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Effect of Ion Species and Their Concentration on the Iontophoretic Transport of Benzoic Acid through Poly(vinyl acetate) Membrane

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Effects of ion species and their concentration on the iontophoretic transport of benzoic acid through an artificial membrane [poly(vinyl acetate)] were investigated using a 2-chamber diffusion cell equipped with platinum electrodes and a constant current power source. The cathode side of the cell was filled with sodium benzoate solution, and the anode side with potassium chloride, lithium chloride or tetraethylammonium bromide solution. When the molar concentration of sodium benzoate in the cathode side (0.21 M) was the same as the potassium chloride in the anode side, the amounts of benzoate anion and potassium cation permeated through the membrane were greater with increasing current. With an increase in the concentration of benzoate anion in the cathode side and a constant concentration of potassium cation in the anode side, the amount of the former that permeated was proportional to the concentration applied, while permeation of potassium cation remained almost constant at a constant current of 0.2 mA. Conversely, an increase in the concentration of potassium cation in the anode side with a constant concentration of benzoate anion in the cathode side resulted in an increase of the permeation of potassium cation and a decrease of that of benzoate anion, respectively, at the same constant current. When potassium chloride in the anode side was replaced by tetraethylammonium bromide, the amount of benzoate anion permeated was increased at a constant current of 0.2 mA. These results may be explained by the following theory on the transport number of each ion through the artificial membrane: Current density calculated from ion flux through the membrane was almost the same as that measured by observed current density. The results appeared to indicate that not only an ion species and its concentration in the donor solution but also ion species in the receiver solution should be considered when evaluating the iontophoretic transport phenomena.

Keywords iontophoresis; permeability; poly(vinyl acetate) membrane; transport number; sodium benzoate

Potent ionizable drugs such as peptides and proteins can now be easily synthesized by biotechnology, and it is important that a suitable dosage form and delivery route are designed for each drug. Peptide and protein drugs are highly susceptible to degradation in the gastrointestinal environment and hepatic "first-pass" metabolism when taken orally. Frequent injections are needed because of their short biological half life when taken parenterally. Transdermal administration is thus interesting to avoid "first-pass" metabolism and obtain typical sustained absorption by this administration route. The permeation rate of such drugs, however, is generally so poor that only low bioavailability is obtained through the skin. This poor permeability can be overcome by the use of one of several methods which include chemical enhancers and the application of external energy. Ultrasound or electric current is used as external energy to enhance the percutaneous absorption of several drugs. The two general categories of energy used are phonophoresis and iontophoresis. Iontophoresis can be defined as a process or method in which the permeation rate of ionic species into the body is enhanced by applying an electric current between viable tissues. Many studies have confirmed its usefulness in chemotherapy[1)] and diagnostics,[2)] and recent efforts have concentrated on understanding and elucidating the mechanism involved.[3)] Few studies have been reported, however, on power sources, electrodes, composition of vehicles or pharmaceutical additives used with these iontophoretic systems.[4)] The purpose of the present study was to gain basic information on the effect of ion species and ionic concentration on the iontophoretic transport of benzoic acid as a model drug using a 2-chamber diffusion cell. Poly(vinyl acetate) membrane was used as an artificial membrane to prevent the effect of biological ions on the transport phenomenon of the drug. The poly(vinyl acetate) membrane is useful because of its adequate permeation property against ions, adequate hydrophilicity (or lipophilicity) and comparative rigidity.

Experimental

Materials Sodium benzoate (Japanese Pharmacopeia grade) was obtained from Yamada Pharmaceutical Co., Ltd. (Ibaraki, Japan). Poly(vinyl acetate) (monomer unit = 1400—1600) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Cold and radiolabelled tetraethylammonium bromide ([1-^{14}C]-, specific activity; 0.15 GBq/mmol, purity; 99%) were obtained from Wako Pure Chemical Industries, Ltd. and Du Pont New England Nuclear (Boston, MA, U.S.A.), respectively. 2,5-Diphenyloxazole was obtained from Wako Pure Chemical Industries, Ltd. and *p*-bis[2-phenyloxazolyl)]-benzene was obtained from Du Pont New England Nuclear to make a scintillation cocktail. Platinum wire (99.9% purity, 1 cm × 1 mm) was obtained from Tokuriki (Tokyo, Japan) for the 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 6.74 and an electric conductivity of 0.39 μS cm^{-1}. Silicone-coated poly(ethylene terephthalate) (PET) film was a gift from Nichiban Co., Ltd. (Tokyo, Japan).

Membrane Preparation Poly(vinyl acetate) (3.7 g) was dissolved in ethyl acetate to get a 37% poly(vinyl acetate) solution. A sheet of silicone coated PET film (30 cm × 25 cm × 25 μm) was placed on a flat glass plate (with silicone-coated side up) and secured in place with silicone tape. The polymer solution (*ca.* 4 g) was poured carefully onto the silicone-coated PET film. An applicator (Ueshima, Tokyo, Japan) was then gently passed through the silicone-coated PET film to produce a 5 ± 1 μm thick membrane. The solvent (ethyl acetate) was allowed to evaporate for 3 min under 130 °C in a laboratory dryer. The resulting poly(vinyl acetate) membrane was measured by a dial thickness gauge (limit, 0.1 μm), and membrane with a thickness of 5 ± 1 μm was used in the permeation study.

Membrane Permeation Study A sheet of poly(vinyl acetate) membrane

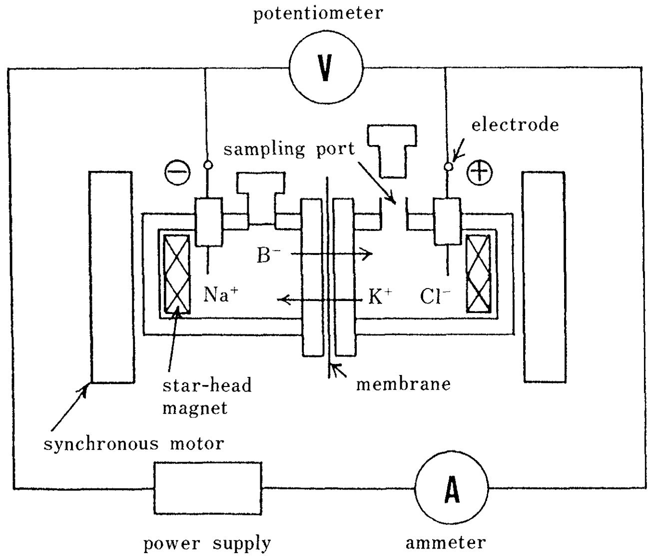

Fig. 1. Schematic Diagram of Ion Permeation Study

B^- means benzoate anion: the same as below.

was mounted between the cathode and anode sides of a 2-chamber diffusion cell which was equipped with platinum electrodes (Fig. 1). The surface area of the membrane available for ion permeation was 0.95 cm^2. A pair of platinum electrodes was immersed in the solutions in both sides of the cell. These electrodes were connected to a constant current power source (DMR-20-2, Metronix, Tokyo, Japan). Current was measured with a digital multimeter (TR6843, Takeda Riken, Tokyo, Japan) in series with the 2-chamber diffusion cell and constant current power source. The cathode side of the cell was filled with 4 ml of sodium benzoate solution (0.069, 0.21 or 0.35 M) and the anode side of the cell with the same volume of potassium chloride, lithium chloride or tetraethylammonium bromide solution (0.021, 0.21 or 2.1 M). A sample of 0.05 ml was withdrawn at predetermined intervals from either side of the cell, and the amount of ions permeated through the poly(vinyl acetate) membrane was determined.

Determination of Permeated Ion A high performance liquid chromatography system consisting of a pump (LC-6A, Shimadzu, Kyoto, Japan), an ultraviolet detector (SPD-6A, Shimadzu) and an integrator (C-R6A, Shimadzu) was used for analyzing benzoate anion concentration. Acetonitrile: 0.05 M phosphate buffer solution (pH 2.5) (1 : 1, v/v) was used as a mobile phase at a flow rate of 1.0 ml min^{-1}. Benzoate anion was resolved using a 4.6 x 250 mm stainless-steel column packed with Nucleosil $5C_{18}$ (Macherey Nagel, Germany) and detected at a wavelength of 230 nm. Ten μg ml^{-1} of *p*-ethylbenzoate in methanol solution was used as an internal standard. A clinical ion meter (CIM-104A, Shimadzu) was used for analyzing potassium cation, and a flame emission spectrophotometer (detector AA-200, gas control unit JAG-5D, Nippon Jarrell-Ash Co., Ltd., Kyoto, Japan) to analyze lithium cation. Analytical conditions were as follows: Peak height method; oxidant, air 6.5 l min^{-1} at 2.45 kg cm^{-2}; fuel, C_2H_2 1.25 l min^{-1} at 0.5 kg cm^{-2}; wavelength, 670.5 nm. Cold tetraethylammonium bromide solution was spiked with hot (185 kBq) tetraethylammonium bromide solution in the anode side of the cell. A 0.05 ml sample was withdrawn at predetermined intervals from the cathode side and poured into vials containing the scintillation cocktail. Radioactivity was measured by a liquid scintillation counter (LSC-703, Aloka, Tokyo, Japan).

Results

Effect of Applied Current on Ion Permeation First, the cathode side was filled with 4 ml of 0.21 M sodium benzoate and the anode side was filled with the same volume of 0.21 M potassium chloride. Figure 2a shows the cumulative amount of benzoate anion permeated through the poly(vinyl acetate) membrane from the cathode side to the anode side, and Fig. 2b shows that of potasssium cation permeated through the membrane from the anode side to the cathode side.

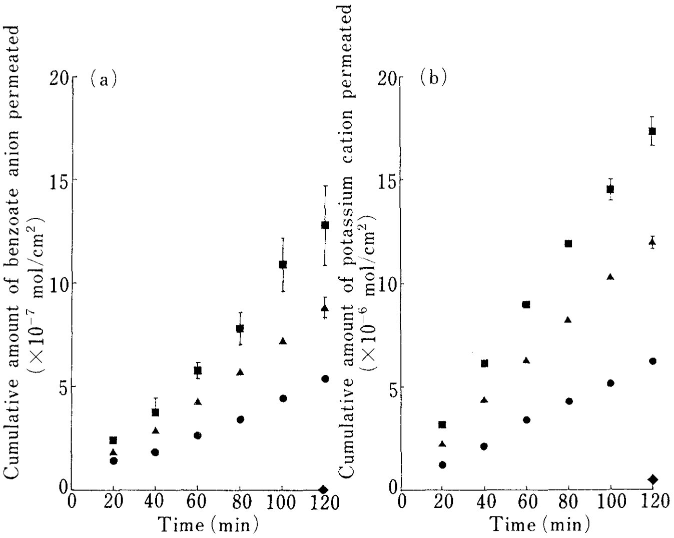

Fig. 2. Effect of Applied Current on the Benzoate Anion (a) and Potassium Cation (b) Permeated through the Poly(vinyl acetate) Membrane

Control; ◆, 0.1 mA; ●, 0.2 mA; ▲, 0.3 mA; ■. Cathode and anode sides are filled with 0.21 M sodium benzoate and potassium chloride solution, respectively. Each point represents the mean ± S.E. of three experiments.

Table I. Effect of Applied Current on the Enhancement Factor of Benzoate Anion and Potassium Cation[a)]

Applied current (mA)	Cumulative amount of ion permeated ($\times 10^{-7}$ mol/cm²)		Enhancement factor[b)]	
	B^-	K^+	B^-	K^+
0	0.0537	5.05		
0.1	5.39	62.2	100.4	12.3
0.2	8.84	120.0	164.6	23.8
0.3	12.8	173.0	238.4	34.3

a) Cathode and anode sides were filled with 0.21 M sodium benzoate and potassium chloride solution, respectively.

b) Enhancement factor $= \dfrac{\text{cumulative amount of ion (iontophoresis)}}{\text{cumulative amount of ion (passive)}}$.

Table I shows the enhancement factor of the permeation of benzoate anion and potassium cation, which was calculated by dividing the cumulative amount of the two ions permeated in the first 2 h of the experiment by those of the control experiments (no applied current) (see legend of Table I). The permeations of benzoate anion and potassium cation were increased with an increase in the applied current.

Effect of Sodium Benzoate Concentration in the Cathode Side on Ion Permeation Next, the cathode side was filled with 4 ml of 0.069, 0.21 or 0.35 M sodium benzoate and the anode side with 4 ml of 0.21 M potassium chloride. Figure 3a shows the cumulative amount of benzoate anion permeated through the membrane from the cathode to the anode side, and Fig. 3b shows that of potassium cation permeated from the anode to the cathode side at a constant current of 0.2 mA. Table II shows the enhancement factor of the permeation of the two ions. When the concentration of benzoate anion in the cathode side was increased with the concentration of 0.21 M potassium cation remaining constant in the anode side, the amount of benzoate anion permeated was proportional to its applied concentration

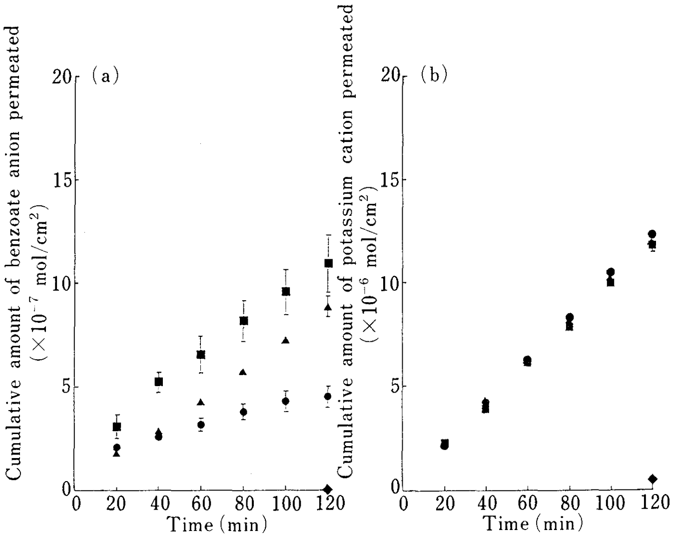

Fig. 3. Effect of Sodium Benzoate Concentration in the Cathode Side on the Benzoate Anion (a) and Potassium Cation (b) Permeated through the Poly(vinyl acetate) Membrane at a Constant Current of 0.2 mA

Control; ◆, 0.069 M; ●, 0.21 M; ▲, 0.35 M; ■. Anode side is filled with 0.21 M potassium chloride solution. Each point represents the mean ± S.E. of three experiments.

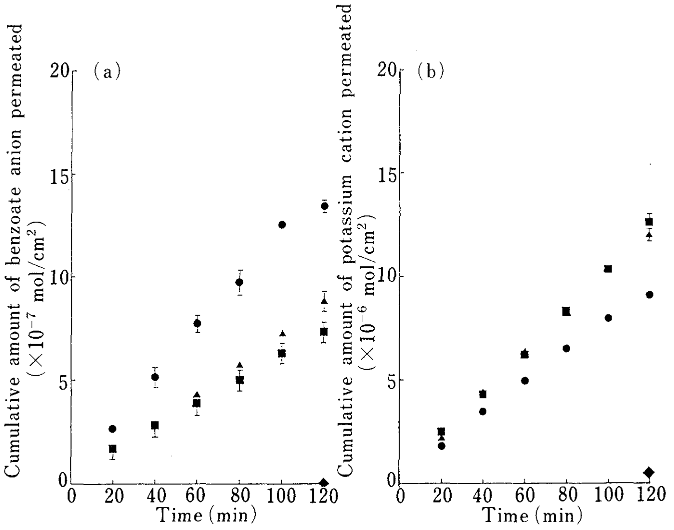

Fig. 4. Effect of Potassium Chloride Concentration in the Anode Side on the Benzoate Anion (a) and Potassium Cation (b) Permeated through the Poly(vinyl acetate) Membrane at a Constant Current of 0.2 mA

Control; ◆, 0.021 M; ●, 0.21 M; ▲, 2.1 M; ■. Cathode side is filled with 0.21 M sodium benzoate solution. Each point represents the mean ± S.E. of three experiments.

TABLE II. Effect of Ion Concentration of Cathode Side[a] on the Enhancement Factor of Benzoate Anion and Potassium Cation

Sodium benzoate conc. (M)	Cumulative amount of ion permeated (× 10^{-7} mol/cm^2)		Enhancement factor[b]	
	B$^-$	K$^+$	B$^-$	K$^+$
Control	0.0537	5.05	—	—
0.069	4.49	123.0	83.6	24.4
0.21	8.84	120.0	164.6	23.8
0.35	10.9	118.0	203.0	23.4

a) Anode side was filled with 0.21 M potassium chloride solution. b) See the legend of Table I.

TABLE III. Effect of Ion Concentration of Anode Side[a] on the Enhancement Factor of Benzoate Anion and Potassium Cation

Potassium chloride conc. (M)	Cumulative amount of ion permeated (× 10^{-7} mol/cm^2)		Enhancement factor[b]	
	B$^-$	K$^+$	B$^-$	K$^+$
Control	0.0537	5.05	—	—
0.021	13.3	90.2	247.7	17.9
0.21	8.84	120.0	164.6	23.8
2.1	7.31	126.0	136.1	25.0

a) Anode side was filled with 0.21 M potassium chloride solution. b) See the legend of Table I.

and the amount of potassium cation permeated was almost constant at the constant current of 0.2 mA. Table II shows the enhancement factor of the two ions.

Effect of Potassium Chloride Concentration in the Anode Side on Ion Permeation The cathode side was then filled with 4 ml of 0.21 M sodium benzoate and the anode side with 4 ml of 0.021, 0.21 or 2.1 M potassium chloride. Figure 4a shows the cumulative amount of benzoate anion permeated through the membrane from the cathode to the anode side, and Fig. 4b shows that of potassium cation permeated from the anode to the cathode side at a constant current of 0.2 mA. Table III shows the enhancement factor for the two ion permeations. When the concentration of potassium cation in the anode side was increased while retaining a constant concentration of benzoate anion in the cathode side, the permeating amounts of these two ions were respectively increased and decreased.

Effect of Ion Species in the Anode Side on the Ion Permeation The cathode side was next filled with 4 ml of 0.21 M sodium benzoate and the anode side with 4 ml of 0.021 M potassium chloride, lithium chloride or tetraethylammonium bromide. Figure 5a shows the cumulative amount of benzoate anion permeated through the mem-

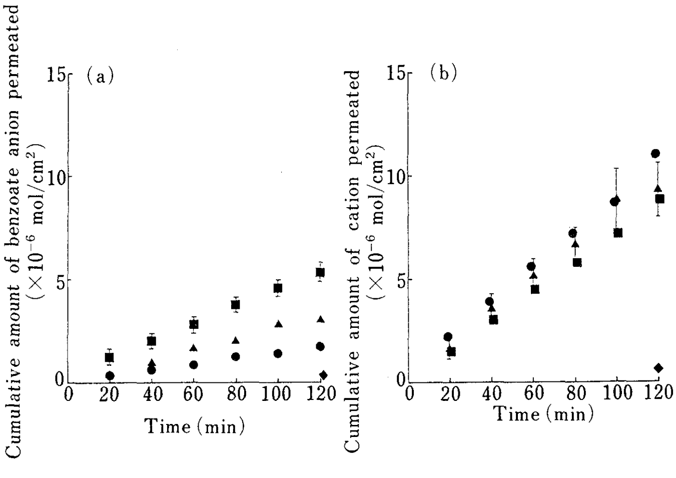

Fig. 5. Effect of Ion Mobility in the Anode Side on the Benzoate Anion (a) and Each Cation (b) Permeated through the Poly(vinyl acetate) Membrane at a Constant Current of 0.2 mA

◆, control; ●, potassium cation; ▲, lithium cation; ■, tetraethylammonium cation. Cathode side is filled with 0.21 M sodium benzoate solution and anode side is filled with 0.021 M potassium chloride, lithium chloride or tetraethylammonium bromide solution. Each point represents the mean ± S.E. of three experiments.

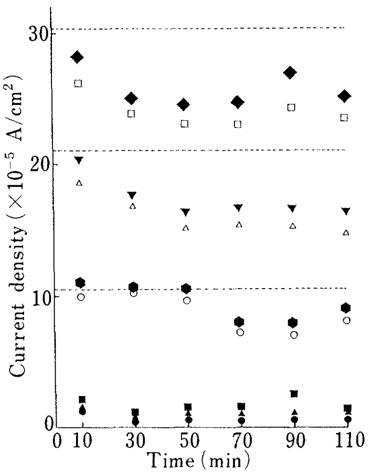

Fig. 6. Time Course of the Total Current Density Calculated from the Amount of Ion Permeated

●, 0.1 mA benzoate anion; ▲, 0.2 mA benzoate anion; ■, 0.3 mA benzoate anion; ○, 0.1 mA potassium cation; △, 0.2 mA potassium cation; □, 0.3 mA potassium cation; ⬟, total current density of 0.1 mA; ▼, total current density of 0.2 mA; ◆, total current density of 0.3 mA. Total current density = current density calculated from benzoate anion permeated + current density calculated from potassium cation permeated.

brane from the cathode to the anode side, and Fig. 5b shows that of potassium, lithium or tetraethylammonium cation permeated from the anode to the cathode side at a constant current of 0.2 mA. When the ion species in the anode side was changed from potassium cation to tetraethylammonium cation, the amount of benzoate anion permeated from the cathode to the anode side and that of the cation permeated from the anode to the cathode side were increased and decreased, respectively, at a constant current of 0.2 mA.

Time Course of Current Calculated from the Amount of Ions Permeated Figure 6 shows the time course of current density calculated from fluxes of benzoate anion from the cathode to the anode side and potassium cation from the anode side to the cathode side through the poly(vinyl acetate) membrane. The time course as calculated in this manner was almost the same as that measured by the observed current density. The difference in the current density calculated from ion flux and observed current density increased with the increase in applied current.

Discussion

In studying iontophoretic membrane permeation using a 2-chamber diffusion cell, not only drug ion (benzoate anion) transport but the transport of other ion species which contribute to the current through the membrane require consideration. Since fluxes of sodium cation from the cathode side to the anode side in the sodium benzoate solution and of chloride anion from the anode to the cathode side in the potassium chloride solution were almost negligible in the present study, the electric current through the poly(vinyl acetate) membrane obeys the following relationship:

$$I_{\mathrm{tot}} = Z_{\mathrm{B}^-} \times F \times C_{\mathrm{B}^-} \times U_{\mathrm{B}^-} \times E \times S + Z_{\mathrm{K}^+} \times F \times C_{\mathrm{K}^+} \times U_{\mathrm{K}^+} \times E \times S \quad (1)$$

where I_{tot} is total current through the membrane, Z, F, C, U, E and S are, respectively, charge of ion, Faraday constant (96485 $\mathrm{C\,mol^{-1}}$), concentration of ion ($\mathrm{mol\,cm^{-3}}$), ionic mobility ($\mathrm{cm^2\,s^{-1}\,V^{-1}}$), potential gradient ($\mathrm{V\,cm^{-1}}$) and surface area of membrane available for ion permeation ($\mathrm{cm^2}$).[5] Subscripts B^- and K^+ show benzoate and potassium ions, respectively. In general, flux of an ion i through membrane can be obtained using the following equation:

Table IV. Effect of Sodium Benzoate Concentration in the Cathode Side on the Transport Number

Sodium benzoate conc. (M)	Transport number	
	B^-	K^+
0.069	0.025	0.975
0.21	0.071	0.929
0.35	0.114	0.886

Anode side was filled with 0.21 M potassium chloride solution.

$$J_i = C_i \times U_i \times E \quad (2)$$

where J_i is flux of ion$_i$ ($\mathrm{mol\,cm^{-2}\,s^{-1}}$). Using Eq. 3, Eq. 1 is transformed to:

$$I_{\mathrm{tot}}/S = Z_{\mathrm{B}^-} \times F \times J_{\mathrm{B}^-} + Z_{\mathrm{K}^+} \times F \times J_{\mathrm{K}^+} \quad (3)$$

where I_{tot}/S is total current density and $Z_{\mathrm{B}^-} \times F \times J_{\mathrm{B}^-} + Z_{\mathrm{K}^+} \times F \times J_{\mathrm{K}^+}$ is current density due to the transport of benzoate anion from the cathode to the anode side and that of potassium cation from the anode to the cathode side. Total current density (left side of Eq. 3) can be determined by measuring the current in the iontophoretic membrane permeation experiment, and $\Sigma\, Z_i \times F \times J_i$ (right side of Eq. 3) can be calculated by fluxes of K^+ and B^-. Transport number of each ion species is defined as:

$$t_{\mathrm{B}^-} = Z_{\mathrm{B}^-} \times F \times J_{\mathrm{B}^-} / (Z_{\mathrm{B}^-} \times F \times J_{\mathrm{B}^-} + Z_{\mathrm{K}^+} \times F \times J_{\mathrm{K}^+}) \quad (4)$$

$$t_{\mathrm{K}^+} = Z_{\mathrm{K}^+} \times F \times J_{\mathrm{K}^+} / (Z_{\mathrm{B}^-} \times F \times J_{\mathrm{B}^-} + Z_{\mathrm{K}^+} \times F \times J_{\mathrm{K}^+}) \quad (5)$$

where t_{B^-} and t_{K^+} are the transport numbers of benzoate anion and potassium cation, respectively. The flux of each ion contributes to the transport number of that ion. Concentration of ion (C_i) and ionic mobility (U_i) also contributes to the transport number since they influence the ion flux as shown in Eq. 2.

The amount of benzoate anion and potassium cation permeated through the membrane was extremely low without current (passive diffusion alone) (Fig. 2). The current flow from one electrolyte through a membrane to another electrolyte is usually the result of ion transfer. As the applied current increased, the amounts of benzoate anion and potassium cation permeated through the membrane were increased (Fig. 2).

When the concentration of benzoate anion in the cathode side was increased, the amount of ion permeated was proportional to its concentration and the amount of potassium cation permeated was almost constant (Fig. 3). The ionic mobility of potassium cation was about 13 times greater than that of benzoate anion when the molar concentration of sodium benzoate in the cathode side was the same as that of potassium chloride in the anode side (Fig. 6). Table IV shows transport numbers of benzoate anion and potassium cation calculated by Eqs. 4 and 5. Figure 7 shows the relationship between sodium benzoate concentration in the cathode side and the transport number

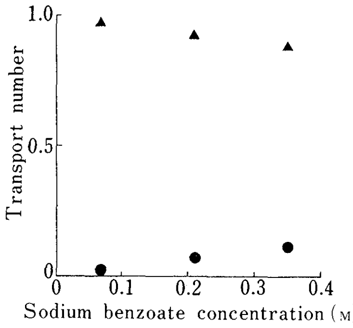

Fig. 7. Relationship between Sodium Benzoate Concentration in the Cathode Side and Transport Number of Benzoate Anion and Potassium Cation at a Constant Current of 0.2 mA

●, sodium benzoate; ▲, potassium chloride. Anode side is filled with 0.21 M potassium chloride solution.

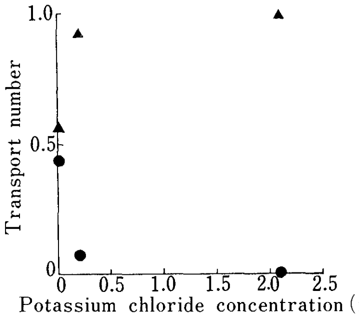

Fig. 8. Relationship between Potassium Chloride Concentration in the Anode Side and Transport Number of Benzoate Anion and Potassium Cation at a Constant Current of 0.2 mA

●, sodium benzoate; ▲, potassium chloride. Cathode side is filled with 0.21 M sodium benzoate solution.

Table V. Effect of Potassium Chloride Concentration in the Anode Side on the Transport Number

Potassium chloride conc. (M)	Transport number	
	B^-	K^+
0.021	0.435	0.565
0.21	0.071	0.929
2.1	0.008	0.992

Cathode side was filled with 0.21 M sodium benzoate solution.

of B^- and K^+. The amount of potassium cation permeated was not affected when the molar concentration of sodium benzoate in the cathode side was changed due to the much greater mobility of potassium cation than that of benzoate anion.

As the concentration of potassium cation in the anode side was increased, the amounts of benzoate anion and potassium cation permeated were decreased and increased, respectively (Fig. 4). Table V shows transport numbers of benzoate anion and potassium cation calculated by Eqs. 4 and 5. Figure 8 shows the relationship between potassium chloride concentration and transport number. The amount of benzoate anion permeated was increased when the molar concentration of potassium chloride in the anode side was decreased, because the ion mobility of potassium cation is greater than that of benzoate anion.

When potassium cation in the anode side was replaced by another cation such as lithium or tetraethylammonium cation, the amount of benzoate anion permeated from the cathode side to the anode side and the amount of cation permeated were increased and decreased, respectively (Fig. 5). It was thought that the amount of benzoate anion permeated was affected by the mobility of ions in the anode side. The ion mobility contributes also to the total current, and hence the transport number of benzoate anion was increased, especially when ion with low mobility (tetraethylammonium cation) was applied in the anode side.

The time course of current density calculated by Eq. 3 was almost the same as the observed current density (Fig. 6). The difference between the current density calculated by total ion flux (ion influx + efflux) and observed current density measured by an ammeter was greater with the increase in applied current. This difference may be due to a drop in voltage between the two platinum electrodes and an increase in electrolysis in both half cells with the increase in applied current.

Conclusion

The results of these studies suggested that not only the ion species and their concentration in the donor solution but also ion species in the receiver solution should be taken into consideration in any evaluation of the iontophoretic transport phenomenon. Clear understanding about the effects of ion species, ionic mobility and their concentration on the iontophoretic transport will lead to the design and discovery of well-assemblied iontophoretic devices.

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