Equilibrium Study on the Ion Association of Monovalent and Divalent Naphtholsulfonates with Tetrabutylammonium Ion in an Aqueous Solution by Capillary Zone Electrophoresis

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The ion-association properties of monovalent and divalent naphtholsulfonate ions were investigated with tetrabutylammonium ion (TBA⁺) as a pairing ion in an aqueous solution. The ion-association constants were obtained by analyzing the change in the electrophoretic mobility of naphtholsulfonate ions in the presence of TBA⁺ by capillary zone electrophoresis; also, the contribution of a hydroxyl group to the ion associability of monovalent and divalent naphtholsulfonate ions, as well as the electrophoretic mobility of the ions, was investigated. The obtained ion-association constants indicate that the positional isomers possessing anionic groups at the β -position of the naphthalene ring are more associable with TBA⁺ than those at the α -position, and that the ion associability of the divalent naphtholsulfonates is larger than those of the monovalent ones, except for 1-naphthol-2-sulfonate. The abnormal associability of 1-naphthol-2-sulfonate can be explained by a synergistic increase in the hydrophilicity of the divalent ion. The difference in the ion associability between the monovalent and divalent naphtholsulfonates, 0.16 in log unit on the average, was smaller than that between the naphthalenesulfonate and naphthalenedisulfonate ions, 0.30 log unit on the average. The electrophoretic mobility of the naphtholsulfonate ions obtained in the absence of TBA⁺ is compared with each other, and the contribution of the hydroxyl group is discussed on the basis of the hydration behavior of the naphtholsulfonates.

Keywords Ion association, aqueous solution, naphtholsulfonate, capillary zone electrophoresis, tetrabutylammonium ion

Capillary electrophoresis (CE) is a powerful separation system, especially for charged molecules. Since such anionic micelles as dodecyl sulfate micelles were used as a modifier of migrating buffers¹, the usefulness of CE has been recognized rapidly and strongly in various fields concerning separation chemistry, and a number of electrically neutral substances have been separated favorably. Recently, various kinds of modifiers of migrating buffers have been used to improve the separability by CE; they are, for example, cyclodextrin derivatives as a chiral recognition modifier², cationic polyelectrolytes as an ion recognition^{3,4} and organic onium ions as an ion association.^{5,6}

In recent years, Takayanagi *et al.* reported on the usefulness of ion association in aqueous solution on the separation of positional isomers of organic anions by capillary zone electrophoresis (CZE). The main factors concerning the motive interactions for the ion association were found to be the hydrophobicity of pairing ions^{7,8} and the amount of charge.⁹ In previous studies⁷⁻⁹, it was proved that CZE was a useful tool for the analysis of the equilibrium of ion-association reactions in aqueous solutions. In an equilibrium analysis by a CZE method, the electrophoretic mobility of a given UV-absorbing analyte ion was measured in the presence of large excess amounts of a pairing ion, such

as quaternary ammonium ions, where the concentrations of the analyte ions were as low as 10^{-5} mol dm⁻³. The unique advantages of the equilibrium analysis by the CZE method are as follows: (1) applicability to easy-to-precipitate ion associates; (2) no need to accurately know the concentrations of the analyte ions (the accurate mobility and the concentrations of pairing ions in the migrating solution are necessary for the determination of equilibrium constants); (3) the possibility of simultaneous measurements of the mobility of analyte ions (impurities existing in the analyte ions do not interfere with the determination); (4) an accurate analysis of the reaction of a very weak interaction. By using the CZE method, the ion-association constants of monovalent organic anions with monovalent organic cations were successfully determined for the first time.⁹

In the present study, the ion associability of eight kinds of naphtholsulfonate anions was examined using the tetrabutylammonium ion (TBA⁺) as a pairing cation. These anions exist as monovalent and/or divalent ions at a certain pH condition; also, the difference in the ion associability of the anions between the two ionic forms is of great interest with respect of the hydrophilicity of the anionic groups, the position of the ionic site and the separability by capillary zone electrophoresis. Furthermore, information concerning the contribution of a hydroxyl group to the ion associability is essentially important because the hydroxyl group is

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commonly present in organic compounds, especially in various kinds of biomolecules.

Experimental

Apparatus and reagents

The capillary electrophoresis system and silica capillary used in this work were the same as those used in previous work.⁹ Eight kinds of positional isomers of *m*-naphthol-*n*-sulfonate (abbreviated as *mNnS*; 1N2S, 1N3S, 1N4S, 1N5S, 1N8S, 2N6S, 2N7S and 2N8S), where *m* and *n* are the number of positions in the naphthalene ring, were purchased from Tokyo Kasei Kogyo. They were all sodium salts and were used as analyte anions without further purification. The protonated and deprotonated forms of the hydroxyl group of analyte anions were represented as HA⁻ and A²⁻, respectively. The salts of the analyte anions were dissolved in purified water. Migrating buffer solutions containing 2-morpholinoethanesulfonic acid (MES)-NaOH (pH 6.0), KH₂PO₄-Na₂HPO₄ (pH 6.0), Na₂B₄O₇-HCl or -NaOH (pH 8–10) and NaOH (pH 12.0) were used. Tetra-

butylammonium ion (bromide salt, TBA⁺·Br⁻) was used as an ion-association reagent, which was added to the migrating solutions.

Procedure for the CZE measurement

A migrating solution was filled in a cathodic and anodic reservoir, as well as in a capillary. A sample solution containing 2×10^{-5} mol dm⁻³ of a certain analyte anion was introduced from the anodic end of the capillary for 3 s (injection volume: about 9 nl). A voltage of 20 kV was then applied, and electrophoresis was started. The electric currents were less than 20 μ A under any of the migrating conditions examined, and it was suitable for the present purpose. All analyte anions were photometrically detected at 225 nm. The electroosmotic flow (EOF) was monitored by detecting the peak of ethanol (3% (v/v)) added to the sample solution. The electrophoretic mobility, as well as the velocity of EOF, was calculated in an ordinary manner. During the experiment, the capillary was held in a thermostated compartment kept at 35°C.

Results and Discussion

Separation behavior of analyte anions with and without an ion-association reagent

Electropherograms of the analyte anions are shown in Fig. 1. Since the analyte anions examined were positional isomers, the mutual separation of each anion was not sufficient when the migrating solution contained only buffer components, as can be seen from Figs. 1 (a) and (c). The separation of the positional isomers was well improved when TBA⁺ was used as a pairing ion under both pH conditions of 6.0 and 12.0 {Figs. 1 (b) and (d)}.

Acid-dissociation property of naphtholsulfonates

The acid-dissociation constants (pK_a) of naphtholsulfonates were examined by measuring the electrophoretic mobility of the analyte anions using various migrating buffers (Na₂B₄O₇-HCl or -NaOH) at different pHs. Equilibrium analyses gave the acid dissociation constants; the obtained pK_a values are summarized in Table 1. Most of the obtained values agree well with the reported values.^{10,11} In the case of 1N8S, the mobility change with changing pH was too small to determine the pK_a value, because its pK_a was too high and about 13. The acid-dissociation constants obtained in this work seem to be more reliable than the reported values, because the present method is not interfered from UV-absorbing impurities probably included in the analyte anions.

Mobility changes of naphtholsulfonates present as monovalent anions by the addition of an ion-association reagent

When the pH of the migrating solution was 6.0, all of the naphtholsulfonate ions existed as monovalent

Fig. 1 Typical electropherograms of naphtholsulfonate anions in the absence and presence of TBA⁺. Sample solution: eight kinds of 2×10^{-5} mol dm⁻³ anions + 3% (v/v) ethanol. Migrating solution: a) 1×10^{-2} mol dm⁻³ MES buffer (pH 6.0); b) 1×10^{-2} mol dm⁻³ MES buffer (pH 6.0) + 2×10^{-2} mol dm⁻³ TBA⁺·Br⁻; c) 1×10^{-2} mol dm⁻³ NaOH (pH 12.0); d) 1×10^{-2} mol dm⁻³ NaOH (pH 12.0) + 1×10^{-2} mol dm⁻³ TBA⁺·Br⁻. CE conditions are cited in the text. 1, 1N2S; 2, 1N3S; 3, 1N4S; 4, 1N5S; 5, 1N8S; 6, 2N6S; 7, 2N7S; 8, 2N8S.

Table 1 Equilibrium constants and electrophoretic mobilities obtained in this study

Analyte anions ^a	Equilibrium constant			Electrophoretic mobility ^c / 10 ⁻⁴ cm ² V ⁻¹ s ⁻¹		
	pK _a	log K _{ass,HA} ^b	log K _{ass,A} ^b	-μ _{ep,HA}	-μ _{ep,A}	-μ _{epIA,A}
1N2S	9.50 (9.47) ^d	1.30±0.08	1.04±0.11	3.18 (3.21)	5.59 (5.56)	1.79
1N3S	8.53 (8.7) ^e	1.35±0.07	1.42±0.14	2.89 (2.90)	5.27 (5.27)	1.64
1N4S	8.11 (8.2) ^e	1.22±0.10	1.28±0.16	2.82 (2.84)	5.37 (5.32)	1.76
1N5S	8.87 (9.11) ^d	1.24±0.09	1.37±0.14	2.86 (2.90)	5.16 (5.20)	1.60
1N8S	— ^f (13.02) ^d	1.20±0.10	— ^f	3.14 (3.12)	— ^f	— ^f
2N6S	9.02 (9.14) ^d	1.29±0.09	1.59±0.12	2.85 (2.86)	5.23 (5.19)	1.58
2N7S	9.17 (9.35) ^d	1.32±0.06	1.59±0.16	2.90 (2.91)	5.22 (5.14)	1.57
2N8S	9.50 (9.48) ^d	1.19±0.09	1.30±0.17	2.87 (2.89)	5.52 (5.58)	1.70
1-NS		1.08±0.11 ^g		3.49 ^h		
2-NS		1.16±0.05 ^g		3.52 ^h		
1-NO		0.92±0.11 ^g		3.38 ^h		
2-NO		1.00±0.12 ^g		3.28 ^h		
1,5-NDS			1.39±0.07 ⁱ		5.01 ^j	2.07 ^j
2,6-NDS			1.44±0.07 ⁱ		5.02 ^j	2.08 ^j

a. NS, naphthalenesulfonate ion; NO, naphtholate ion; NDS, naphthalenedisulfonate ion. b. Error: 3σ. c. Calculated values. Values in parentheses are experimentally obtained values. d. Values in parentheses are reported values (ref. 10). e. Values in parentheses are reported values (ref. 11). f. Not determined. g. Values correspond to the 1 : 1 ion associate, which were cited from ref. 9. h. Values of -μ_{ep} of monovalent anions (ref. 9). i. Values correspond to the 1 : 1 ion associate, which were cited from ref. 8. j. Ref. 8.

anions, as could be expected from the pK_a values given in Table 1. The effect of the concentrations of TBA⁺ was examined using the migrating solution adjusted to pH 6.0 with the MES-NaOH buffer. The change in the apparent electrophoretic mobility (-μ_{ep,HA}') is shown in Fig. 2. The electrophoretic mobility of monovalent anions (-μ_{ep,HA}) is also summarized in Table 1. As can be seen from Table 1, the values of -μ_{ep,HA} are smaller than those of monovalent naphthalenesulfonate (NS) and naphtholate (NO) ions, and the decreases in the mobility are much larger than those expected from the increase in the molecular weight of HA⁻. The electrophoretic measurement provides information about the hydration of analyte ions; that is, the hydrated ion increases its apparent molecular mass, which reduces its electrophoretic mobility. Considering the -μ_{ep,HA} values obtained in this work, these results indicate that the hydroxyl group in HA⁻ is greatly hydrated. It is also noticed that the -μ_{ep,HA} values of 1N2S and 1N8S are larger than those of other isomers. These results suggest that the hydration behavior of these two anions is different from that of the other isomers. Since the sulfonate group of 1N8S is placed near to the hydroxyl group, hydrogen bonding occurs between the -SO₃⁻ and -OH groups. As a result, the number of hydrated water molecule are fewer than those in other naphtholsulfonate anions. The extremely higher pK_a value of 1N8S also indicates the presence of hydrogen bonding. In 1N2S, since the hydroxyl group is present at a position adjacent to the sulfonate group, hydrogen bonding can not be formed between the hydroxyl and sulfonate groups. However, hydration around these adjacent groups is decreased due to a steric hindrance.

The values of -μ_{ep,HA}' decreased with increasing the

concentrations of TBA⁺ added to the migrating solutions. Such decrease is correlated to the ion-association reaction of the analyte anions with TBA⁺, which is discussed in a later section.

The magnitude of the decrease in -μ_{ep,HA}' was almost identical when a phosphate buffer (pH 6.0) was used instead of the MES-NaOH buffer. This result indicates that the buffer components do not interfere with the ion-association reaction in an aqueous solution under

Fig. 2 Change in the electrophoretic mobility of HA⁻ with an increase in the TBA⁺ concentrations. Migrating solution: 1×10⁻² mol dm⁻³ MES-NaOH (pH 6.0)+(0–2)×10⁻² mol dm⁻³ TBA⁺·Br⁻. Sample solution and CE conditions are the same as in Fig. 1. ○, 1N2S; △, 1N3S; □, 1N4S; ◇, 1N5S; ▽, 1N8S; ●, 2N6S; ▲, 2N7S; ■, 2N8S.

the present experimental conditions.

Mobility changes of naphtholsulfonates present as divalent anions by the addition of an ion-association reagent

When 0.01 mol dm^{-3} NaOH was used as a migrating buffer, the analyte anions, except for 1N8S, existed as divalent anions, as expected from the $\text{p}K_{\text{a}}$ values. The electrophoretic mobility of divalent anions ($-\mu_{\text{ep,A}}$) obtained at a pH of about 12 is summarized in Table 1, from which it can be seen that the values of $-\mu_{\text{ep,A}}$ are larger than those present in the forms of the monovalent anions, $-\mu_{\text{ep,HA}}$. This is quite reasonable, because the charges of the anions increased from -1 to -2 . The values of $-\mu_{\text{ep,A}}$ of the divalent naphtholsulfonate anions are larger than those of the naphthalenedisulfonate (NDS) ions. This is probably because the molecular weight of the naphtholsulfonate ions is smaller than that of the NDS ions. The separability of the divalent isomers was slightly improved, even in the absence of TBA^+ , compared with that of monovalent isomers, as can be seen from the electropherogram in Fig. 1 (c). This suggests that the difference in the number of hydrated water molecules among the isomers increased when the charge changed from monovalence to divalence. In 1N8S, the migrating time was very short compared to that of the other isomers, as can be seen in Fig. 1 (c). This is quite reasonable because most of the anions of 1N8S exist in the monovalent form at 0.01 mol dm^{-3} NaOH, and the mobility is much smaller than that of the other isomers. The hydration behavior or difference in the number of hydrated water molecules of analyte ions is also discussed in later sections concerning the electrophoretic mobility.

The apparent electrophoretic mobility of the divalent anions ($-\mu_{\text{ep,A}}'$) changed upon the addition of TBA^+ in the migrating solution, as shown in Fig. 3. The decrease in $-\mu_{\text{ep,A}}'$ of divalent 1N2S with increasing TBA^+ concentration was very small compared with that of other divalent isomers. A similar phenomenon was observed for the 1,2-phthalate ion.⁸ This phenomenon is attributed to the lower hydrophobicity in adjacent anionic groups than that in apart anionic groups.

Ion-association constants

The decrease in the apparent electrophoretic mobility of analyte anions can be correlated to the ion associability in an aqueous solution between TBA^+ and the analyte anions. The following ion-association reactions occur in the anions, HA^- and A^{2-} :

The corresponding constants, $K_{\text{ass,HA}}$ and $K_{\text{ass,A}}$, can be represented as

$$K_{\text{ass,HA}} = \frac{[\text{TBA}^+ \cdot \text{HA}^-]}{[\text{TBA}^+][\text{HA}^-]}, \quad (3)$$

$$K_{\text{ass,A}} = \frac{[\text{TBA}^+ \cdot \text{A}^{2-}]}{[\text{TBA}^+][\text{A}^{2-}]}. \quad (4)$$

The apparent electrophoretic mobility of HA^- or A^{2-} , $-\mu_{\text{ep,HA}}'$ and $-\mu_{\text{ep,A}}'$, respectively, can be expressed as

$$-\mu_{\text{ep,HA}}' = \frac{1}{1 + K_{\text{ass,HA}}[\text{TBA}^+]} \times (-\mu_{\text{ep,HA}}) + \frac{K_{\text{ass,HA}}[\text{TBA}^+]}{1 + K_{\text{ass,HA}}[\text{TBA}^+]} \times (-\mu_{\text{epIA,HA}}), \quad (5)$$

$$-\mu_{\text{ep,A}}' = \frac{1}{1 + K_{\text{ass,A}}[\text{TBA}^+]} \times (-\mu_{\text{ep,A}}) + \frac{K_{\text{ass,A}}[\text{TBA}^+]}{1 + K_{\text{ass,A}}[\text{TBA}^+]} \times (-\mu_{\text{epIA,A}}), \quad (6)$$

where $-\mu_{\text{epIA,HA}}$ and $-\mu_{\text{epIA,A}}$ are the electrophoretic mobility of the ion associates, $\text{TBA}^+ \cdot \text{HA}^-$ and $\text{TBA}^+ \cdot \text{A}^{2-}$, respectively. To obtain the ion-association constants, a non-linear least-squares method was used in a similar manner as in previous works.^{8,9} In a calculation of $K_{\text{ass,HA}}$, the ion associate, $\text{TBA}^+ \cdot \text{HA}^-$, was expected to be electrically neutral, and a value of zero was used for the value of $-\mu_{\text{epIA,HA}}$. In a calculation of $K_{\text{ass,A}}$, the values of $-\mu_{\text{epIA,A}}$ were also optimized, as well as $-\mu_{\text{ep,A}}$; they are summarized in Table 1. The ion-association constants obtained in this study are also summarized in Table 1.

Fig. 3 Change in the electrophoretic mobility of A^{2-} with an increase in the TBA^+ concentrations. Migrating solution: $1 \times 10^{-2} \text{ mol dm}^{-3}$ NaOH (pH 12.0) + $(0 - 1) \times 10^{-2} \text{ mol dm}^{-3}$ $\text{TBA}^+ \cdot \text{Br}^-$. Sample solution and CE conditions are the same as in Fig. 1. $\circ$, 1N2S; $\triangle$, 1N3S; $\square$, 1N4S; $\diamond$, 1N5S; $\bullet$, 2N6S; $\blacktriangle$, 2N7S; $\blacksquare$, 2N8S.

Comparison of the ion associability of naphtholsulfonate ions

It can be seen from the ion-association constants given in Table 1 that the ion associability of the monovalent naphtholsulfonate ions with TBA^+ is larger than that of 1- and 2-naphthalenesulfonates (1- and 2-NS).⁹ The increase in the ion associability of naphtholsulfonates can be attributed to the hydroxyl group introduced into the naphthalenesulfonates. In naphtholsulfonates, the hydroxyl group acts as an electron-donating group; therefore, the electron density of the sulfonate group is increased and the ion associability of monovalent naphtholsulfonates is large compared with that of the naphthalenesulfonates.

The ion associability of the each analyte anion was compared with each other from the view point of the position of the anionic groups. In the monovalent form, the values of $K_{\text{ass,HA}}$ were larger when a sulfonate group was present at the β -position of the naphthalene ring; that is, the values of 1N2S, 1N3S, 2N6S and 2N7S (average $\log K_{\text{ass,HA}}$ with $\pm$ deviation: 1.32 ± 0.08) were larger than those of 1N4S, 1N5S, 1N8S and 2N8S (average $\log K_{\text{ass,HA}}$ with $\pm$ deviation: 1.21 ± 0.10). Similarly, in the case of the divalent form, the order of the ion associability was 2N6S and 2N7S (β, β) > 1N3S and 2N8S (α, β or β, α) > 1N4S and 1N5S (α, α). These results are in good agreement with the order of the basicity, β -position > α -position, reported in a previous study.⁹

In a comparison of the monovalent and divalent naphtholsulfonates, the ion associability of the divalent anions was larger than that of the monovalent ones, except for 1N2S. This increase in the ion associability is attributed to an increase in the charge. However, the difference in the ion-association constants between the monovalent and divalent ions was about 0.16 in log unit on the average, which was smaller than the difference between the naphthalenesulfonate and naphthalenedisulfonate ions, 0.30 in log unit on the average. It is noticed that the increase in the ion associability of 1N2S is reversed compared with the other isomers, even when the anion changes from the monovalent to the divalent form. This fact seems to be quite interesting with respect to the electrostatic interaction between a cation and an anion. The abnormal ion associability of the divalent 1N2S can be explained as follows. When the two adjacent anionic groups are placed at a close position, the electron density around these anionic

groups is higher synergistically, and therefore the hydrophilicity of the groups becomes larger. As a result, the hydration shell around the anionic groups becomes thicker than that of the other anions in which the two anionic groups were present apart. The increase in the thickness of the hydration shell results in a lowering of the hydrophobic ion association with TBA^+ . A similar phenomenon was observed previously: naphthalene-2,3-dicarboxylate and 1,2-phthalate were less associable with TBA^+ than other corresponding divalent anions.⁸

In conclusion, the ion-association constants of naphtholsulfonates with TBA^+ were successfully determined, and the ion associability and electrophoretic mobility of naphtholsulfonates could be reasonably explained on the basis of the number of charge, the positions of the anionic groups, and hydration behavior related to the electrophoretic mobility.

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