



Effect of pH on Iontophoretic Transport of Pramipexole Dihydrochloride across Human Epidermal Membrane

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ABSTRACT

Purpose Drugs with higher molecular charges generally show higher flux enhancement when electromigration is the main mechanism in transdermal iontophoresis. This study evaluated the effect of decreasing the formulation pH to increase the positive charges of pramipexole dihydrochloride (PXCl) on its iontophoretic transport across skin.

Methods *In vitro* transdermal iontophoresis of PXCl in buffer solution isotonized with either sodium chloride or mannitol were performed in a pH range of 3.0–7.0. Experiments of iontophoresis under symmetric condition with respect to donor and receiver pH and passive transport of the drugs after pretreatment with iontophoresis were conducted to investigate the transport mechanism involved.

Results Iontophoretic permeation of PXCl was pH-dependent in drug solution isotonized with mannitol. The iontophoretic flux of PXCl with valence $z = +2$ at pH 3.0 was half of that of PXCl with $z = +1$ at pH 7.0. The results suggest that the decrease in PXCl delivery at higher valence at pH 3 was mainly due to pH-dependent selectivity of PX ion permeation across the skin and not electroosmosis.

Conclusions Skin permselectivity is a significant factor for iontophoretic transport of PXCl, and reducing formulation pH to increase the positive charges on PX ions did not enhance PXCl delivery.

ABBREVIATIONS

AP	Acetaminophen
c	Concentration for ion
J_{EM}	Electromigration flux
J_{EO}	Electroosmosis flux
μ	Electrophoretic mobility
HPLC	High performance liquid chromatography
HEM	Human epidermal membrane
HCl	Hydrochloric acid
pI	Isoelectric point
LOD	Limit of detection
LOQ	Limit of quantitation
$J_{Passive}$	Passive delivery flux
P	Permeability coefficient
PBS	Phosphate buffered saline
PXCl	Pramipexole dihydrochloride
PX	Pramipexole
J	Steady-state flux
NaCl	Sodium chloride
NaOH	Sodium hydroxide
v	Solvent flow velocity due to electroosmosis
SD	Standard deviation
J_{total}	Total iontophoretic flux
t_i	Transference number
z	Valence for ion

KEY WORDS iontophoresis · pH · pramipexole · transdermal · skin permselectivity

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INTRODUCTION

Pramipexole (PX), an aminothiazole derivative, is a selective dopamine receptor agonist. It has been widely used as a first-line drug for the treatment of early and advanced Parkinson's disease due to its long-term efficacy, safety, and tolerability (1,2). PX dihydrochloride (PXCl) is the salt form of PX in commercial drug products. Oral administration of dopamine agonists often cause fluctuations in plasma concentration resulting in pulsatile dopaminergic stimulation and poor

patient compliance in Parkinson's disease patients suffering from motor symptoms and dysphagia (3). Therefore, an alternative route for PX is desired for continuous and more patient-friendly drug delivery to improve symptomatic treatment of the drug and patient compliance (4,5). PX contains two ionizable amine groups (primary amine and secondary amine with pKa of 5.0 and 9.6, respectively) on its structure (6). It has a molecular weight of 211.33 g/mol and is available as dihydrochloride salt possessing good aqueous solubility and a net positive charge at physiological pH. Thus, PX cannot permeate across the skin effectively unless penetration enhancement is accomplished in the delivery of the drug. Among the skin penetration enhancement techniques (7,8) such as chemical penetration enhancer, prodrug approach, carrier systems and iontophoresis, iontophoresis is a promising method due to its significant flux enhancement on ionic drugs (9) and the potential of programmable delivery taking advantage of the multiple charges on PX ion.

Transdermal iontophoresis is an effective method to deliver ionized or charged compound across skin using an electric field as an external driving force (9). For iontophoresis, transport enhancement of ionic permeants is a result of electromigration, electroosmosis and electric field-induced skin alteration (10). Several factors affecting iontophoretic drug transport include pH of the donor solution, drug concentration, solution ionic strength, current intensity and type of current (9,10). In theory, the transport efficiency depends on the fraction of the applied current carried by the ion of interest. The fraction of current carried by a particular ion is described by its transference number (t_i) and can be calculated by Eq. 1 (11):

$$t_i = \frac{\mu_i |z_i| c_i}{\sum_j \mu_j |z_j| c_j} \quad (1)$$

where μ is the electrophoretic mobility, z is the valence, and c is the concentration for ions i and j , respectively.

Formulation pH has a significant influence on the ionization of drug and skin related to transport enhancement due to electromigration and electroosmosis in transdermal iontophoresis. A change in solution pH in transdermal iontophoresis affects the net positive valence of PX ions and the degree of skin ionization and subsequently the contribution of electromigration, electroosmosis and skin permselectivity to the iontophoretic drug transport (12–14). Previously, Kalaria *et al.* (15,16) investigated the mechanism of iontophoretic transport of PXCl across pig and human skin and found that electromigration is a predominant transport mechanism for PXCl iontophoresis with minimum contribution from electroosmosis. In theory, when electromigration is the main mechanism in transdermal iontophoresis with small contribution from electroosmosis, drugs with higher molecular charges are

expected to provide higher flux enhancement due to the increase in their electrophoretic mobilities. In other words, for drugs with the multiple charges such as PX ion (positive charges of +1 and +2 with respect to the two values of pKa), it is expected that PX ion with a net valence of +2 at pH 3 would provide higher flux than PX ion with a net valence of +1 at pH 7. Therefore, it is reasonable to investigate whether adjusting the pH of PX solution to increase its valence would enhance the iontophoretic delivery of PXCl, which has not been systematically studied.

The objective of this study was to determine the influence of pH on iontophoretic transport of PXCl due to drug ionization (molecular charges of +1 and +2). As would be shown in the present study, the iontophoretic flux of PXCl with net valence of +2 at pH 3.0 was lower than that of PXCl with net valence of +1.5 at pH 5.0, which in turn was lower than that with net valence of +1 at pH 7.0. Therefore, another objective was to investigate the mechanism of the slower PX ion transport observed at pH 3.0 and the faster transport at pH 7.0 under the influence of formulation pH. In this study, anodal iontophoresis experiments at pH ranging from 3.0 to 7.0 were conducted with constant direct current of 0.5 mA/cm². Passive permeation experiments without iontophoresis were the control for comparison. As the ion composition of drug formulation affects the transference numbers of ions and subsequently transdermal iontophoretic transport of PXCl, iontophoresis experiments were performed as a function of pH in buffer solution isotonized with sodium chloride (NaCl) in comparison with that in buffer solution isotonized with mannitol to investigate the ion competition effect. Acetaminophen (AP), a neutral drug permeant, was concomitantly delivered with PXCl by iontophoresis to determine the contribution of electromigration and electroosmosis to the iontophoretic transport of PXCl. Passive transport of PXCl and AP was measured at different pH conditions after iontophoresis pretreatment to evaluate skin permselectivity to PX ions and its effect on pH-dependent iontophoretic transport.

MATERIALS AND METHODS

Materials

PX and AP were purchased from Xi'an Lyphar Biotech Co. (Shaanxi, China) and Bengbu Bayi Pharmaceutical Co., Ltd. (Anhui, China), respectively. The hydrochloride salt of PX was prepared by a procedure described by Balicki *et al.* (17) with some modifications. PXCl monohydrate with a molecular weight of 302.06 g/mol were analyzed by elemental

analysis and high-performance liquid chromatography (HPLC). PXCl monohydrate was used in this study. NaCl and trisodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) were supplied by Carlo Erba Reagent (Milan, Italy). Citric acid ($C_6H_8O_7$) and sodium azide (NaN_3) were obtained from Sigma-Aldrich (St. Louis, Missouri). D-mannitol, sodium dihydrogen orthophosphate (NaH_2PO_4) and disodium hydrogen orthophosphate (Na_2HPO_4) were available from Ajax Finechem (New South Wales, Australia). HPLC grade acetonitrile was supplied by Honeywell Burdick and Jackson (Ulsan, Korea). Triethylamine was obtained from Merck (Hesse, Germany). Phosphoric acid was provided by J.T.Baker[®] (Central Valley, Pennsylvania). Agarose was purchased from Life Technologies Corporation (Carlsbad, California). Silver sheet (99% purity) was purchased from a local precious metal alloy manufacturer in Bangkok, Thailand. Potassium chloride was provided by VWR Prolabo BDH chemicals (Flemish Brabant, Belgium). Buffer solutions of 0.01 M citrate buffer (pH 3.0 and 5.0) and 0.01 M phosphate buffer (pH 6.0 and 7.0) were prepared by mixing appropriate amounts of buffering agents in water: $C_6H_8O_7$ and $C_6H_5Na_3O_7 \cdot 2H_2O$ for citrate buffer and NaH_2PO_4 and Na_2HPO_4 for phosphate buffer, respectively. These buffer solutions were utilized to prepare the drug solution. Phosphate-buffered saline pH 7.4 (PBS) consisting of 0.01 M phosphate buffer and 0.142 M NaCl was used to prepare skin and serve as a simulated physiological fluid in the receiver chamber for transport study. All buffer solutions were preserved by 0.02% (w/v) NaN_3 . The pH of each buffer solution was checked with a pH meter (Inlab[®]; Mettler-Toledo International Inc., Columbus, Ohio).

Skin Preparation

Human skin of female aged 22–68 years old was obtained from abdominoplastic surgical operations (Department of Surgery, Yanhee International Hospital, Thailand). Human epidermal membrane (HEM) was prepared by heat separation technique (18). Briefly, all residual subcutaneous fat was removed with surgical scissors. Subsequently, the skin was immersed in PBS at 60°C for 1 min. The epidermis was carefully peeled off from the underlying dermis using plastic forceps. The HEM was soaked in PBS, blotted dry, wrapped in aluminum foil and stored at –20°C for later use. This protocol was approved by the committee on human rights related to human experimentation, Mahidol University, Thailand (COE. No. MU-DT/PY-IRB 2018–058.2911).

Preparation of Isotonic Solution of PXCl

Briefly, either 1 or 10 mg/ml PXCl isotonic solution was prepared by dissolving PXCl monohydrate in each buffer solution (i.e., 0.01 M citrate buffer pH 3.0, 0.01 M citrate buffer

pH 5.0, 0.01 M phosphate buffer pH 6.0 and 0.01 M phosphate buffer pH 7.0). NaCl or mannitol was added in each solution to adjust the tonicity. The concentrations of NaCl in the NaCl isotonized PXCl solutions of pH 5.0, 6.0 and 7.0 were 0.142, 0.145 and 0.143 M, respectively. The concentrations of mannitol in the mannitol isotonized PXCl solutions of pH 3.0, 5.0 and 7.0 were 0.269, 0.256 and 0.257 M, respectively. The pH of the drug solution was adjusted to the desired pH by adding 1 M NaOH or 1 M HCl. PXCl was freely soluble in the buffer solutions of citrate buffer pH 3.0 and 5.0 and phosphate buffer pH 6.0 and 7.0 (solubility from 140 mg/ml to >300 mg/ml at pH 7.0 to pH 3.0, respectively) determined in a separate PXCl solubility study.

Transport Studies: General Setup

In vitro drug transport of PXCl across HEM was studied in vertical Franz diffusion cells. Prior to the experiments, HEM samples were cut into $\sim 4 \times 4$ cm² and allowed to hydrate in PBS at 4°C in a refrigerator overnight. HEM was mounted on the diffusion cells with an effective area of 1.77 cm². The stratum corneum faced upward to the donor chamber and the viable epidermis faced the receiver chamber of the diffusion cells. A regenerated cellulose membrane (Spectra/Por[®] 4 MWCO 12,000–14,000, Spectrum Laboratories, Inc., Rancho Dominguez, California) was placed between the viable epidermis side of the HEM sample and the receiver chamber. Prior to starting the experiment, the skin was equilibrated with PBS for 1 h. The integrity of HEM was ensured by electrical resistance measurement according to Ohm's law. The electrical resistance of the supporting cellulose membrane was negligible compared to the skin; cellulose membrane resistance was comparable to that of the buffer solution and had no significant effect on the reading of skin resistance. In addition, there was no significant difference of drug permeability coefficient between transport experiment across HEM with and without supporting cellulose membrane in a preliminary study. HEM with electrical resistance ≥ 15 –20 k Ω ·cm² was reported to have good integrity (19,20). Therefore, only the HEM with initial electrical resistance ≥ 20 k Ω ·cm² was used in the experiment. After assembly of the diffusion cells, 1 ml of drug solution was pipetted into the donor chamber. The precise amount of buffer solution (~ 5 ml) was added into the receiver chamber and continuously stirred with a magnetic stirrer. HEM temperature was maintained at 32 ± 1 °C by a thermostat heater (Safety Thermostat 911 model, PolyScience, Niles, Illinois).

Anodal iontophoresis experiments (anode electrode in the donor) were performed by applying electric current across the HEM with an iontophoresis device (ActivaDose[®] II model, ActivaTek[™], ActivaTek, Inc., Gilroy, California). Ag and AgCl electrodes were placed in each glass scintillation vial containing 0.3 M NaCl. The electrodes were connected to

donor and receiver chambers by salt bridges to avoid the interactions between PXCl and the electrodes during iontophoresis (electrochemical reactions at the electrodes). The salt bridges were prepared by dissolving 2% (w/v) agarose in 0.3 M NaCl and filling a silicone tube with the agarose solution (21). A direct current of 0.5 mA/cm² was applied continuously for 6 h and post-iontophoresis study was continued until 24 h.

During the transport experiments, samples of 300 µl were withdrawn from the receiver chamber at predetermined time intervals (i.e., 0, 1, 2, 3, 4, 5, 6, 9, 12, 18 and 24 h) unless stated otherwise. The same volume of fresh PBS was added back to the receiver chamber to maintain a constant volume. The samples were diluted with the mobile phase and then analyzed by HPLC. The integrity of HEM was also determined at the end of each experiment.

Table I summarizes the conditions of the permeation experiments investigated in the present study. The skin permeation studies consisted of iontophoretic transport experiments and passive transport studies after skin pretreated with iontophoresis. The details for each experiment were described in the following sections.

Transport Studies: pH Effect

The iontophoretic drug transport experiments under the asymmetric pH conditions (donor and receiver chambers had the different pH, Table I) were conducted in the Franz diffusion cells as described in the “Transport studies: general setup” section. The asymmetric condition provided data mimicking the situations in transdermal delivery in practice. Iontophoretic skin permeation of PXCl was first studied using 1 mg/ml PXCl in citrate buffer pH 5.0, phosphate buffer pH 6.0 and phosphate buffer pH 7.0 isotonized with NaCl as the donor solution to investigate the effect of pH on iontophoretic transport of PXCl. In another experiment, the donor solutions contained 1 mg/ml PXCl and 2.27 mg/ml AP in citrate buffer pH 3.0, citrate buffer pH 5.0 and phosphate buffer pH 7.0 isotonized with mannitol to examine the effect

of ion composition in the drug solution on the transport hindrance/enhancement of PXCl as a function of pH. AP was included in the PXCl solution to determine the electromigration and electroosmosis contributing to iontophoretic transport of PXCl (15). The receiver solution was PBS. Passive diffusion experiments of 1 mg/ml PXCl in citrate buffer pH 5.0, phosphate buffer pH 6.0 and phosphate buffer pH 7.0 isotonized with NaCl were performed to serve as the baseline references to determine iontophoretic transport enhancement. PXCl was found to be stable with no detectable degradation under current application of 0.5 mA/cm² for 6 h and storage at pH 3.0–7.0 for a week as shown by the greater than 90% recovery of PXCl after current application and storage in a separate study (data not shown).

Transport Studies: Iontophoresis Pretreatment

To investigate PXCl transport across iontophoresis-altered skin without the influence of iontophoresis as the driving force, passive transport experiments were performed after HEM was pretreated with iontophoresis under the asymmetric condition (Table I). Another purpose of the iontophoresis pretreatment was to provide passive PXCl fluxes that might otherwise be difficult to measure (e.g., undetectable) without the pretreatment. In the pretreatment experiment, the donor solutions of pH 3.0 and pH 7.0 were studied to examine the influence of skin permselectivity on drug transport. The donor chamber was filled with a buffer solution isotonized with mannitol at pH 3.0 or pH 7.0 without the drug. Anodal iontophoresis with constant current of 0.5 mA/cm² was applied for 6 h in the same manner as the iontophoretic transport experiment. After switching off the current, the donor solution was removed and replaced by drug solution of the same pH isotonized with mannitol. The drug solutions used in the study contained either 1 mg/ml or 10 mg/ml PXCl and 2.27 mg/ml AP. PBS was used as the receiver solution. Passive delivery of PXCl and AP after iontophoresis pretreatment was then measured. As the passive transport of

Table I Permeation Conditions Investigated in the Transport Studies for PXCl and AP

Permeation conditions	Drug concentration in donor solution		Tonicity agent	pH of donor solution	pH of receiver solution
	PXCl	AP			
<i>Anodal iontophoresis</i>					
Asymmetric	1 mg/ml	- ^a	NaCl	5.0, 6.0, 7.0	7.4
Asymmetric	1 mg/ml	2.27 mg/ml	mannitol	3.0, 5.0, 7.0	7.4
Symmetric	1 mg/ml	2.27 mg/ml	mannitol	3.0	3.0
<i>Passive transport after skin treated with iontophoresis</i>					
Asymmetric	1, 10 mg/ml	2.27 mg/ml	mannitol	3.0, 7.0	7.4

^a Not applicable

PXCl was expected to be low, the amounts of PXCl permeated across the skin from the drug solutions were determined at predetermined time intervals: (i) 36, 40, 44 and 48 h for the drug solution containing 1 mg/ml PXCl and (ii) 12, 15, 18, 21 and 24 h for the drug solution containing 10 mg/ml PXCl after pretreatment.

Transport Studies: Symmetric Condition

In iontophoretic transport study of PXCl under the symmetric condition (donor and receiver chambers had the same pH), pH 3.0 condition in both donor and receiver chambers was studied (Table I). The symmetric condition provided the data to study the transport mechanism of PXCl when the pH across HEM (from the donor to receiver) was constant. In the experiment, citrate buffer pH 3.0 isotonized with mannitol and citrate buffer pH 3.0 isotonized with NaCl were added in the donor and receiver chambers, respectively, to equilibrate HEM for 2 h. After equilibration, 1 ml of drug solution in citrate buffer pH 3.0 isotonized with mannitol containing 1 mg/ml PXCl and 2.27 mg/ml AP was added in the donor chamber. A precise amount of citrate buffer pH 3.0 isotonized with NaCl (~5 ml) was added into the receiver chamber. Anodal iontophoresis with constant direct current of 0.5 mA/cm² was conducted for 6 h. The data from the experiment under the symmetric condition were compared with the asymmetric condition (i.e., pH 3.0 and pH 7.4 in donor and receiver chambers, respectively).

Determination of Electromigration and Electroosmosis Contribution to the Iontophoretic Transport of PXCl as a Function of pH

The electromigration and electroosmosis contribution to the iontophoretic transport of PXCl were determined by co-iontophoretic delivery of PXCl with AP (15). The steady-state flux (J) and permeability coefficient (P) were determined by using Eq. 2 and 3 (8,22):

$$J = \frac{dQ}{dt} \times \frac{1}{A} \quad (2)$$

$$P = \frac{dQ}{dt} \times \frac{1}{AC} = \frac{J}{C} \quad (3)$$

where J was calculated from the straight line portion of the cumulative permeated amount (Q) curve of either PXCl or AP per unit area (A) against time (t) over the 6-h iontophoresis period and C is the donor solution concentration of drug.

The total iontophoretic flux (J_{total}) of a drug molecule is related to the fluxes due to electromigration (J_{EM}), electroosmosis (J_{EO}), and passive delivery (J_{passive}). For the small value of passive delivery of PXCl due to its polar nature, J_{passive} was considered to be negligible. Thus, J_{EM} and J_{EO} of PXCl were determined by Eq. 4 and 5, respectively (15):

$$J_{\text{EM}} = J_{\text{total}} - J_{\text{EO}} \quad (4)$$

$$J_{\text{EO}} = v \times C_{\text{PXCl}} = \frac{J_{\text{AP}}}{C_{\text{AP}}} \times C_{\text{PXCl}} \quad (5)$$

where v is the average solvent flow velocity due to electroosmosis, J_{total} and J_{AP} are steady-state fluxes of PXCl and AP across HEM and C_{PXCl} and C_{AP} are the donor solution concentration of PXCl and AP, respectively. It should be noted that J_{EO} calculated by eq. 5 is an apparent value. For anodal iontophoresis with skin at pH 5.0 and 7.0 (electroosmosis enhanced transport), J_{EO} is an accurate representation of PXCl electroosmotic flux. For anodal iontophoresis with skin at pH 3.0, J_{EO} for PXCl is expected to be a negative value (from cathode to anode) that can be described by the Peclet number related to the apparent value obtained from Eq. 5 (see electroosmotic flux discussed in Results and Discussion).

Drug Content Analysis

Modified reverse phase HPLC methods (23) were used to assay PXCl and AP. The HPLC system consisted of an Agilent 1200 series quaternary pump, degasser, autosampler, UV-Vis diode array detector (Agilent Technologies, Inc., Santa Clara, California) and Hypersil™ BDS C18 column with a guard column (5 μm particle size, 150 mm × 4.6 mm; Thermo Fisher Scientific Inc., Waltham, Massachusetts). The mobile phase comprised acetonitrile and aqueous phase at the ratio of 3:97 (v/v). The aqueous phase contains 0.5% (v/v) triethylamine in water with its pH adjusted to 6.0 by phosphoric acid. The flow rate and injection volume were 1.0 ml/min and 50 μl, respectively. The HPLC assay was performed at room temperature. The detection wavelengths for PXCl and AP were set at 262 and 245 nm, respectively. Standard solutions at appropriate concentration ranges were used to prepare the calibration curve in the mobile phase. The linearity of calibration curves for PXCl and AP were in the range of 0.20–6.41 μg/ml ($R^2 = 0.999$) and 0.16–10.01 μg/ml ($R^2 = 1.000$). The limit of detection (LOD) and limit of quantitation (LOQ) for PXCl were 0.03 and 0.10 μg/ml, respectively. For AP, the LOD and LOQ were 0.02 and 0.07 μg/ml, respectively.

Data Analysis

The results were expressed as the mean \pm standard deviation (SD). The statistical comparison was evaluated using either independent t-test or one-way ANOVA followed by multiple comparisons (e.g., Turkey and Dunnett method). The level of significant difference was considered at p value <0.05 .

RESULTS

Effect of pH on PXCl Iontophoretic Delivery

PX has two pKa (i.e., 5.0 and 9.6 for primary amine substituted in thiazole ring and secondary amine on the structure, respectively) indicating that PX acquires valence of +1 and +2 when the drug ionizes with decreasing pH. Therefore, the valence of PX ions in the drug solution was controlled by the solution pH (as shown in the equilibrium conditions in Fig. 1). The extent of ionization of PX ions at each pH was calculated using Eqs. 6–8 (24) and summarized in Table II. Consequently, the pH of drug solution would affect PX iontophoretic transport across skin.

$$\%[\text{PX}^{2+}] = \frac{[\text{H}^+]^2}{[\text{H}^+]^2 + \text{Ka1}[\text{H}^+] + \text{Ka1Ka2}} \times 100 \quad (6)$$

$$\%[\text{PX}^+] = \frac{\text{Ka1}[\text{H}^+]}{[\text{H}^+]^2 + \text{Ka1}[\text{H}^+] + \text{Ka1Ka2}} \times 100 \quad (7)$$

$$\%[\text{PX}] = \frac{\text{Ka1Ka2}}{[\text{H}^+]^2 + \text{Ka1}[\text{H}^+] + \text{Ka1Ka2}} \times 100 \quad (8)$$

Figure 2a shows the permeation profiles of PXCl across HEM from different pH solutions isotonized with NaCl by iontophoresis in comparison with passive diffusion. The data show that passive permeation of PXCl across the HEM was low and iontophoresis significantly enhanced drug transport at each pH studied ($p < 0.05$; independent t-test). After 24 h passive permeation, the amounts of PXCl permeated across HEM from the drug solutions at pH 5.0, 6.0, and 7.0 were 0.58 ± 0.88 , 1.38 ± 2.11 , and $0.29 \pm 0.27 \mu\text{g}/\text{cm}^2$, respectively. Using iontophoresis, the amounts of PXCl permeated across HEM at pH 5.0, 6.0, and 7.0 were 90 ± 22 , 84 ± 14 ,

and $102 \pm 34 \mu\text{g}/\text{cm}^2$ after the 6 h current application, corresponding to $J(1-6 \text{ h})$ of 15.3 ± 3.0 , 13.9 ± 4.4 , and $18.9 \pm 6.7 \mu\text{g}/\text{cm}^2\cdot\text{h}$, respectively. The amounts of PXCl permeated across HEM during iontophoresis were at least two orders of magnitude larger than those of passive permeation. The results in the figure also show no significant difference in permeated amounts and $J(1-6 \text{ h})$ of PXCl at pH 5.0, 6.0, and 7.0 during iontophoresis ($p > 0.05$; one-way ANOVA).

Figure 2b presents the permeation profiles of PXCl across HEM during iontophoresis with the drug solution isotonized with mannitol. PXCl fluxes increased significantly when the concentrations of the current-competing background ions were reduced by replacing NaCl with mannitol, a neutral molecule for adjusting the tonicity. The data also show the effect of drug solution pH on iontophoretic transport of PXCl. Although no pH effect was observed for the drug solution isotonized with NaCl (Fig. 2a), solution pH significantly affected iontophoretic transport of PX ions from the drug solution isotonized with mannitol in a pH-dependent manner. With the mannitol isotonized system, iontophoretic transport of PXCl in pH 3.0 solution was performed because 99% of PX ions have valence of +2 under this condition (Table II); this provided a closer examination of the pH effect on iontophoretic transport of PX ion at the higher net +2 valence. In the figure, the highest $J(1-6 \text{ h})$ occurred in the pH 7.0 solution ($69.7 \pm 7.9 \mu\text{g}/\text{cm}^2\cdot\text{h}$) followed by pH 5.0 solution ($46.6 \pm 5.6 \mu\text{g}/\text{cm}^2\cdot\text{h}$) and pH 3.0 solution ($34.3 \pm 3.6 \mu\text{g}/\text{cm}^2\cdot\text{h}$), respectively. This result was different from the prediction of electrotransport theory that reducing solution pH to increase the positive charges on PX ions should enhance its flux in anodal iontophoresis.

To compare the data with electrotransport theory, Fig. 3 presents the theoretical calculations using Eq. 1 (11,25,26). The plots show the estimated transference numbers normalized by valence (t_i/z) of PX ions, co-ions and counterions in the drug solution versus pH. The theoretical calculations show that the use of NaCl for isotonicity adjustment significantly reduces the t_i/z values of PX ions in the drug solution. When replacing NaCl with mannitol, the t_i/z values of PX ions in the drug solution increase, which is consistent with the experimental data in Fig. 2a and b. However, different from the experimental data, the calculations show that the highest t_i/z values of PX ions should occur at pH 3. This is investigated later in the “Discussion” section.

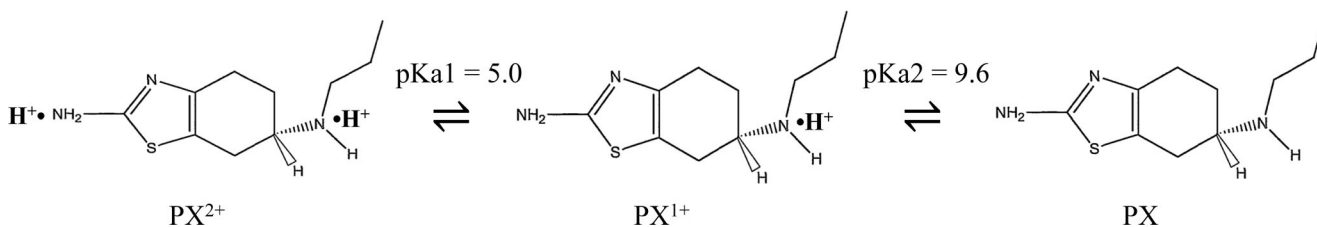


Fig. 1 Scheme of PX ionization in equilibrium conditions.

Table II Percent of Ionized Species of PX in Various pH Solutions

pH	%species		
	PX ²⁺	PX ⁺	PX
Citrate buffer 3.0	99.01	0.99	0.00
Citrate buffer 5.0	50.00	50.00	0.00
Phosphate buffer 6.0	9.09	90.89	0.02
Phosphate buffer 7.0	0.99	98.76	0.25

Contribution of Electromigration and Electroosmosis to Iontophoretic Transport of PXCl

The contributions of J_{EO} and J_{EM} to the iontophoretic flux of PXCl as a function of pH using drug solution isotonized with mannitol were summarized in Table III. The highest values of

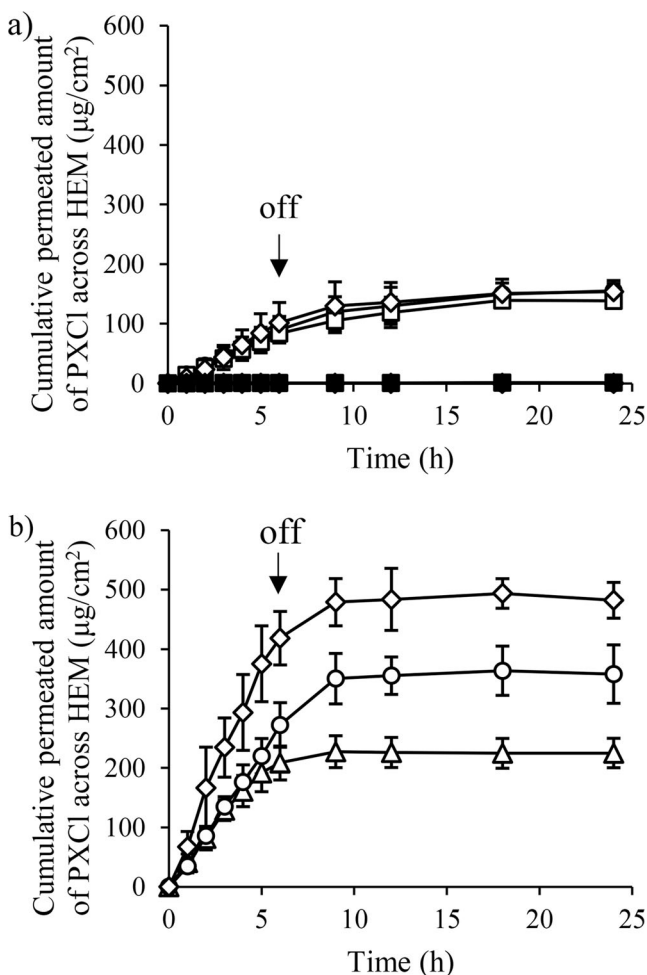


Fig. 2 Cumulative amount of PXCl permeated across HEM from 1 mg/ml drug solution isotonized with (a) NaCl and (b) mannitol. Symbols: pH 3.0 (triangles); pH 5.0 (circles); pH 6.0 (squares) and pH 7.0 (diamonds). Closed symbols and open symbols with solid line represent the permeation profiles of PXCl transported by passive diffusion and iontophoresis, respectively. Direct current density of 0.5 mA/cm² was applied continuously for 6 h to PXCl solution. Each point and bar represent the mean \pm SD ($n = 3-4$).

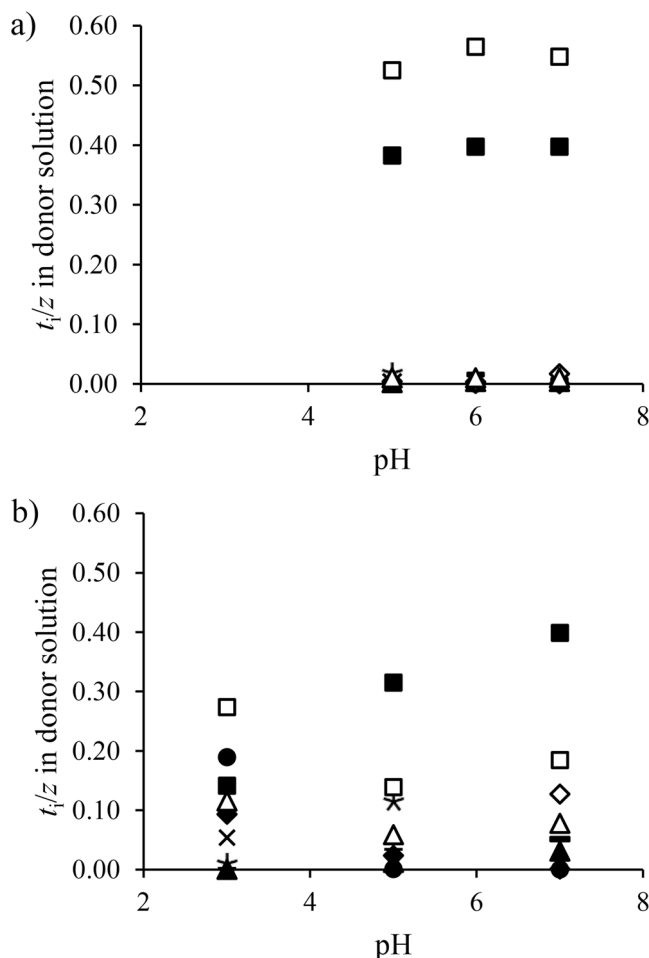


Fig. 3 Transference number of PX ions, co-ions and counterions present in the drug solution isotonized with (a) NaCl or (b) mannitol versus pH. Symbols: ●, H⁺; ▲, PX⁺; ◆, PX²⁺; ■, Na⁺; ×, C₆H₇O₇⁻; +, C₆H₆O₇²⁻; *, C₆H₅O₇³⁻; -, H₂PO₄⁻; ◇, HPO₄²⁻; □, Cl⁻; △, N₃⁻. Electrophoretic mobilities in Eq. 1 were calculated using $\mu = DzF/RT$ and $\mu_i/\mu_j = D_{zi}/D_{zj}$, where μ , D and z are the electrophoretic mobility, diffusion coefficient and valence of the ion, F is the Faraday constant, R is the universal gas constant, T is temperature, and subscripts i and j represent ion species i and H⁺ ion, respectively (25). The diffusion coefficients of the ions and H⁺ ion electrophoretic mobility were obtained from references (25, 26). PX diffusion coefficient was estimated using the diffusion coefficients of molecules with similar molecular sizes based on the relationship $D = kT/6\pi\eta r$, where r is the hydrodynamic radius, k is the Boltzmann constant and η is the solvent viscosity (25)

both J_{EO} and J_{EM} were obtained at pH 7.0 ($2.01 \pm 0.52 \mu\text{g}/\text{cm}^2\cdot\text{h}$ and $67.7 \pm 7.5 \mu\text{g}/\text{cm}^2\cdot\text{h}$, respectively) followed by pH 5.0 ($1.20 \pm 0.25 \mu\text{g}/\text{cm}^2\cdot\text{h}$ and $45.4 \pm 5.6 \mu\text{g}/\text{cm}^2\cdot\text{h}$, respectively) and pH 3.0 ($0.11 \pm 0.06 \mu\text{g}/\text{cm}^2\cdot\text{h}$ and $37.8 \mu\text{g}/\text{cm}^2\cdot\text{h}$, respectively). Electromigration was 97 to 110% of the total flux of PXCl during iontophoresis, and electroosmotic flux was not greater than 10% of electromigration flux in the pH range studied. The result is in agreement with the earlier studies by Kalaria *et al.* (15,16) at pH 5.3 under similar anodal iontophoresis condition that electromigration is the main mechanism for transdermal iontophoretic delivery of PXCl.

Table III Contribution of Electroosmosis (J_{EO}) and Electrorrepulsion (J_{EM}) to the Iontophoretic Flux of PX in Different pH Media Isotonized with Mannitol

pH	J_{total} ($\mu\text{g}/\text{cm}^2\cdot\text{h}$) ^a	J_{EO} ($\mu\text{g}/\text{cm}^2\cdot\text{h}$) ^a	J_{EM} ($\mu\text{g}/\text{cm}^2\cdot\text{h}$) ^a	% J_{EO} ^d	% J_{EM} ^e
3.0	34.3 ± 3.6	0.11 ± 0.06 (−3.54) ^b	37.8 ^c	9.4	110.2
5.0	46.6 ± 5.6	1.20 ± 0.25	45.4 ± 5.6	2.6	97.4
7.0	69.7 ± 7.9	2.01 ± 0.52	67.7 ± 7.5	3.0	97.1

^a mean ± SD (n = 4)^b apparent and calculated values (calculated value in parentheses). The calculated value was from the relationships $E = Pe/(1 - \exp(-Pe))$ and $E = J_{EO,pH}/J_{EO,pl}$, where E is the enhancement factor due to electroosmosis, Pe is the Peclet number (33,34), $J_{EO,pH}$ is the J_{EO} at pH 3, and $J_{EO,pl}$ is the J_{EO} at skin pl. $J_{EO,pl}$ was estimated by interpolation at pl of 4.8 using the J_{EO} vs. pH data^c calculated using $J_{EO} = -3.54 \mu\text{g}/\text{cm}^2\cdot\text{h}$ for cathodal iontophoresis^d calculated using % $J_{EO} = |J_{EO}/J_{EM}| \times 100$ ^e calculated using % $J_{EM} = |(J_{total} - J_{EO})/J_{total}| \times 100$

Passive Transport of PXCl and AP after Skin Pretreated with Iontophoresis

Table IV summarizes the passive delivery of PXCl and AP across HEM from donor solutions of pH 3.0 and 7.0 after the membrane was pretreated with iontophoresis to investigate skin permselectivity, i.e., the partitioning of positively charged PX ions into positively and negatively charged HEM at pH 3.0 and 7.0, respectively. Assuming that the barrier properties of HEM after iontophoresis pretreatment were similar to those during iontophoresis, the results can be used to investigate the enhancement mechanisms of PXCl iontophoretic transport. In the experiment using 1 mg/ml PXCl, passive transport of PXCl at pH 3.0 was undetectable. Consequently, the LOD for PXCl was used to estimate the maximum value possible of PXCl permeation at this pH for comparison. To analyze the data, the flux and permeability coefficient of AP were used as the internal reference under each condition and the ratio of PXCl to AP permeability coefficients was determined. A significant change in the difference in permeability coefficients between PXCl and AP (i.e., the P ratio) with respect to pH changes would indicate the effect of membrane permselectivity for PXCl due to

charge-charge interaction at pH 3.0 and 7.0. The data show that the passive transport of PXCl at pH 7.0 was faster than that at pH 3.0 for both 1 mg/ml and 10 mg/ml conditions ($p < 0.05$, independent t -test). When donor pH increased from 3.0 to 7.0, the P ratios increased by at least 13-fold and approximately 4-fold for the 1 mg/ml and 10 mg/ml PXCl solutions, respectively.

Iontophoretic PXCl Transport across HEM under Symmetric Condition

Table V summarizes the iontophoretic transport of PXCl and AP in citrate buffer pH 3.0 isotonized with mannitol in the asymmetric and symmetric permeation studies. The iontophoretic transport of PXCl (J and J_{EM}) under symmetric condition ($18.4 \pm 2.4 \mu\text{g}/\text{cm}^2\cdot\text{h}$ and $17.6 \pm 1.4 \mu\text{g}/\text{cm}^2\cdot\text{h}$, respectively) was approximately 2-fold smaller than that under asymmetric condition ($34.3 \pm 3.6 \mu\text{g}/\text{cm}^2\cdot\text{h}$ and $34.2 \pm 3.6 \mu\text{g}/\text{cm}^2\cdot\text{h}$, respectively) ($p < 0.05$, independent t -test). No change in the AP transport and J_{EO} was observed between the asymmetric ($0.24 \pm 0.14 \mu\text{g}/\text{cm}^2\cdot\text{h}$ and $0.11 \pm 0.06 \mu\text{g}/\text{cm}^2\cdot\text{h}$, respectively) and symmetric ($0.25 \pm 0.09 \mu\text{g}/\text{cm}^2\cdot\text{h}$ and $0.10 \pm 0.04 \mu\text{g}/\text{cm}^2\cdot\text{h}$, respectively) conditions.

Table IV Effect of pH on the Passive Delivery of PXCl and AP Across HEM after Iontophoresis Pretreatment

Permeation protocol ^a	pH	J ($\mu\text{g}/\text{cm}^2\cdot\text{h}$)		P ($\times 10^{-7}$ cm/s)		P ratio of PXCl to AP
		PXCl	AP	PXCl	AP	
I	3.0	0.01 ^b	0.39 ± 0.02	0.04 ^b	0.48 ± 0.03	0.08 ^b
	7.0	0.40 ± 0.20	0.97 ± 0.43	1.18 ± 0.60	1.16 ± 0.51	1.02
II	3.0	0.26 ± 0.16	0.38 ± 0.04	0.07 ± 0.05	0.46 ± 0.05	0.15
	7.0	2.51 ± 1.31	0.91 ± 0.52	0.70 ± 0.37	1.15 ± 0.66	0.61

mean ± SD (n = 3)

^a The concentrations of PXCl in solution for protocols I and II were 1 and 10 mg/ml, respectively^b Below detection limit; values are maximum possible values estimated using the limit of detection of HPLC assay for PXCl

Table V Iontophoretic Transport of PXCl and AP in Drug Solution pH 3.0 Isotonized with Mannitol under Asymmetric and Symmetric Permeation Studies with Constant Direct Current of 0.5 mA/cm² for 6 h

Permeant	Permeation study condition	J ($\mu\text{g}/\text{cm}^2\cdot\text{h}$)	J_{EO} ($\mu\text{g}/\text{cm}^2\cdot\text{h}$)	J_{EM} ($\mu\text{g}/\text{cm}^2\cdot\text{h}$)
PXCl	Asymmetric	34.3 ± 3.6	0.11 ± 0.06	34.2 ± 3.6
	Symmetric	18.4 ± 2.4	0.10 ± 0.04	17.6 ± 1.4
AP	Asymmetric	0.24 ± 0.14	- ^a	- ^a
	Symmetric	0.25 ± 0.09	- ^a	- ^a

mean ± SD (n = 4–6)

^a Not applicable

DISCUSSION

Iontophoresis-Enhanced Transdermal Delivery of PXCl and Effect of pH

Transdermal iontophoresis is a promising technique which utilizes an electric field as the driving force to enhance the transport of charged and uncharged drugs across skin. Iontophoretic drug transport is influenced by several factors such as pH and ion composition of the drug solution. The pH of a solution has a significant effect on iontophoretic transport of ionic drugs because it governs the degree of drug ionization as well as the charges in skin membrane (9). PX is a weak base with two pKa values (i.e., 5.0 and 9.6). The drug completely ionizes at pH below 7.6 and the ability of PXCl to permeate across skin is low by passive transport (Fig. 2a). Therefore, the application of electric current can increase skin permeation of PXCl. The result in this study is consistent with iontophoresis-enhanced transport for effective transdermal delivery of a charged molecule, and iontophoretic transport of PXCl is mainly a result from electromigration whereas the effects of electroosmosis and passive transport are small (9).

The efficiency of an ion as a charge carrier during an electrotransport process can be evaluated by the transference number of the ion (11). Increasing the valence of the target ion (Eq. 1) provides a higher transference number and subsequently more efficient iontophoretic transport. Thus, increasing the valence of positively charged PX, a function of the donor pH (Fig. 1 and Table II), could improve its iontophoretic transport. To compare the results between the drug solutions isotonized with NaCl and mannitol, the low transference number of PX ion in the solution isotonized with NaCl (Fig. 3a), which contained relatively high concentration of Na⁺ (~0.16 M) and Cl⁻ ions (~0.15 M) to PX ions (3.31 mM) is in general agreement with electrotransport theory; i.e., drug transference number is usually low when there is a relatively high concentration of current-competing ion compared to that of the drug in iontophoretic delivery (10,11). The fraction of current carried by PX ions during iontophoresis is low and inadequate to observe a significant difference in iontophoretic transport of PXCl as a function of pH under these conditions (Fig. 2a). In addition to the ion competition effect, solution ionic strength impacts the electrophoretic mobilities of ions. The higher ionic

strength of the drug solution isotonized with NaCl would decrease ion electrophoretic mobilities (Onsager theory on ion conductivity) and affect ions of larger sizes and higher valence more than small monovalent ions (27,28). Avoiding extraneous electrolyte for tonicity adjustment allows cationic PX to be delivered efficiently by iontophoresis. In the present study, pH-dependent iontophoretic transport of PXCl was observed when NaCl was replaced by mannitol for isotonicity adjustment (Fig. 2b) supporting that the insignificant pH effect on the iontophoretic transport of PXCl in the solution isotonized with NaCl could be attributed to these factors.

An interesting finding in the present study was the opposite effect of pH on iontophoretic transport of PXCl in drug solution isotonized with mannitol (Fig. 2b) compared to the trend predicted by electrotransport theory (Fig. 3b). The theory suggests that the iontophoretic transport of PXCl should be most efficient in the drug solution of pH 3.0 among the pH studied when the valence of PX ion increases. In this respect, the following factors are considered: (i) the decrease in iontophoretic transport due to the change in electroosmosis (29,30) and (ii) the hindrance of drug transport due to the decrease in partitioning of drug into the charged skin membrane as a result of charge-charge interactions (skin cation permselectivity) (30–32). PXCl was stable with no detectable degradation under the conditions studied as shown by the greater than 90% recovery of PXCl after iontophoresis application (data not shown), which could be attributed to the use of the salt bridges in the present study.

Contribution of Electroosmosis to the Iontophoretic Transport of PXCl as a Function of pH

Since the human skin has pI value of approximately 4.8 (14), the skin becomes net positively charged at solution pH lower than the pI (different from its negatively charged state at physiological pH above the pI) (14). The charge density of the skin transport pathways is a continuous function with respect to pH. When the pH decreases from pH 7.0 to 3.0, the skin transport pathways change from net negatively charged to positively charged. For anodal iontophoresis, decreasing donor solution pH would decrease the electroosmosis contribution to iontophoretic enhancement of cationic drug transport (29). At pH below the pI value, electroosmosis has a negative

impact on anodal iontophoretic transport enhancement. This phenomenon is supported by the results of AP showing larger electroosmosis contribution at pH 7.0 compared to that at pH 5.0 and the retardation effect of electroosmosis at pH 3.0 (Table III). Assuming skin pI of 4.8 and interpolation to estimate the AP flux at this pH, the Peclet numbers of electroosmosis were determined to be -3.5 and 1.6 at pH 3.0 and pH 7.0, respectively, using the electroosmosis enhancement theory (33,34). Although this observation is in agreement with the smaller PXCl flux enhancement at pH 3.0 compared with that at pH 7.0 in the present study, the effect of electroosmosis in the studied pH range is relatively small as compared to electromigration and cannot be used to explain the pH-dependent PXCl data. The present AP results are consistent with (i) the transport theory that the contribution of electroosmosis is generally small for a small ionic permeant in transdermal iontophoresis (10,35) and (ii) the findings at pH 5.3 in previous studies (15,16). It should be noted that the present data interpretation did not take into account specific interactions between PX and skin (e.g., PX binding) and assumed that the ions are point charges. Therefore, the iontophoretic transport behaviors of other multivalent cationic drugs could be different from those of PX observed in this study.

PX Ion Permeation at pH below Skin pI and Mechanism of pH-Dependent PX Permeation

Skin behaves as a permselective membrane to ionic compounds (36). This skin property is one of the determinants for ion permeation across skin during iontophoresis (37). The skin can be positively or negatively charged depending on the pH and composition of formulation in contact with the skin (pI = 4.8). PX, as a cationic drug, can interact with the skin via charge-charge interactions. Considering the charge-charge interactions between the skin and cationic PX when the solution pH is below or above skin pI, skin permselectivity could significantly hinder the iontophoretic transport of PX at pH 3 (when solution pH < skin pI) or facilitate iontophoretic transport at pH 7 (when solution pH > skin pI). To investigate the charge-charge interactions in skin transport, passive transport experiments (without the impact of the electric field on PX permeation) were performed at pH 3.0 and pH 7.0 after the skin was pretreated with iontophoresis. The pH 3.0 and 7.0 conditions were selected so the transport of PXCl across the negatively and positively charged skin (at pH 7.0 and pH 3.0, respectively) could be compared. The lower permeability of skin to PXCl at pH 3.0 than pH 7.0 under both PXCl concentrations (Table IV) can be attributed to the decrease in drug partitioning into the skin via charge-charge interaction between PXCl and the positively charged skin. The larger effect observed at the lower PXCl concentration (increase in P ratio difference of >13-fold at 1 mg/ml from

~4-fold at 10 mg/ml) is consistent with the stronger charge-charge interactions (thicker Debye–Huckel layer) at the lower ionic strength in 1 mg/ml drug solution (28,38). The data are consistent with the negatively charged skin providing preferential permeation of PX^+ ions at pH 7. When the skin becomes positively charged at pH 3.0, the membrane repels the positively charged PX, resulting in transport hindrance. Such charge-charge interactions and the corresponding effects are commonly observed in ion transport across biological membranes (11,39). Although the exact locations of the iontophoretic transport pathway and charge-charge interactions between the cationic PX and skin are not known, previous studies have provided information on this topic (40–43). In the present study, the decrease in electromigration (iontophoretic transport) with the decrease in donor solution pH is consistent with this skin permselectivity effect, which is likely to be a key factor in the iontophoretic transport of PXCl.

Conducting iontophoresis experiment under a symmetric condition with respect to the donor and receiver chambers can provide additional insights into the transport mechanisms during iontophoresis. Under the asymmetric condition, membrane pH could be influenced by the pH in the donor and receiver solutions. As membrane pH is the determining factor of PX ionization and membrane iontophoretic transport, the pH of the receiver solution (pH 7.4) could affect membrane transport and mask the pH effect of the donor solution in the present study (32,44). Under the symmetric condition, the data can be interpreted without the complication of a pH gradient in skin membrane under the asymmetric condition (i.e., donor pH 3.0, receiver pH 7.4, and skin pH between 3.0 and 7.4). The present study showed that PXCl transport decreased when the pH of the environment in the membrane decreased due to the symmetric condition of pH 3.0 (i.e., due to the decrease in the pH of the receiver solution), whereas the AP transport and J_{EO} in both asymmetric and symmetric permeation studies were essentially the same (Table V). The results in the present study suggest that electroosmosis in the membrane was not significantly affected by the receiver solution pH under the conditions studied, similar to those observed in a previous study (32). Electroosmosis was mainly controlled by the donor solution pH. The decrease in the pH in the membrane, if any, due to the decrease in pH of the receiver solution under the symmetric condition (*vs.* the asymmetric condition) leads to a further decrease in the flux of PXCl; this effect is consistent with the decrease of PXCl permeation due to skin permselectivity when pH decreases. This rejects the notion that the lack of iontophoretic enhancement under the asymmetric condition (pH 3.0 donor solution and pH 7.4 receiver solution) was a result of the membrane pH being significantly influenced by the receiver solution. At the same time, this supports the conclusion that skin permselectivity due to charge-charge interactions between cationic PX and the skin could be a main factor contributing to the observed

iontophoresis results of PXCl. When the skin gradually changed from net negatively charged to slightly negatively charged and then positively charged, the highest J_{total} was observed at pH 7.0 followed by 5.0 and 3.0, respectively, as a result from the different magnitudes of cation permselectivity of skin to cationic PX.

CONCLUSION

The present study demonstrates that the iontophoretic transport of PXCl was influenced by the pH and the presence of competing ions for the electric current in the donor solution. Iontophoresis of PXCl solution buffered at pH 7.0 and isotonized with mannitol provided the highest PX flux among the conditions studied. Decreasing the solution pH to increase the net valence of positively charged PX ion has a negative impact on its iontophoretic transport. The results from the analyses of electroosmosis, iontophoretic drug transport under symmetric condition, and the pH effect on passive permeation of PXCl after iontophoresis pretreatment suggest that the pH-dependent characteristic of PXCl transport during iontophoresis was mainly related to cation-permselectivity of the skin rather than the effect of electroosmosis. Skin permselectivity, consisting of drug to membrane pathway partitioning via charge-charge interactions, could play a major role in the electrotransport of PXCl at pH below the skin pI. This observation is different from the belief that electroosmosis is the major cause of the slower anodal iontophoretic transport of cationic drugs when the pH is below the skin pI.

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