

## Analysis of *in Vitro* Iontophoretic Skin Permeation of Sodium Benzoate by Transport Numbers of Drug and Additives

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Effect of species and concentrations of pharmaceutical additives on the iontophoretic transport of benzoate anion through excised hairless rat skin was investigated using a 2-chamber iontophoretic diffusion cell equipped with platinum electrodes at 0 mA for 4 h (control) followed by a constant direct current of 0.5 mA for another 4 h. One cell facing the stratum corneum was filled with sodium benzoate solution, and the other cell facing the dermis with lithium chloride (LiCl), potassium chloride (KCl) or tetraethylammonium bromide (TEA-Br) solution. Iontophoretic delivery rate of benzoate anion that permeated through skin increased with an increase in the sodium benzoate concentration while maintaining a constant KCl concentration. In contrast, a flux of benzoate anion decreased with an increase in KCl concentration and a constant concentration of sodium benzoate. When KCl was replaced by LiCl or TEA-Br, the flux of benzoate anion was almost the same. These phenomena were evaluated by a concept of transport numbers: theoretical values of benzoate anion flux were very close to the observed data. Potential difference between the skin during the permeation study was also measured between two salt bridges which were connected *via* calomel electrodes to a potentiometer. It gradually decreased to a certain level in each case, but increased again in some cases. This gradual decrease and increase in the potential difference, in spite of a constant current, were theoretically explained by a gradual increase of ion concentration in the skin membrane and depletion of the cation in the receiver cell, respectively. Analysis of ionic mobility and concentration of penetrants gave a great deal of information on iontophoretic drug permeation through skin.

**Key words** iontophoresis; sodium benzoate; hairless rat skin; transport number; pharmaceutical additive

Transdermal drug delivery overcomes several problems such as hepatic "first-pass" metabolism and side effects of a drug in the gastrointestinal tract after oral administration. Several shortcomings for transdermal drug delivery were found, however, despite such advantages: the greatest being low drug permeation. This can be overcome by the use of chemical enhancers,<sup>1,2)</sup> pro-drugs,<sup>3,4)</sup> and the application of external energy.<sup>5,6)</sup> Electroporation,<sup>7)</sup> electro-incorporation<sup>8)</sup> and iontophoresis are methods to enhance the skin permeation of a drug using electric power as external energy. Iontophoresis can be defined as a process or method in which the permeation rate of ionic species into the body is increased by applying a current between different sites of viable tissue.<sup>5)</sup> Various reports have been published on chemotherapy<sup>9)</sup> and diagnosis,<sup>10)</sup> and recent efforts have concentrated on understanding and elucidating the mechanism of the iontophoretic transport involved.<sup>11)</sup> From a formulation technological point of view, when designing a well-assembled iontophoretic device, one needs to evaluate materials and shape of active and passive electrodes and to investigate the effect of the kind and concentration of drugs and pharmaceutical additives, including polymers in dermal patch vehicles consisting of those electrodes. Effect of variations in ionic composition in passive electrode patch on flux of a drug across skin may be investigated using a 3-chamber iontophoretic diffusion cell which consists of donor, center and receiver compartments. Most of the *in vitro* iontophoretic skin permeations, however, have been done using a 2-chamber iontophoretic diffusion cell which consists of only donor and receiver compartments.<sup>12–14)</sup> Bellantone *et al.* have already reported that the effect of ionic compositions of donor solution on the skin permeation of benzoate, as a model permeant, using 2-chamber

as well as 3-chamber diffusion cells. They mentioned in their report that 2-chamber cells could be applied instead of 3-chamber cells to evaluate the effect of the ionic compositions in the passive electrodes. We earlier reported that ion species and their concentration in donor and receiver solutions had an effect on the iontophoretic transport of benzoic acid through poly(vinyl acetate) membrane.<sup>15)</sup> These phenomena can be explained by a concept of the transport number of each ion.

The purpose of this present study was to gain basic information on the effect of species and concentrations of pharmaceutical additives on the iontophoretic transport of benzoate anion as a model drug through hairless rat skin. Three monocations, potassium, lithium and tetraethylammonium cations ( $K^+$ ,  $Li^+$ ,  $TEA^+$ ) were selected as model pharmaceutical additives. These results will lead to not only the exact evaluation of *in vitro* iontophoretic skin permeation but also the design and discovery of well-assembled devices.

### Theoretical

Fluxes of benzoate anion from donor to receiver side,  $J_b$ , and a monovalent cation from receiver to donor side,  $J_c$ , through a biological membrane (Fig. 1) are expressed as follows<sup>16)</sup>:

$$J_b = \beta_b U_b C_b E \quad (1)$$

$$J_c = \beta_c U_c C_c E \quad (1')$$

where  $\beta$ ,  $U$ ,  $C$  and  $E$  are partition coefficient (biological membrane/aqueous solution), ionic mobility, concentration in the donor or receiver cell of penetrant and potential difference across the membrane, respectively, and the subscripts b and c represent the benzoate anion and the

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monovalent cation. Equations 1 and 1' can be applied when concentration differences of  $b$  and  $c$  between the donor and receiver solutions are negligible.  $\beta$  may be related to a volume or area fraction of aqueous porosity in the biological lipoidal membrane, because no ions generally are able to penetrate into the lipid phase of the membrane.

In a case of cathodal iontophoresis, transport of a cation from the donor to receiver and that of an anion from the receiver to donor through a membrane can be negligible.<sup>15)</sup> Transport number of benzoate anion ( $t_b$ ) is thus expressed as follows<sup>16)</sup>:

$$(t_b)_{AC=0} = J_b / (J_b + J_c) = \beta_b U_b C_b / (\beta_b U_b C_b + \beta_c U_c C_c) \quad (2)$$

Under a constant current application, sum of the fluxes of benzoate anion from donor to receiver and a monovalent cation from receiver to donor become a constant,  $k$ , after a short lag time, as follows:

$$J_b + J_c = 1/ZF = k \quad (3)$$

where  $Z$  and  $F$  are charge of ion ( $Z=1$  in our case) and Faraday constant, respectively. Using Eq. 3, Eq. 2 is transformed to:

$$J_b = k \beta_b U_b C_b / (\beta_b U_b C_b + \beta_c U_c C_c) = k / (1 + R_\beta R_{UC}) \quad (4)$$

$$R_\beta = (\beta_c / \beta_b), \quad R_{UC} = (U_c C_c / U_b C_b)$$

The following equation is finally obtained:

$$k/J_b = 1 + R_\beta R_{UC} \quad (4')$$

A plot of  $k/J_b$  as an ordinate against the  $R_{UC}$  (which is calculated by the concentration and the ionic mobility of benzoate anion and cation used in the iontophoretic permeation experiment) as an abscissa may give a straight line with a slope of  $R_\beta$  and an intercept of 1. The obtained  $R_\beta$  is a good index for selecting a candidate drug and pharmaceutical additive for iontophoretic drug delivery through skin.

### Experimental

**Materials** Sodium benzoate (Japanese Pharmacopoeia grade) was obtained from Yamada Pharmaceutical Co., Ltd. (Ibaraki, Japan). Lithium chloride (LiCl), potassium chloride (KCl) and TEA-Br were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Platinum wire (99.9% purity, 1 cm  $\times$  1 mm) was obtained from Tokuriki (Tokyo, Japan) for electrodes. Other chemicals were of reagent grade. All reagents were used without further purification and all solutions were made with deionized water which had been passed through a water purifier (Eyela ER, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). The resulting water had a pH of 6.4 and electric conductivity of  $0.39 \mu\text{S cm}^{-1}$ .

**Animals** Male hairless rats (WBN/ILA-Ht strain, weighing about 180 g, Life Science Research Center, Josai University, Saitama, Japan) were used.

**In Vitro Skin Permeation Study** Figure 1 shows the entire apparatus for the *in vitro* permeation study. Excised abdominal full-thickness skin of the hairless rat was mounted between donor and receiver sides of the 2-chamber iontophoretic diffusion cell equipped with platinum electrodes. The donor (epidermis) side was filled with 4 cm<sup>3</sup> of deionized water and the receiver (dermis) side with the same volume of LiCl, KCl or TEA-Br solution. Both sides of the cell were stirred for 12 h by a star-head bar activated by a constant-speed synchronous motor (MC-301, Scinics, Tokyo, Japan) to sufficiently hydrate the skin so that the potential difference across the skin immediately after current application was much lower, and there was less skin sample-to-sample difference than that without the 12 h-skin hydration treatment. After this pretreatment, only the donor side was removed and filled with 4 cm<sup>3</sup> of sodium benzoate solution. The surface area of the skin available for the drug permeation was 0.95 cm<sup>2</sup>. Pretreatment and permeation experiments were carried out at 37 °C. Both electrodes were immersed in the solution of both half-cells, and were then connected to a constant current power source (Phoresor, PM 600, Iomed, Inc., Salt Lake City, UT, U.S.A.). The donor side of the cell was filled with 4 cm<sup>3</sup> of sodium benzoate solution (0.069, 0.21 or 0.35 M) and the receiver side of the cell with the same volume of LiCl, KCl or TEA-Br solution (0.021, 0.21 or 2.1 M). A passive transport study (0 mA) was carried out during the first 4 h of the permeation experiment. Thereafter, a cathodal iontophoretic permeation study was performed for 4 h at a constant current of 0.5 mA. At predetermined times, 0.5 cm<sup>3</sup> of the receiver solution was removed and the same volume of receiver solution was added to maintain a constant volume throughout the permeation experiment. Permeated benzoate anion was assayed by HPLC.<sup>12)</sup>

**Measurement of Potential Difference across the Skin** Potential difference across the skin at iontophoresis was measured between two salt bridges (3% agar in 3 M KCl solution) which were connected *via* calomel electrodes (HC-205C, TOA Electric Ltd., Tokyo, Japan) to a digital multimeter (TR6843, Takeda Riken, Tokyo, Japan).

### Results

**Effect of Donor Concentration** To measure the effect of sodium benzoate concentration on the skin permeation of its anion, the donor side was filled with 0.069, 0.21 or 0.35 M sodium benzoate and the receiver side with 0.21 M KCl. Figure 2a shows permeation profiles of benzoate anion through hairless rat skin without (0 mA, 4 h) and

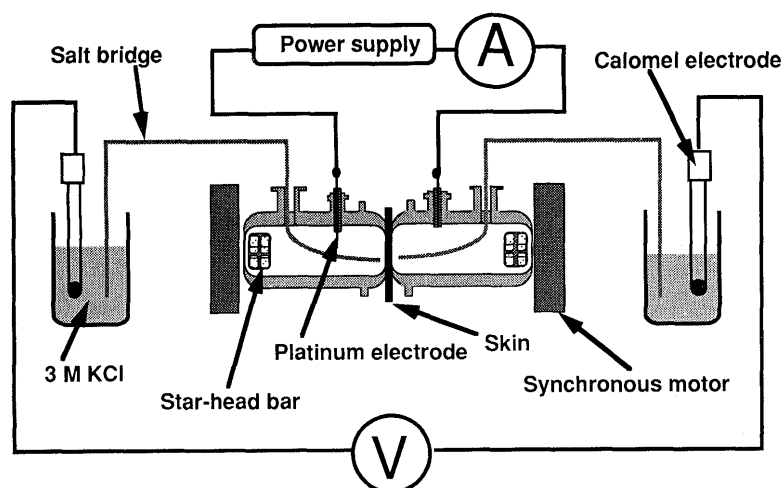


Fig. 1. Schematic Diagram of Iontophoretic Permeation Apparatus

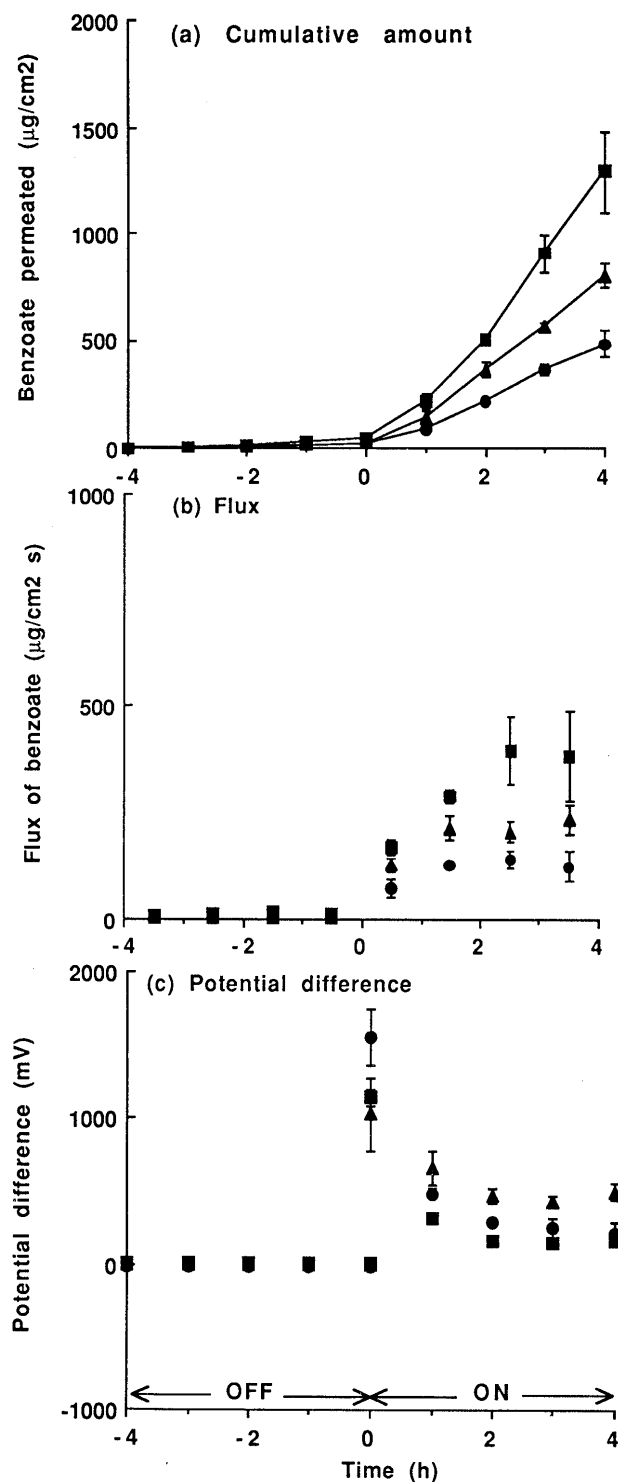


Fig. 2. Effect of Sodium Benzoate Concentration in the Donor Side on Benzoate Anion Permeated through Hairless Rat Skin and Change in Voltage Drop

(a) Cumulative amount, (b) flux, (c) voltage drop. ●, 0.069 M; ▲, 0.21 M; ■, 0.35 M. Each point represents the mean  $\pm$  S.E. of 4 experiments.

with (0.5 mA, 4 h) cathodal iontophoresis. Rank order of the cumulative amount of benzoate anion permeated over each period was the same as the applied concentration of sodium benzoate. Figure 2b shows each flux of benzoate anion during the permeation experiment. The flux increased after the current application to reach a constant value at about 2 h after the current application. Figure 2c shows time course of potential difference across the skin.

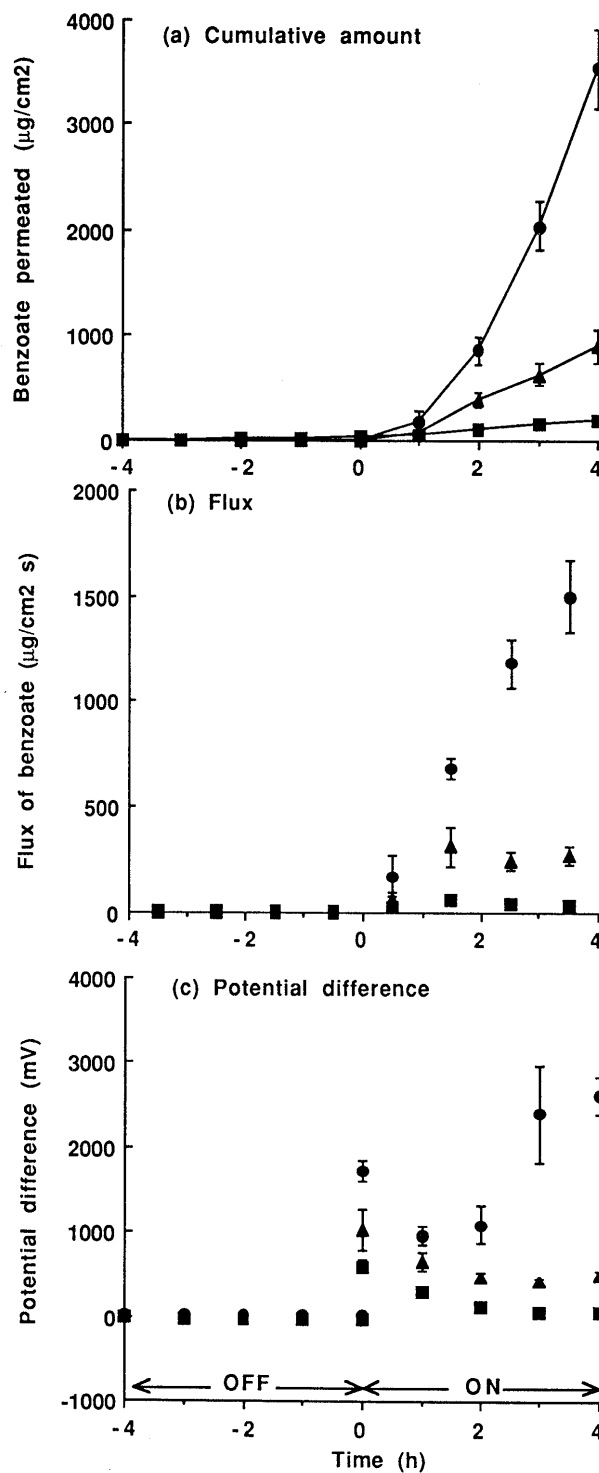


Fig. 3. Effect of KCl Concentration in the Receiver Side on Benzoate Anion Permeated through the Hairless Rat Skin and Change in Voltage Drop

(a) Cumulative amount, (b) flux, (c) voltage drop. ●, 0.021 M; ▲, 0.21 M; ■, 2.1 M. Each point represents the mean  $\pm$  S.E. of 4 experiments.

The potential differences gradually decreased with passage of time to a certain level.

**Effect of Receiver Concentration** The receiver side was filled with 0.021, 0.21 or 2.1 M KCl and the donor side with 0.21 M sodium benzoate. The cumulative amount of benzoate anion permeated from -4 to 0 h by passive diffusion (0 mA) was almost the same among the three treatments, whereas that from 0 to 4 h decreased in spite

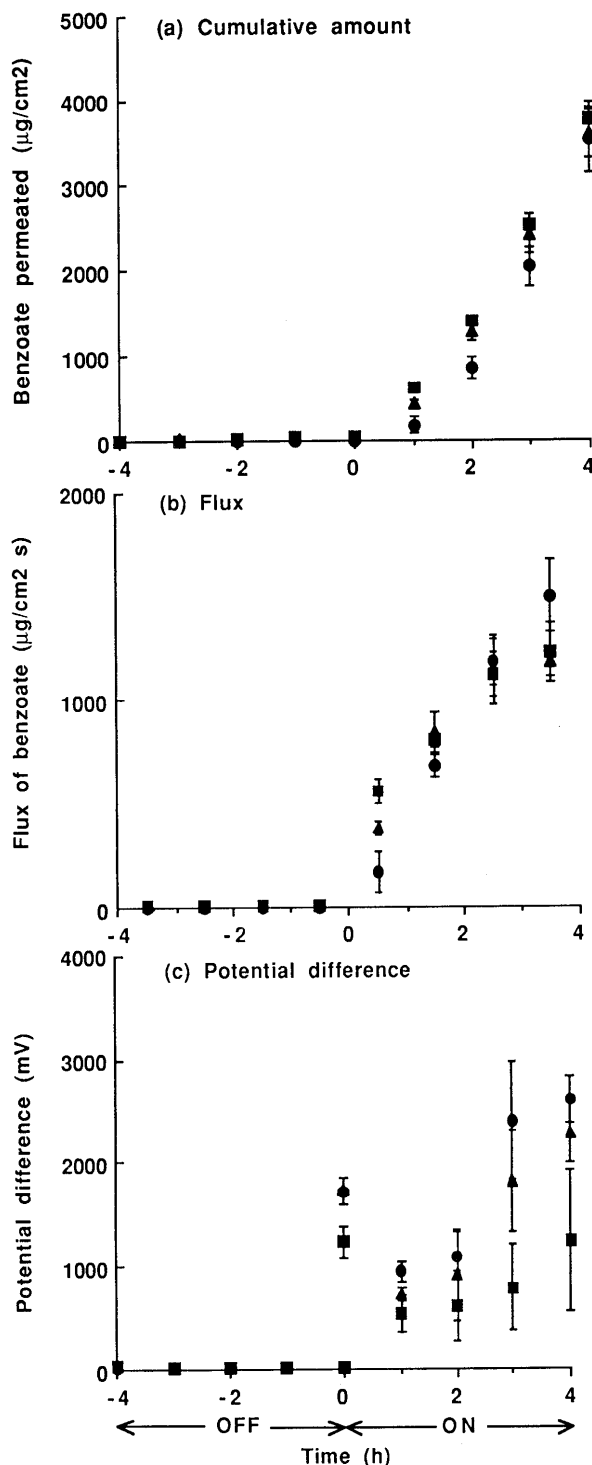


Fig. 4. Effect of Ionic Mobility in the Receiver Side on the Benzoate Anion Permeated through Hairless Rat Skin and Voltage Drop

(a) Cumulative amount, (b) flux, (c) voltage drop. ●, 0.021 M K<sup>+</sup>; ▲, 0.021 M Li<sup>+</sup>; ■, 0.021 M TEA<sup>+</sup>. Each point represents the mean  $\pm$  S.E. of 4 experiments.

of there being the same concentration of sodium benzoate in the donor side when the K<sup>+</sup> concentration in the receiver side was increased (Fig. 3a). The flux of benzoate anion increased immediately after application of the current (Fig. 3b). No steady-state flux was observed for 0.021 M KCl, nor for 0.21 or 2.1 M KCl. A higher KCl concentration resulted in a lower potential difference across the skin. The potential difference became a constant value with 0.21 and 2.1 M KCl, whereas it decreased and

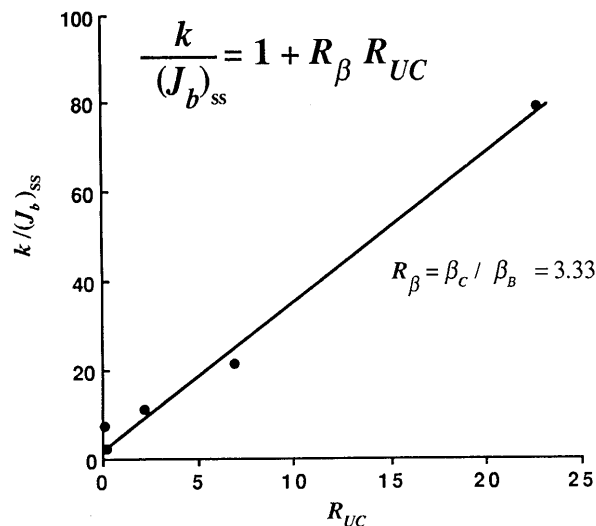


Fig. 5. Relationship between  $k/J$  and  $R_{UC}$

Average flux ( $J$ ) was calculated by the permeation data at 6, 7 and 8 h.

then increased with 0.021 M KCl.

**Effect of Ionic Mobility** To measure the effect of ionic mobility of the pharmaceutical additive in the receiver side on the flux of benzoate anion, the donor side was filled with 0.21 M sodium benzoate and the receiver side with 0.021 M LiCl, KCl or TEA-Br. The cumulative amount of benzoate anion permeated over 4 h by passive diffusion (0 mA) was almost the same, but increased immediately after the current was applied (Fig. 4a). No significant difference in the flux of benzoate anion was found among LiCl, TEA-Br and KCl, although each flux increased with passage of time. Time course of voltage drop for LiCl or TEA-Br (Fig. 4c) decreased and increased the same as for 0.021 M KCl (Fig. 3c).

**Analysis Using  $\beta$  Ratio** A ratio of partition coefficient of benzoate anion ( $\beta_b$ ) and monocation ( $\beta_c$ ) between skin membrane and aqueous solution,  $R_\beta$  ( $R_\beta = \beta_c / \beta_b$ ) was determined by fitting 5 sets of average flux of benzoate anion over 2–4 h obtained in the iontophoresis experiments when KCl was used as a pharmaceutical additive in the receiver side (Figs. 2b and 3b) to Eq. 4'. The obtained  $R_\beta$  was 3.33 (slope in Fig. 5), which meant that K<sup>+</sup> distributed 3.33 fold more easily into the skin membrane than benzoate anion.

The solid line in Fig. 6a indicates a theoretical relationship between benzoate anion flux and its concentration in the donor side, calculated by Eq. 4. This simulation line was close to the observed data points. The same kind of simulation was done in Fig. 6b, where the solid line indicates a theoretical relationship between benzoate anion flux and KCl concentration in the receiver side. The simulated line was also close to the observed data points.

$R_\beta$  of each ion was then evaluated:  $R_\beta$  of Li<sup>+</sup> and TEA<sup>+</sup> against benzoate anion was calculated to be 13.0 and 14.0, respectively, using permeation data as shown in Fig. 4 and ionic mobility (Li<sup>+</sup> and TEA<sup>+</sup> is 4.008 and 3.385  $\times 10^{-4}$  cm<sup>2</sup> V<sup>-1</sup> s<sup>-1</sup>, respectively).<sup>17)</sup> Interestingly, a higher  $R_\beta$  was obtained with a lower ionic mobility of the cation in the receiver side.

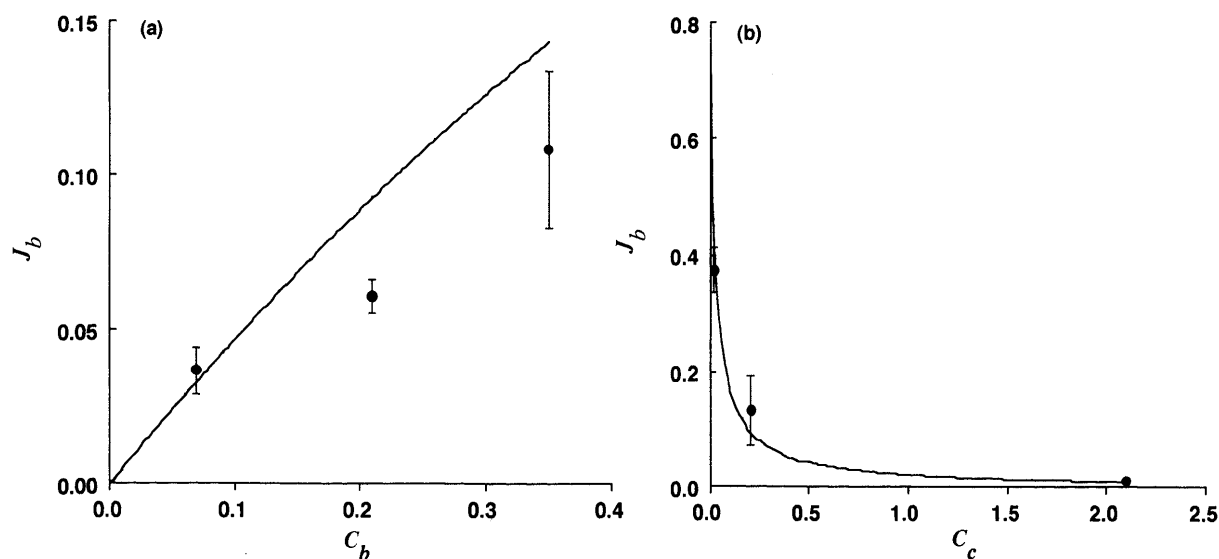


Fig. 6. Simulation of a Relationship between Benzoate Anion Flux and Concentration in the Donor or Receiver Side Solution  
(a) Sodium benzoate in the donor side, (b) potassium chloride in the receiver side.

### Discussion

Flux of benzoate anion during iontophoresis increased over 2 h to reach a steady-state when a different concentration of sodium benzoate in the donor side and 0.21 M KCl in the receiver side were applied (Fig. 2b). In contrast, no steady-state flux was observed when 0.021 M KCl, TEA-Br or LiCl was applied (0.021 M KCl in Figs. 3b and 4b). Potential difference across the skin during the iontophoresis became constant only when a steady-state flux of benzoate anion was observed (Fig. 2c). It is considered that the variation in the skin sample-to-sample potential difference across the skin is due to the difference of permeation resistance and/or amount of ions in individual skins. It increased with an increase in the flux of benzoate anion (0.021 M KCl in Figs. 3c and 4c).

The total current through the skin membrane may obey the following relationship<sup>15)</sup>:

$$I_t = Z_b F J_b S + Z_c F J_c S \quad (5)$$

where  $I_t$  is total current (mA) and  $S$  is effective area of ion permeation ( $\text{cm}^2$ ). The sum of fluxes of benzoate anion and monocation contributes to the total current through the membrane. The flux of monocation contributes to most of the total current, since each ionic mobility of the monocations used in the present study is higher than that of the benzoate anion. When ions with high ionic mobility were depleted in the receiver side, a flux of benzoate anion with low ionic mobility was increased and compensated for the total current (0.021 M KCl in Figs. 3b, 4b). A low permeation of an ion through skin or permeation of an ion with low ionic mobility results in the incremental change in the electrical resistance of skin. Potential difference across the skin is increased with increasing electric resistance of the skin at a constant current iontophoresis in support of Ohm's law. It was suggested that these increments of potential difference were probably due to the incremental change in transport number of the benzoate anion.

Figure 6a shows the relationship between flux and concentration of benzoate anion in the donor side, and

Fig. 6b shows flux of benzoate anion and concentration of KCl in the receiver side. The resulting good fittings to Eq. 4 suggested that the effect of ionic concentrations in the donor or receiver side on the permeation of benzoate anion through the skin could be analyzed by the transport numbers of their ions.

Flux of benzoate anion and  $\text{K}^+$ ,  $\text{Li}^+$  or  $\text{TEA}^+$  through the skin during iontophoresis,  $J_b$  and  $J_c$ , obeys Eqs. 1 and 1'. With an increase in the concentration of sodium benzoate in the donor side ( $C_b$ ) under a constant KCl concentration in the receiver side ( $C_c$ ) the flux of benzoate anion that permeated ( $J_b$ ) was increased (Fig. 2b). Both fluxes ( $J_b$  and  $J_c$ ) contribute to the total current through the skin. Since ionic mobility of potassium cation ( $U_c$ ) is much larger than that of the benzoate anion ( $U_b$ ) however,  $J_c$  mainly contributes to the total current. Consequently, potential difference across the skin ( $E$ ) immediately after the voltage application was almost the same, independent of  $C_b$  (Fig. 2c). As the  $E$ ,  $U_b$ , and  $\beta_b$  at that 4 h time period are constant,  $J_b$  is proportional to  $C_b$  as shown in Eq. 1. The present results (Fig. 2a) supported this theory.

With an increase in the KCl concentration in the receiver side, the flux of benzoate anion that permeated through the skin decreased exponentially, as shown in Figs. 3b and 6a. This phenomenon is due to a decrease in the transport number of benzoate anion across the skin. Potential difference was the highest when the lowest concentration of KCl was applied in the receiver side (Fig. 3c), which can be explained by the decrease in total ions in the skin.

No change in the cumulative amount or flux of benzoate anion immediately after application of iontophoresis was observed when the KCl solution in the receiver side was replaced by LiCl or TEA-Br solution (Fig. 4). Under constant  $C_i$  and  $E$  in Eq. 1 or 1',  $J_i$  is decreased with a decrease in  $U_i$ . A decrease in ion flux means an increase in electric resistance of the skin. Under constant current application, i.e., the flux of ion  $i$  ( $J_i$ ) does not change, the potential difference ( $E$ ) across the membrane increases since the electric resistance of the skin increases. That is,

the potential difference across the skin increases when the ion in the receiver solution changes to a low ionic mobility ion under constant current iontophoresis. Potential difference across the membrane is a driving force of ion permeation. The flux of benzoate anion increased when a low ionic mobility ion was applied in the receiver side (Fig. 4b). However, potential difference across the skin after application of iontophoresis was almost the same when the ion in the receiver was changed (Fig. 4c). This is the reason that the benzoate anion flux was not affected by ionic mobility of ions in the receiver side. An increase in the potential difference across the skin at the end of iontophoretic permeation study may be due to ion depletion in the receiver side.  $R_b$  was increased with a decrease in ionic mobility of a cation in the receiver side.  $\beta_c$  was increased when the large Stokes radius ion, *i.e.*, ion with low ionic mobility was applied in the receiver side. If the larger Stokes' radius ion requires larger pore radius for ion permeation,  $\beta$  may be regarded as the pore occupation coefficient.

### Conclusion

In the present studies, the effect of ion concentration in the donor or receiver side on the skin permeation of benzoate anion was analyzed by transport number of their ions. Ion concentration (or mobility), as well as partition coefficient ( $\beta$ ) between ionic solution and the hairless rat skin affected the skin permeation. It was suggested that the *in vitro* permeation of the drug increased when an ion with low ionic mobility ( $U$ ) and low partition coefficient ( $\beta$ ) was applied in the receiver side. In the *in vivo* application, however, endogenous ions correspond-

ing to the receiver side cannot be changed. We finally proposed criteria for selecting a candidate drug and an additive (stabilizer, enhancer and solubilizer) for iontophoretic drug delivery: the drug must have high ionic mobility ( $U$ ) and high partition coefficient ( $\beta$ ), while the additive should have low ionic mobility and low partition coefficient.

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