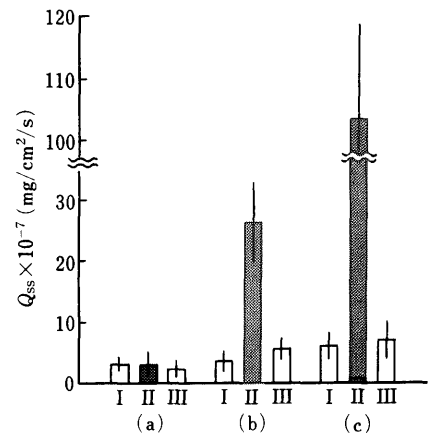

Fig. 6. The Steady-State Flux (Q_{ss}) in Treatments I, II and III

The vertical lines indicate the mean ± S.D. I, treatment I; II,. treatment II; III, treatment III. The applied electrical potential (E_a) was (a) 1.675 V, (b) 3.35 V, and (c) 5.025 V.

If the impedance of the solution is constant during current exposure, it is possible to evaluate the skin impedance, an indicator of the electrical barrier of the skin,[7] by determining the Er value. Figure 5 shows the relationship between Er value and time of current exposure. At each applied potential, Er was approximately constant. This fact indicates that the skin impedance as well as the driving force for transfer of the charged drug (electrical potential) may not be changed during iontophoresis.

The Steady-State SIX Flux in Each Treatment (Exp. No. 1) The steady-state SIX fluxes (Q_{ss}) in treatments I, II, and III are shown in Fig. 6. When the applied potential was 3.35 and 5.025 V, Q_{ss} in treatment II was about five and fifteen times greater than Q_{ss} in treatment I respectively, though no change of Q_{ss} was not observed at 1.675 V because the transdermal potential difference was very small. There was no significant difference ($p<0.05$) between Q_{ss} in treatment I and the Q_{ss} in treatment III. This fact indicates that alteration in the skin permeability characteristics did not occur at the applied electrical potentials (1.675, 3.35, 5.025 V) employed.

Assuming that the charged drug permeation is independent of other ion species, the steady-state drug flux in treatment II across the skin (the ratio of $Q_{ss,ion,calc}$) is described by Goldman's equation,[6] as shown in Eq. 6.

$$Q_{ss,ion,calc}=\frac{DKC_0}{L}\frac{-ErZF/RT}{1-\exp(ErZF/RT)} \quad (6)$$

where Z is the charge number, F is the Faraday constant, R is the gas constant and T is the temperature in K. The iontophoretic enhancement ratio (Q_{ss} in treatment I/treatment II) is given by Eq. 7.

$$\text{enhancement ratio}=\frac{-ErZF/RT}{1-\exp(ErZF/RT)} \quad (7)$$

In the present study, $Z=-1$, and $T=305$ K. Theoretical values for enhancement ratio were calculated. The results are shown in Fig. 7a. The plotted points show the observed data on the iontophoretic enhancement ratio and the solid line shows calculated values according to Eq. 7. A close agreement between experimental data and calculated values was observed. Figure 7b shows the correlation between calculated value and observed value of Q_{ss} in treatment II. The correlation coefficient (r^2) was 0.978.

The Effect of Current-Induced Volume Flow on the Drug Permeation across the Skin (Exp. No. 2—6) The apparent

Table II. The Flux of SIX and the Volume Flow under Various Conditions

Er (V)	Anodal side	Q_{ss} (I)[a]	Q_{ss} (II)[a]	$Vf_{e\to d}$[b]
3.35	Epidermal	2.82	0.43	11.41
3.35	Dermal	4.35	26.18	−5.22[c]
5.025	Epidermal	1.65	0.10	50.14
5.025	Dermal	4.21	103.45	−30.64[c]

a) Steady-state flux in treatment I $\times 10^{-7}$ (mg/cm²/s). *b*) Steady-state flux in treatment II $\times 10^{-7}$ (cm/s). *c*) A minus sign indicates that overall volume flow occurred from dermis to epidermis.

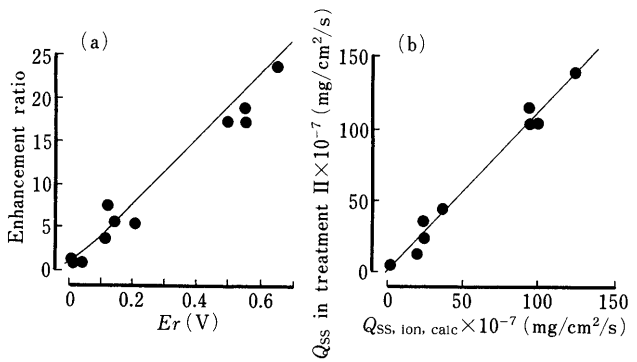

Fig. 7. The Enhancement of SIX Flux by Iontophoresis

(a) The relationship between transdermal potential difference (Er) and the iontophoretic flux enhancement ratio is shown. The solid curve represents the flux enhancement ratio calculated by using transdermal potential difference. (b) The relationship between observed and calculated steady-state flux (Q_{ss}) during iontophoresis is shown. The solid curve represents the line obtained by a linear least squares method ($r^2=0.978$).

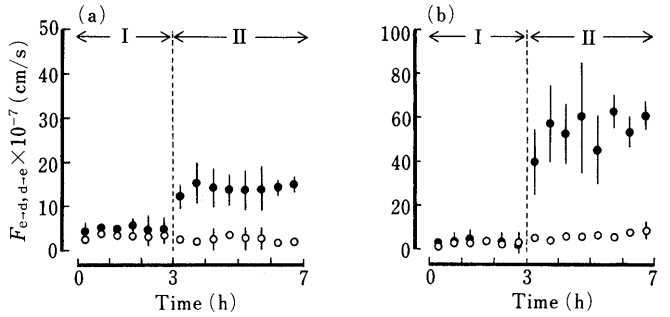

Fig. 8. Time Course of Apparent Volume Flow ($F_{e\to d}$, $F_{d\to e}$) in Treatments I and II

The anode was placed on the epidermal side. The applied electrical potential (E_a) was (a) 3.35 V, and (b) 5.025 V. ●, apparent volume flow in the direction of the cathodal side ($F_{e\to d}$); ○, apparent volume flow in the direction of the anode side ($F_{d\to e}$).

volume flow ($F_{e\to d}$, $F_{d\to e}$) *versus* time is shown in Fig. 8 (exp. No. 3—4). Before current exposure, no significant difference between the apparent volume flow to the dermal side ($F_{e\to d}$) and the epidermal side ($F_{d\to e}$) was observed. During current exposure (anode on the epidermal side), $F_{e\to d}$ increased and then achieved a steady state, whereas $F_{d\to e}$ was not changed significantly. This result suggests that current-induced volume flow occurs to the cathodal side when an external electric potential exists.

Miyamoto *et al.*[8] suggested that there is a power coupling process between hydraulic power and electric power *via* ionic power. That is, the potential difference applies the driving force to cations, and to anions in the opposite direction. Each force is transmitted to the volume flow by friction between ions and the solvent. The direction of volume flow is determined by the ionic species and the character of the membrane. Since the volume flow was observed to the direction of anode to cathode in the present study, it was considered to be caused by cation (Na^+) flow. Our observation is consistent with the results of Miyamoto *et al.*

In order to clarify the relationship between the skin permeation of SIX and the current-induced volume flow, SIX permeation studies were performed in the reversed polarity condition, namely with the anode on the epidermal side (exp. No. 2). Figure 9 shows the cumulative amount of SIX *versus* time in exp. No. 2. Although passive transport

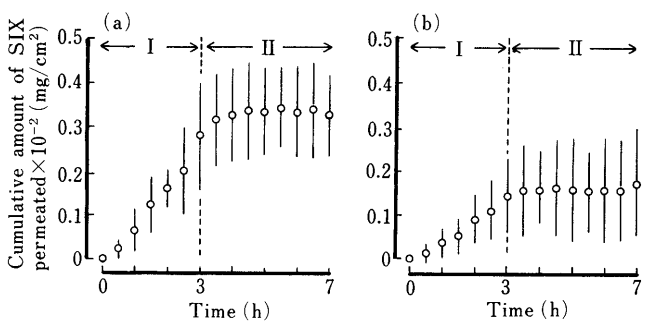

Fig. 9. Time Course of Cumulative Amount of SIX before Current Exposure (Treatment I) and during Iontophoresis (Treatment II) in Exp. No. 2

The applied electrical potential (E_a) was (a) 3.35 V and (b) 5.025 V. Results are expressed as the mean ± S.D. of 3—5 experiments.

caused by the concentration gradient occurred before current exposure (treatment I), drug permeation was not observed after current exposure (−3.35, −5.025 V). Under these experimental conditions, it is assumed that current-induced volume flow would occur with current exposure, in the same direction as the passive transport of SIX. This result indicated that the driving force applied to the drug caused by electric power is much larger than that by volume flow or the concentration gradient.

The total volume flow ($Vf_{e\to d}$) and the steady-state drug flux (Q_{ss} in treatments I and II) under all experimental conditions are summarized in Table II. These data indicate that the volume flow was induced by current in the same direction as cation flow and in the opposite direction to SIX flux. As seen in the glomerular filtration of several drugs (mannitol, inulin and *etc.*), the drug flux as a result of volume flow is given by the product of the concentration of the drug (C_0) and flow rate ($Vf_{e\to d}$).[9] Consequently, the contribution of current-induced volume flow to the drug permeation should be taken into account; however, under these experimental conditions, the current-induced volume flow did not affect the SIX permeation.

In conclusion, we clarified the following three points. (1) During iontophoresis, the permeability characteristics of the skin were not changed under a given external potential (5.025 V), (2) the flux of SIX (Q_{ss}) across the skin increased with current exposure and (3) the contribution of the current-induced volume flow was negligible.

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