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Effect of polymer ion concentrations on migration velocities in ion-exchange electrokinetic chromatography

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ABSTRACT

Ion-exchange electrokinetic chromatography was developed for the separation of analyte ions having identical electrophoretic mobilities in capillary electrophoresis. The separation principle is based on the differential ion-pair formation of the analyte ion with a polymer ion. Polybrene and poly(diallyldimethylammonium chloride) were employed as polymer ions and some isomeric acids as analytes. The dependence of the relative migration velocities of the analytes on the concentration of the polymer ions was studied experimentally, and the results are discussed in comparison with a theoretically derived equation.

INTRODUCTION

Electrokinetic chromatography (EKC)[1] is an electrophoretic separation technique based on the separation principle of chromatography. Micellar EKC[2,3] has been developed for the separation of electrically neutral analytes by electrophoresis with an ionic micellar solution as a separation solution. The ionic micelle, which corresponds to the stationary phase in conventional liquid chromatography, incorporates the analyte and migrates with a different velocity from that of the surrounding aqueous medium by electrophoresis. Accordingly, differential partition and differential migration constitute the separation principle of EKC, similar to that of chromatography. All of the advantages of high-performance capillary electrophoresis (HPCE)[4,5] can also be realized for EKC, and plate numbers more than 100 000 are easily obtained[6].

Micellar EKC has become a popular technique in HPCE. Although it is also called micellar electrokinetic capillary chromatography (MECC)[7], EKC techniques other than micellar are available. For example, in cyclodextrin EKC[1,8], cyclodextrin derivatives having ionizable groups are employed instead of a micelle. As inclusion-complex formation is the partition mechanism in cyclodextrin EKC, isomeric aromatic

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compounds and many chiral compounds have been successfully separated. It is noteworthy that ordinary cyclodextrins can modify the electrophoretic mobility of ionic analytes[9], but electrically neutral cyclodextrins themselves do not separate electrically neutral analytes.

HPCE has been proved to be a highly efficient separation method[4,5] and it is still a subject to extensive studies from the viewpoints of instrumentation and separation techniques. A disadvantage of HPCE is that it can separate only charged substances, but this has been overcome by the development of EKC. As the separation principle of HPCE is based on differences in electrophoretic mobilities, it is not effective for the separation of analytes having very similar or identical mobilities. This is often the case with isotopic ions or isomeric ions, such as positional isomers of aromatic compounds. Oxygen isotopic benzoic acids have been successfully resolved by optimizing the experimental conditions, in particular by carefully adjusting the pH of the separation buffer to the calculated optimum value[10]. The choice of pH is thus very important in separate analytes having very similar dissociation constants.

In order to increase its selectivity for isomeric ions, we have developed ion-exchange EKC, in which the separation mechanism is based on differential ion-pair formation of analyte ions with a polymer ion added to the separation solution[1,11]. A successful application of the technique was recently demonstrated with the separation of five isomeric naphthalenedisulphonates, which could not be resolved by HPCE alone[11].

In ion-exchange EKC, a polymer ion having a charge opposite to that of the analyte ion is employed as a modifier of the electrophoretic mobility. Both the analyte and the polymer ions are subject to electrophoresis, but the migration directions differ. Accordingly, an analyte ion bonded to the polymer ion through ion-pair formation migrates in the opposite direction of the free analyte ion, as shown in Fig. 1. It is consequently possible that even analytes having identical electrophoretic mobilities will migrate with different velocities if their ion-pair formation constants are different. The separation principle is based on differences in ion-pair formation constants, but

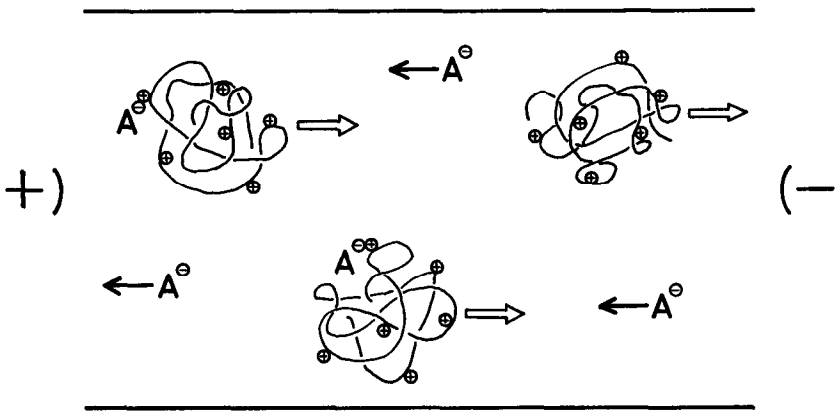


Fig. 1. Schematic diagram of the separation principle of ion-exchange EKC, where polymer cations are used. A^- = analyte ion; open and filled arrows = electrophoretic migration of the polymer ion and that of the analyte ion, respectively.

not on those in electrophoretic mobilities; therefore, the technique should be classified as a chromatographic method.

In this study, two different polymer cations, polybrene and poly(diallyldimethylammonium chloride) (PDDAC), were employed. The effect of the concentration of PDDAC on the relative migration velocities of various analytes was investigated mainly from the viewpoint of selectivity. The experimental data are explained according to a theoretically derived equation.

EXPERIMENTAL

Apparatus and procedures

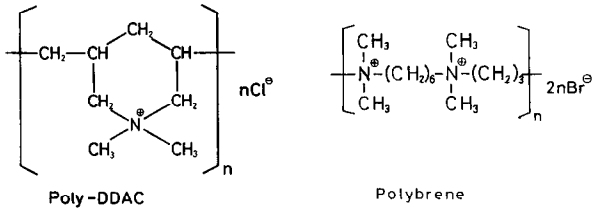
HPCE equipment similar to that described previously[2,10] was employed. Fused-silica tubing of 50 μm I.D. (Polymicro Technologies, Phoenix, AZ, U.S.A., or Scientific Glass Engineering, Ringwood, Victoria, Australia) was used as separation capillaries. The HPCE instrument consisted of a regulated high-voltage d.c. power supply (LG-40R-3.5, Glassman, Whitehouse Station, NJ, U.S.A.), which delivered up to 40 kV, a variable-wavelength UV detector for high-performance liquid chromatography (HPLC) (Uvidec-100-V, Jasco, Tokyo, Japan), the cell holder of which was modified to accommodate the 50-μm I.D. fused-silica capillary for on-column detection, and a data processor (C-R3A Chromatopac, Shimadzu, Kyoto, Japan). A sample solution was introduced manually into one end of the capillary by the hydrostatic or siphoning method, as described previously[2]. HPCE experiments were performed at ambient temperature (*ca.* 25°C).

Reagents

The two polymer cations employed were commercial products: polybrene (Aldrich, Milwaukee, WI, U.S.A.) and PDDAC as a solution of 15% solid in water (Polyscience, Warrington, PA, U.S.A.) (Fig. 2). Disodium 1,6- and 1,7-napthalenedisulphonates were gifts from Sumitomo (Osaka, Japan). All other reagents were of analytical-reagent grade and purchased from Wako (Osaka, Japan). All the reagents were used as received. Water was purified with a Milli-Q system (Nihon Millipore, Tokyo, Japan).

RESULTS

As the electroosmotic velocity was significantly dependent on the experimental conditions, especially on the concentration of polymer cations, relative velocities were



Poly-DDAC Polybrene

Fig. 2. Molecular structures of PDDAC and polybrene.

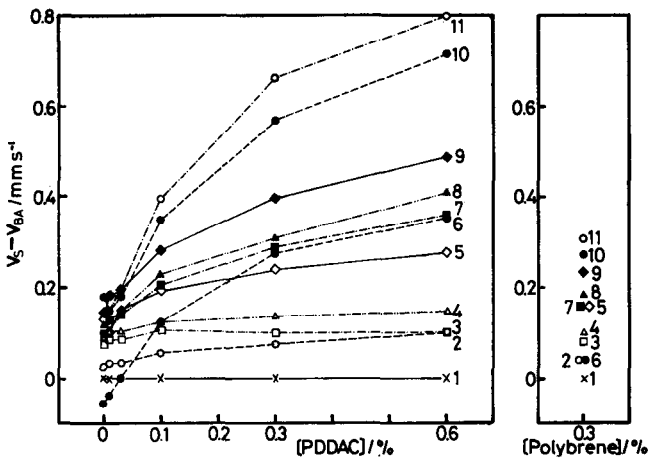


Fig. 3. Dependence of relative migration velocities of monobasic acids on the concentration of PDDAC. 1 = benzoic acid (BA, the standard solute of the migration velocity); 2 = *o*-; 3 = *m*-; 4 = *p*-aminobenzoic acids; 5 = 1-naphthoic acid; 6 = *o*-; 7 = *m*-; 8 = *p*-hydroxybenzoic acids; 9 = 2-naphthoic acid; 10 = 2-; 11 = 1-naphthalenesulphonic acids. The relative velocities in a 0.3% polybrene solution are also shown. Capillary, 750 mm × 50 μm I.D., 500 mm to the detector; buffer solution, 50 m*M* phosphate buffer (pH 7.0); applied voltage, 20 kV. See text regarding the ordinate.

employed to evaluate the effect of the polymer cations. The variable electroosmotic velocity was probably due to the change in the surface condition of the capillary wall, depending on the polymer concentration. The adsorption of the polymer cation on the negatively charged wall must have changed its zeta potential substantially. The electroosmotic flow was actually positive or toward the negative electrode in the absence of the polymer cation, but toward the positive electrode in its presence. We shall take the velocity to be positive when it is toward the negative electrode.

Benzoic acid was chosen as the standard solute for the migration velocity and was added to every sample solution. The difference between the migration velocity of the solute, v_s, and that of benzoic acid, v_{ba}, was measured at different concentrations of PDDAC, and the results are shown in Fig. 3 for monoanionic and in Fig. 4 for dianionic solutes. The differential velocity data in the absence of PDDAC and in the presence of 0.3% polybrene are also given.

The electroosmotic velocity was *ca.* 0.7 mm s^{-1} in the absence of the polymer ions, *ca.* −2.0 mm s^{-1} in 0.01–0.03% PDDAC solutions and *ca.* −1.7 mm s^{-1} in a 0.3% polybrene solution. The use of the differential velocity, $v_s - v_{ba}$, eliminates the effect of the electroosmotic velocity, and hence indicates the effect of just the polymer addition on the apparent electrophoretic velocity.

Benzoic acid had the highest electrophoretic mobility among the monobasic acids in this study, except for *o*-hydroxybenzoic acid, either in the absence or in the presence of the polymer cation. It is noteworthy that the electrophoretic velocities of these solutes and their electrophoretic mobilities were all negative, but we shall consider only the magnitude of these values. The difference in velocities increased with increase in PDDAC concentration, in particular the naphthalene derivatives showed significant dependences. The increase in differential velocities suggests that the

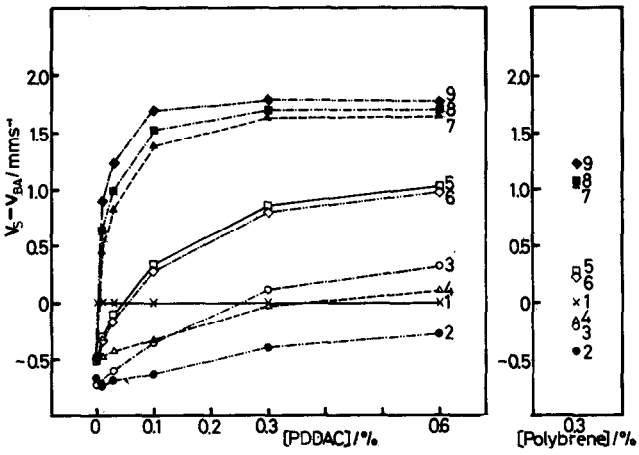


Fig. 4. Dependence of relative velocities of dibasic acids on the concentration of PDDAC. 1 = benzoic acid; 2 = maleic acid; 3 = fumaric acid; 4 = phthalic acid; 5 = isophthalic acid; 6 = terephthalic acid; 7 = 2,6-; 8 = 2,7-; 9 = 1,5-naphthalenedisulphonic acids. Other conditions as in Fig. 3.

naphthalene derivatives, *e.g.*, 1- and 2-naphthalenesulphonate ions, form ion pairs with PDDAC more strongly than benzoic acid. All the solutes employed are considered to be fully ionized at the experimental pH of 7.0. The abnormal behaviour of *o*-hydroxybenzoic acid will be discussed later.

All the dibasic acids employed had electrophoretic mobilities higher than that of benzoic acid in the absence of the polymer cations. This is obvious, because the divalent acids will have charges higher than monobasic benzoic acid at pH 7.0.

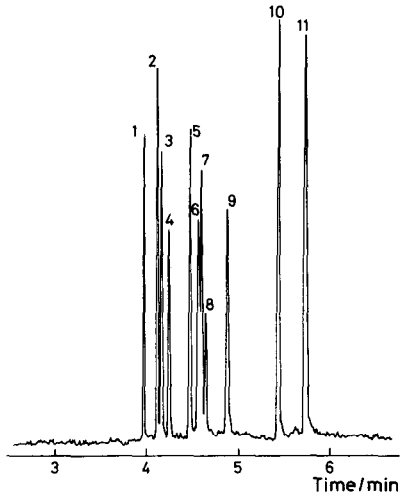
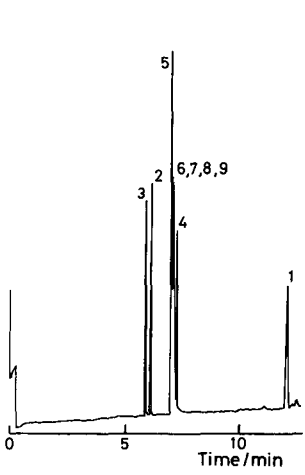


Fig. 5. Ion-exchange electrokinetic chromatogram of monobasic acids. Peak numbers as in Fig. 3. Separation solution, 0.3% PDDAC in 50 m*M* phosphate buffer (pH 7.0); current, 49 μA. Other conditions as in Fig. 3.

However, in the presence of PDDAC, the electrophoretic mobilities decreased rapidly with increase in the PDDAC concentration, and eventually led to lower values than that of benzoic acid, except for maleic acid. It should be noted that the scales of the ordinates are different between Figs. 3 and 4. In particular, the velocities of the naphthalenedisulphonates were most susceptible to the polymer cation addition.

When a 0.3% polybrene solution was used, the results agreed fairly well with those expected at 0.06% PDDAC, as shown in Figs. 3 and 4. Although the concentration effects of PDDAC and polybrene were not identical, the fact that the relative magnitudes of solute velocities were almost the same between 0.06% PDDAC and 0.3% polybrene strongly suggests that PDDAC and polybrene have very similar selectivity in ion-pair formation.

An ion-exchange electrokinetic chromatogram of the mixture of eleven monobasic acids employed in Fig. 3 is shown in Fig. 5, which was obtained with a 0.3% PDDAC solution. Although most of the analytes were also separated well in the absence of PDDAC, as judged from Fig. 3, the use of the polymer cations gave better separations. For example, 1- and 2-naphthalenesulphonates could not be resolved by conventional HPCE, but they were easily separated by use of PDDAC, as shown in Fig. 5. The plate numbers of the peaks were about 250 000. Fig. 6 shows an electropherogram of the mixture of nine dibasic acids employed in Fig. 4. As expected from Fig. 4, the resolution of three naphthalenedisulphonates was unsuccessful. Fig. 7 shows an ion-exchange electrokinetic chromatogram of the same mixture of dibasic acids as in Fig. 6. All the solutes were successfully separated with the addition of 0.01% PDDAC, although the peaks of the three naphthalenedisulphonates were broad and tailed. The successful separation of five isomeric naphthalenedisulphonates, including the three isomers shown in Fig. 7, with a 2% diethylaminoethyldextran was illustrated elsewhere[11]. Although no chromatogram is shown, the use of polybrene instead of PDDAC resulted in chromatograms very similar to those in Figs. 5 and 7.



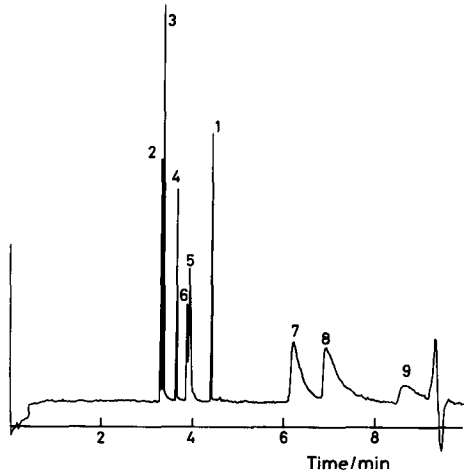


Fig. 6. Capillary electropherogram of dibasic acids. Peak numbers as in Fig. 4. Current, 39 μA; other conditions as in Fig. 3, except for the PDDAC concentration, which was zero in this run.

Fig. 7. Ion-exchange electrokinetic chromatogram of dibasic acids. Peak numbers as in Fig. 4. Separation solution, 0.01% PDDAC in 50 m*M* phosphate buffer (pH 7.0); current, 49 μA. Other conditions as in Fig. 3.

DISCUSSION

If two ionic solutes have identical electrophoretic mobilities and the polymer ion has the opposite charge to the solutes, the differences in migration velocities, Δv, between the two solutes, 1 and 2, in the presence of the polymer ion is derived as[11]

$$\Delta v = \frac{(K_{ip2} - K_{ip1})[pi]_0[v_{ep}(free) - v_{ep}(pi)]}{(1 + K_{ip1}[pi]_0)(1 + K_{ip2}[pi]_0)} \quad (1)$$

where $[pi]_0$ is the total concentration of the polymer ion in the separation solution, $v_{ep}(free)$ and $v_{ep}(pi)$ are the electrophoretic velocities of the solute free from the polymer ion and that of the polymer ion, respectively, and K_{ip1} and K_{ip2} are the ion-pair formation constants of the solutes 1 and 2, as shown in the following equilibrium equation:

$$s + pi \rightleftharpoons s \cdot pi \quad (2)$$

$$K_{ip} = [s \cdot pi]/[s][pi] \quad (3)$$

where s and pi are the solute and polymer ion, respectively. In the derivation of eqn. 1, the electrophoretic velocity of the ion pair is assumed to be equal to that of the polymer ion, or the electrophoretic velocity is independent of ion-pair formation; [pi] in eqn. 3 is presumed to be equal to $[pi]_0$, because $[s \cdot pi]$ may be negligibly low compared with [pi]. Although the polymer ions are considered to have wide distributions of molecular weights, if the equilibrium given in eqn. 2 is rapid in comparison with the migration velocities, the molecular weight distribution may not affect the migration velocities of the solutes.

Eqn. 1 describes the dependence of the differential velocity on the polymer concentration when the solutes have identical electrophoretic velocities in the absence of the polymer ion. Accordingly, some pairs of solutes in Figs. 3 and 4 that are suitable for the evaluation of eqn. 1 are selected and their data are replotted in Fig. 8. Here, Δv for each pair of solutes is zero or close to zero when the polymer ion concentration is zero. Not all the electrophoretic velocities were identical among the solutes shown in Fig. 8, even in the absence of the polymer ion, but the electrophoretic velocities of each pair were identical or nearly equal under such conditions.

The relationship between Δv and $[pi]_0$ does not seem straightforward and, therefore, in order to simplify eqn. 1 we shall assume that K_{ip1} and K_{ip2} are close to each other. Then, we shall discuss the function of P, which is a $[pi]_0$-related part extracted from eqn. 1:

$$f(P) = P/(1 + KP)^2 \quad (4)$$

where P means $[pi]_0$ and K is assumed to be equal to K_{ip1} and K_{ip2} only in the denominator. The dependence of $f(P)$ on P is shown in Fig. 9, where the maximum value of $f(P)$ is obtained at $P = K^{-1}$. The value of $f(P)$ increases from zero to $0.25\,K$ with increase in P from zero to K^{-1}, and then decreases asymptotically to zero with a further increase in P more than K^{-1}.

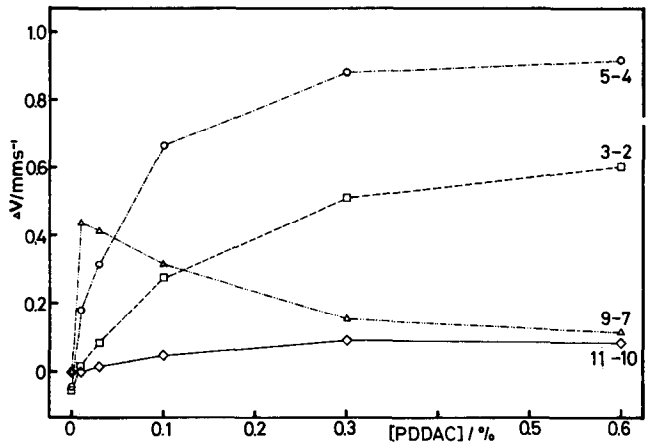


Fig. 8. Dependence of differential velocities between two solutes having identical or very close electrophoretic velocities on the concentration of PDDAC. Each curve was obtained by plotting the differential velocity between the pair of solutes shown on each curve. The numbers are the same as in Fig. 4, except for 10 and 11, which appear in Fig. 3.

The plot denoted 11–10 in Fig. 8 is for 1- and 2-naphthalenesulphonates, and it has the maximum value of Δv at 0.3% PDDAC, although the exact concentration of PDDAC that gives this is not known. If we assume that 0.3% PDDAC gives the maximum Δv, K_{ip} is easily calculated to be 54 l mol^{-1} according to eqn. 4, where [PDDAC] is transformed to the molar concentration of the quaternary ammonium ion in PDDAC. In order to obtain precise K_{ip} values, we have to determine the most appropriate values for K_{ip1} and K_{ip2} that agree best with the observed data of Δv for the pair of solutes.

The maximum value of Δv appears at 0.01% PDDAC fo the pair of 1,5- and 2,6-naphthalenedisulphonates (9–7 in Fig. 8), and this will mean that K_{ip} is much larger

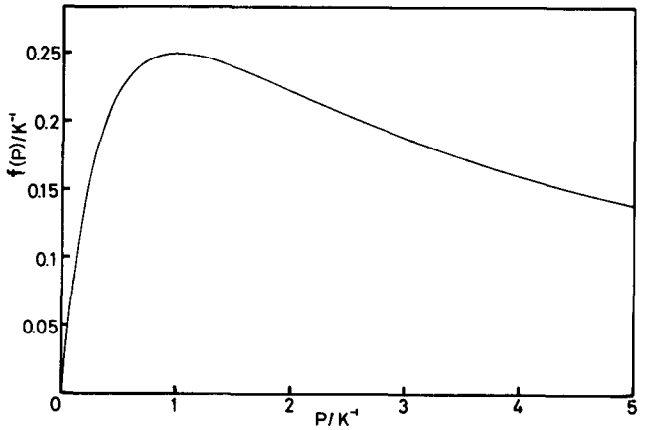


Fig. 9. Dependence of the function f(P) given in eqn. 4 on P.

than that of the naphthalenesulphonates. In the case of the other two pairs denoted 3–2 and 5–4 in Fig. 8, the maximum values of Δv are not realized at concentrations of PDDAC lower than 0.6%. This suggests that the values of K_{ip} for these compounds are smaller than those of the naphthalenesulphonates and that is consistent with the data shown in Fig. 4. However, it should be mentioned that eqn. 4 assumes that $K_{ip1} = K_{ip2}$ in deriving eqn. 1, but the Δv values shown in Fig. 8 seem too large to assume $K_{ip1} = K_{ip2}$ for these pairs of compounds.

Hydrophobic interaction seems to enhance ion-pair formation, because the naphthalenesulphonic and naphthoic acids formed stronger ion pairs than the benzoic acid derivatives, as shown in Fig. 3; this tendency was also observed for the divalent acids in Fig. 4. The sulphonate group may tend to form ion pairs with the quaternary ammonium group more readily than the carboxyl group, as judged by the differences between naphthalenesulphonates and naphthoates, as shown in Fig. 3.

Although we have not extensively explored the use of different polymer cations for ion-exchange EKC, PDDAC and polybrene have very similar selectivities in ion-pair formation. However, diethylaminoethyldextran showed significantly different selectivities, as shown elsewhere[11]. The relationship between selectivity in ion-pair formation and the structure of the polymer ion remains to be extensively studied.

A very different dependence of the differential velocity, $v_s - v_{ba}$, for *o*-hydroxybenzoic acid shown in Fig. 3 is probably due to the effect of intramolecular hydrogen bonding[12]. The high electrophoretic velocity of this compound in the absence of the polymer ion probably means that it is less hydrated than the other benzoic acids, owing to the intramolecular hydrogen bond. The polymer cation will interfere with the intramolecular hydrogen bonding by forming an ion pair, and at about 0.3% PDDAC the intramolecular hydrogen bond seems to be completely broken.

CONCLUSION

Ion-exchange EKC, which is easily performed by adding a polymer ion having charges opposite to the analyte ions to the separation solution, has been shown to be very effective for the separation of analyte ions that cannot be resolved by HPCE alone, because of identical electrophoretic mobilities. The effect of the polymer ion on the migration velocity has been investigated. In particular, the dependence of the differential velocity between two solutes having identical electrophoretic velocities on the concentration of polymer ions has been theoretically treated on the basis of the ion-pair formation equilibrium. It has been predicted that the concentration of the polymer ion producing the maximum difference in velocity is equal to K_{ip}^{-1}, provided that the ion-pair formation constants of the analyte ions are very close. However, it is difficult at present to obtain the precise values of K_{ip}.

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REFERENCES

1 S. Terabe, *Trends Anal. Chem.*, 8 (1989) 129.
2 S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 111.
3 S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 57 (1985) 834.
4 M. J. Gordon, X. Huang, S. L. Pentoney, Jr. and R. N. Zare, *Science*, 242 (1988) 224.
5 R. A. Wallingford and A. G. Ewing, *Adv. Chromatogr.*, 30 (1989) 1.
6 S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 61 (1989) 251.
7 D. E. Burton, M. J. Sepaniak and M. P. Mascarinec, *J. Chromatogr. Sci.*, 24 (1986) 211.
8 S. Terabe, H. Ozaki, K. Otsuka and T. Ando, *J. Chromatogr.*, 332 (1985) 211.
9 A. Guttman, A. Paulus, A. S. Cohen, N. Grinberg and B. L. Karger, *J. Chromatogr.*, 448 (1988) 41.
10 S. Terabe, T. Yashima, N. Tanaka and M. Araki, *Anal. Chem.*, 60 (1988) 1673.
11 S. Terabe and T. Isemura, *Anal. Chem.*, 62 (1990) 650.
12 S. Fujiwara and S. Honda, *Anal. Chem.*, 59 (1987) 487.