The Analyst

Electrophoretic Mobility Study of Ion Association Between Aromatic Anions and Quaternary Ammonium Ions in Aqueous Solution

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The ion association reaction between aromatic anions and quaternary ammonium ions in aqueous solutions was investigated through mobility changes in capillary electrophoresis. The electrophoretic mobility of aromatic anions decreased with increasing amount of quaternary ammonium salt added to the migrating solutions. The change in mobility of anions due to the ion association was found and was treated with a least-squares method, giving ion association constants. The ion associability order of the isomers of aromatic anions was found to be naphthalene-2,6-dicarboxylate > naphthalene-2,3-dicarboxylate, naphthalene-2-sulfonate > naphthalene-1-sulfonate and terephthalate > isophthalate > phthalate. The order of the ion associability of naphthalene-1,5- and -2,6-disulfonate reversed depending on the alkyl chain length of the pairing cation. The order of ion associability of quaternary ammonium ions was found to be tetraamylammonium > tetrabutylammonium > tetrapropylammonium ≈ octyltrimethylammonium ≈ hexyltrimethylammonium > tetraethylammonium > tetramethylammonium, and suggested that ion association in aqueous solution was governed by the hydrophobicity of the pairing cation.

Keywords: *Ion association; aqueous solution; capillary electrophoresis; aromatic anions; quaternary ammonium ions*

Capillary electrophoresis (CE) is a powerful separation system, especially for charged molecules. The separation of positional isomers, however, is still a difficult problem. Many approaches have been made to perform such separations. Cationic polymers,[1] acid dissociation properties[2] and surfactants[3] have been used for the separation of naphthalenesulfonate compounds. Hydrophobic cations have also been used as modifiers of migrating solutions. The use of cationic substances such as quaternary ammonium ions has been mainly investigated in capillary ion analysis (CIA).[4–6] In a CIA system, the addition of long-chain quaternary ammonium ions could be used to reverse and control the electroosmotic flow (EOF), which permitted the detection of small anionic molecules directly and indirectly by spectrophotometry. The interaction between inorganic analyte anions and the modifiers, the cations, was found to be slight in such systems.

The use of hydrophobic cations, however, should cause some interaction, ion association, with relatively bulky organic anions, such as naphthalenesulfonates and naphthalenecarboxylates. The use of ion association reagents have been investigated in the CE separation of anionic metal chelates.[7,8] However, the contribution and steric effect of pairing ions in ion association have never been discussed.

Ion association reactions have often been used in various analytical systems, such as two-phase distributions,[9] precipitation of bulky ions and reversed-phase ion-pair chromatography.[10] In two-phase distribution systems, the contribution of the hydrophobicity of pairing ions has been found and estimated by using a fragmentation method.[11,12] The ion association between inorganic cations and anions has often been investigated by using a conductivity measurement method.[13,14] The ion association between bulky organic ions in aqueous media is very interesting with respect of the reaction between ionic biomolecules. Ion association in aqueous media, however, has not yet been clarified, because ion association is not as stable as chelation, and the ion associates formed are difficult to dissolve in aqueous media at high concentrations. It is very important and interesting to establish the dominant factors governing ion association.

In this work, a fundamental study of ion association reactions in aqueous solutions was carried out to clarify the factors affecting the stability of ion associates. Quaternary ammonium ions were used and aromatic sulfonate and carboxylate ions were adopted as organic bulky anions, because positional isomers were available and were suitable as model reagents.

Experimental

Apparatus

An Applied Biosystems (Foster City, CA, USA) Model 270A capillary electrophoresis system with a UV detector was used. Two types of a capillary were attached to the system: a fused-silica capillary (50 μm id) (GL Sciences, Tokyo, Japan) and a CElect-N capillary (50 μm id) (Supelco, Bellefonte, PA, USA), where silanol groups were chemically coated on the inner wall. They were cut to the required length after a detection window had been made by burning off a small portion of the polyimide coating. By using the CElect-N capillary, the EOF was more suppressed than with an uncoated capillary. The capillaries were of 72 cm total length and 50 cm effective length to the UV detector.

Reagents

Analyte anions, such as sodium naphthalene-1-sulfonate (1-NS), sodium naphthalene-2-sulfonate (2-NS), disodium naphthalene-1,5-disulfonate (1,5-NDS), disodium naphthalene-2,3-disulfonate (2,6-NDS), naphthalene-2,3-dicarboxylic acid (2,3-NDC), naphthalene-2,6-dicarboxylic acid (2,6-NDC), phthalic acid (PH), isophthalic acid (i-PH), and terephthalic acid (t-PH), were purchased from Tokyo Kasei Kogyo (Tokyo, Japan) and were used as received. Carboxylic compounds were dissolved in water with 2 mol equiv of sodium hydroxide.

Migrating buffer solutions were prepared with sodium tetraborate (borax), potassium dihydrogenphosphate and disodium hydrogenphosphate, which were purchased from Wako (Osaka, Japan). Quaternary ammonium salts ($Q^+ \cdot X^-$), such as tetramethylammonium bromide ($TMA^+ \cdot Br^-$), tetraethylammonium bromide ($TEA^+ \cdot Br^-$), tetrapropylammonium bromide ($TPA^+ \cdot Br^-$), tetrabutylammonium bromide ($TBA^+ \cdot Br^-$), tetraamylammonium bromide ($TAA^+ \cdot Br^-$), hexyltrimethylammonium bromide ($HTMA^+ \cdot Br^-$) and octyltrimethylammo-

nium bromide ($OTMA^+ \cdot Br^-$), were purchased from Tokyo Kasei Kogyo. Distilled, de-ionized water was used throughout.

CE Measurement

Migrating solution was transferred into a cathodic and an anodic reservoir, and then filled into a capillary tube before each measurement with a vacuum system. Sample solution was introduced into the capillary from the anodic end with the vacuum system for 3 s (injection volume about 9 nl). A voltage of 25 kV was then applied, and separations were started. Sample solution was introduced into a CElect-N capillary from the cathodic end and a UV detector was placed at the anodic end. The capillary was held in a thermostated room at 35 °C throughout the experiment, and analyte anions were detected photometrically at 230 nm. The EOF was monitored by adding about 3% v/v of ethanol to the sample solution. The EOF could be monitored by measuring the analyte in the method using a fused-silica capillary. However, it was necessary to determine the EOF in an inverse voltage direction when using the CElect-N capillary.

The apparent electrophoretic mobility of an analyte, μ_{ep}', and the electroosmotic flow, μ_{EOF}, were calculated in the usual manner, using the eqns. (1) and (2).

$$\mu_{EOF} = \frac{L_T L_D}{V t_{EOF}} \quad (1)$$

$$\mu_{ep}' = \frac{L_T L_D}{V t} - \mu_{EOF} \quad (2)$$

where L_T and L_D are the total length of the capillary and its effective length to the UV detector, respectively, V is the applied voltage and t_{EOF} and t are the migration times of EOF and an analyte anion, respectively. Three or more measurements were carried out to obtain the mean electrophoretic mobility in each instance.

Determination of Ion Association Constants

Ion association constants, K_{ass}, between quaternary ammonium ions and aromatic anions were determined by measuring the change in electrophoretic mobility with the change in pairing ion concentrations. A least-squares method was applied to the analysis of the correlation of μ_{ep}'. A laboratory-written program in BASIC, which gave both mean values and standard deviations, was developed.

Results and Discussion

Separation of Anions With Quaternary Ammonium Ions

The separation of anions in a silica capillary in the absence and presence of appropriate amounts of quaternary ammonium salts in the migrating solutions is shown in Fig. 1. Anions with the same molecular mass and charge, such as positional isomers of naphthalenesulfonates and naphthalenedisulfonates, could not be separated in the absence of an ion association reagent, Q^+, whereas some of them were well separated with the additional use of Q^+, as shown in Fig. 1(*b*) and (*c*). The anionic isomers were also separated with the use of other Q^+, such as TEA^+, TPA^+, TAA^+, $HTMA^+$ and $OTMA^+$. The separation time was much longer in the presence of TMA^+, $HTMA^+$ and $OTMA^+$ than in the absence of Q^+, because less bulky cations adsorb fairly strongly on the internal surface of the silica capillary tube by an electrostatic interaction that suppresses the EOF, the velocity of which is usually larger than the electrophoretic mobility of the analyte anions.

Changes in the velocity of the EOF with increasing Q^+ concentration are shown in Fig. 2. It was found that the smaller the spherical quaternary ammonium ions, or the longer the alkyl chain in trimethyl-type ammonium ions, the more effectively they suppressed the EOF. Such quaternary ammonium ions, however, are less adsorptive on the silanol surface than tetradecyltrimethylammonium ions[15] and other long-chain quaternary ammonium ions,[16] which were used to reverse the direction of the EOF in the determination of anions by CIA.

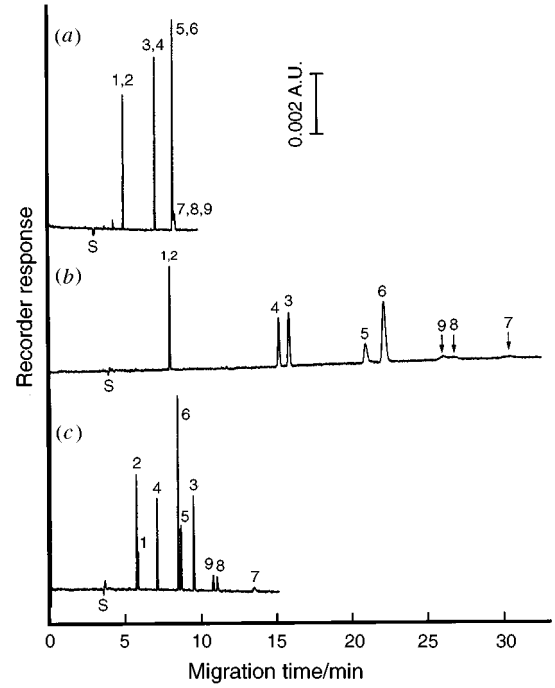

Fig. 1 Electropherograms of anions obtained with a fused-silica capillary in the absence and presence of quaternary ammonium salts. Sample solution: 1×10^{-5} m anions. Migrating solutions: 10 mm borax (pH, 9.2) and a certain amount of quaternary ammonium salt ($Q^+ \cdot X^-$). CE conditions: applied voltage 25 kV, capillary temperature 35 °C, detection wavelength 230 nm. Injection volume, 3 s (about 9 nl). $Q^+ \cdot X^-$ in the migrating solutions: (*a*) none; (*b*) 20 mM $TMA^+ \cdot Br^-$; and (*c*) 20 mM $TBA^+ \cdot Br^-$. 1, 1-NS; 2, 2-NS; 3, 2,3-NDC; 4, 2,6-NDC; 5, 1,5-NDS; 6, 2,6-NDS; 7, PH; 8, i-PH; and 9, t-PH.

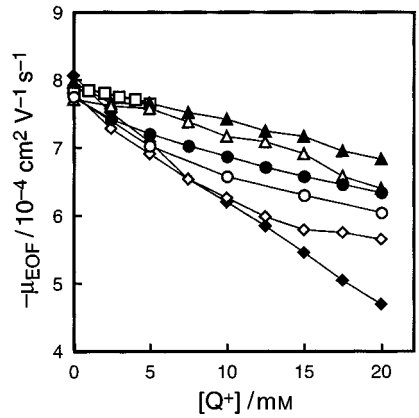

Fig. 2 Change in the velocity of the EOF as a function of the amount of quaternary ammonium salt added in the migrating solution. CE conditions and injection volume as in Fig. 1. The EOF was monitored with 3% v/v ethanol in the sample solution. Migrating solution: 10 mm borax (pH, 9.2) and a certain amount of $Q^+ \cdot X^-$. ○, TMA^+; ●, TEA^+; △, TPA^+; ▲, TBA^+; □, TAA^+; ◇, $HTMA^+$; and ◆, $OTMA^+$.

With $HTMA^+$ and $OTMA^+$, the formation of cationic micelles did not need to be considered, because the critical micelle concentration of $OTMA^+$ was about 1.4×10^{-1} m.[17] In $OTMA^+$ at high concentrations, three phthalate compounds could not be detected within 1 h of measurement. For TAA^+, the baseline became much worse on increasing its concentration beyond 5 mm, because TAA^+ has a high ion associability and forms the precipitates in the capillary.

The migration order of the two isomers of NDS reversed in TPA^+, although the migration order did not change with a concentration change in each Q^+. Such a reversal was not found with other positional isomers. Separation of the two naphthalenesulfonate isomers was not achieved with TMA^+ and TEA^+ even at 20 mm, and was not yet achieved even with TAA^+, because its concentration was too low.

From these results, TBA^+ was found to be the optimum modifier in respect of the separation efficiency and separation time.

Separation of Anions in a Coated Capillary

The separation of the anions was also examined in a polymer-coated capillary, CElect-N, the EOF of which was well suppressed by the coating on the internal wall. The electropherogram obtained with the coated capillary is shown in Fig. 3. In this case, the detection end was the anodic end, and therefore the migration order of analyte anions was the opposite of that obtained in the fused-silica capillary. The separation behaviour was similar in the case of the silica capillary, which indicates that analyte anions will not interact with the capillary wall.

Electrophoretic Mobility Change by Addition of Quaternary Ammonium Salts

Changes in the apparent electrophoretic mobility, μ_{ep}', on addition of TMA^+ and TBA^+ are shown in Fig. 4(*a*) and (*b*). For each analyte anion, μ_{ep}' decreased more significantly when the concentration of Q^+ increased and the bulkiness of Q^+ increased. The change in mobility of all the anions was very slight when TMA^+ was adopted as the pairing ion. This indicates that such small cations find it difficult to form ion associates with the organic anions in aqueous solutions. A smaller change in mobility with an increase in the concentration of TMA^+, as shown in Fig. 4(*a*), also suggests that the Joule heat generated in the capillary can be released during the electrophoresis, and that the electrophoretic mobility of anions is not affected much by the change in ionic strength of Q^+ under the experimental conditions used in this work. As shown in Figs. 1 and 3, μ_{ep}' of isomers decreased in the order: 2-NS > 1-NS, 2,6-NDC > 2,3-NDC and t-PH > i-PH > PH. As indicated in the previous section, the migration order or the mobility of the two naphthalenedisulfonate compounds reversed in TPA^+. These results suggest that the ion associability of the anions is affected by the bulkiness and other properties of Q^+.

Determination of Ion Association Constants

Ion association constants, K_{ass}, were determined by using the change in electrophoretic mobility. The ion association constants of a few anions with TBA^+ have already been examined in our previous work,[18] where the apparent electrophoretic mobility, $-\mu_{ep}'$, was plotted against the term $(-\mu_{ep} + \mu_{ep}')/[Q^+]$. Such plots were also made in this study, as shown in Fig. 5. In this method, the mobility of the ion associates between anions and Q^+ and the K_{ass} values was obtained from the intercept on the ordinate and the slope of the plots, respectively. However, the mobility of anions, μ_{ep}, in the absence of Q^+ must be determined very precisely, because the value of μ_{ep} is used as a standard mobility of the analyte anion in each plot. As can be seen from Fig. 5, plots for anions of low ion associability were dispersed, and the values of K_{ass} could not be determined from the slopes. Hence the determination of ion association constants was limited to relatively stable ion associates formed between TBA^+ and anions such as 1,5-NDS, 2,6-NDS and 2,6-NDC.

In this study, we developed another method for the determination of ion association constants, which can give more reliable constants is applicable to monovalent anions of low ion associability.

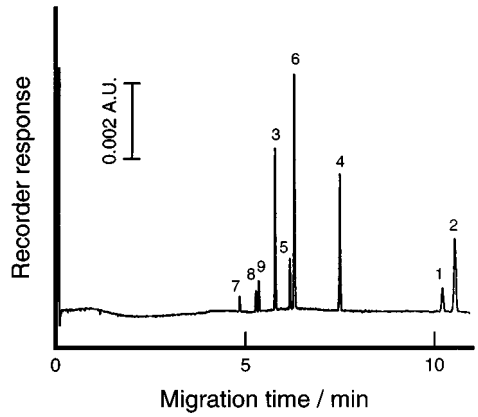

Fig. 3 Electropherogram of anions in a coated capillary. Sample solution, CE conditions and injection volume as in Fig. 1. Migrating solution contained 10 mm phosphate buffer (pH 7.0) and 20 mm $TBA^+ \cdot Br^-$. 1, 1-NS; 2, 2-NS; 3, 2,3-NDC; 4, 2,6-NDC; 5, 1,5-NDS; 6, 2,6-NDS; 7, PH; 8, i-PH; and 9, t-PH.

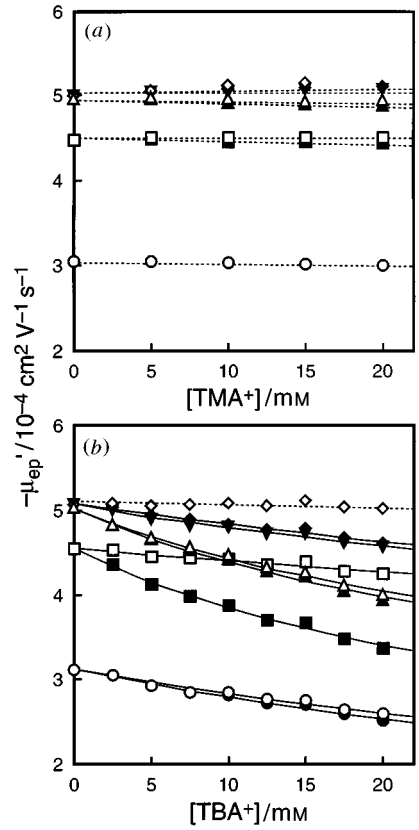

Fig. 4 Change in apparent electrophoretic mobility of anions as a function of the molar concentration of quaternary ammonium salt added to the migrating solution. Conditions, except for $Q^+ \cdot Br^-$ concentrations, as in Fig. 1. (*a*), TMA^+; and (*b*), TBA^+. ○, 1-NS; ●, 2-NS; △, 1,5-NDS; ▲, 2,6-NDS; □, 2,3-NDC; ■, 2,6-NDC; ◇, PH; ◆, i-PH; and ▼, t-PH.

A 1:1 ion associate is assumed to be formed between an analyte anion (A^{n-}) and a quaternary ammonium ion (Q^+) as shown in reaction (3), with an equilibrium constant given by eqn. (4).

$$Q^+ + A^{n-} \rightleftharpoons Q^+ \cdot A^{n-} \quad (3)$$

$$K_{ass} = \frac{[Q^+ \cdot A^{n-}]}{[Q^+][A^{n-}]} \quad (4)$$

The apparent electrophoretic mobility of a particular anion, μ_{ep}', can be represented as follows:

$$\mu_{ep}' = \frac{[A^{n-}]}{[A^{n-}] + [Q^+ \cdot A^{n-}]} \times \mu_{ep} + \frac{[Q^+ \cdot A^{n-}]}{[A^{n-}] + [Q^+ \cdot A^{n-}]} \times \mu_{epIA} \quad (5)$$

where μ_{ep} and μ_{epIA} are the mobility of the analyte anion and that of the 1:1 ion associate, respectively. Eqn. (5) can be converted into eqn. (6) by using the mass balance for A^{n-} and eqn. (4):

$$\mu_{ep}' = \frac{1}{1 + [Q^+]K_{ass}} \times \mu_{ep} + \frac{[Q^+]K_{ass}}{1 + [Q^+]K_{ass}} \times \mu_{epIA} \quad (6)$$

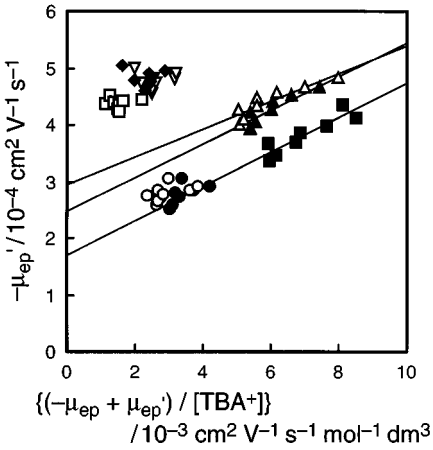

Fig. 5 Plots of μ_{ep}' *versus* $(-\mu_{ep} + \mu_{ep}')/[TBA^+]$. Electrophoretic mobility used was the result obtained with a fused-silica capillary, using TBA^+ as a pairing ion. Slopes for 1,5-NDS, 2,6-NDS and 2,6-NDC correspond to $1/K_{ass}$, which gave ion association constants of $10^{1.6}$, $10^{1.5}$, and $10^{1.5}$, respectively. Other constants could not be obtained. ○, 1-NS; ●, 2-NS; △, 1,5-NDS; ▲, 2,6-NDS; □, 2,3-NDC; ■, 2,6-NDC; ◆, i-PH; and ▽, t-PH.

To calculate K_{ass}, a non-linear least-squares method was applied. In the method, known values of $[Q^+]$ and μ_{ep}' were used, and the values of the constants, μ_{ep}, μ_{epIA} and K_{ass} were obtained after the calculated curves had been optimized. The values of μ_{ep}, μ_{epIA} and K_{ass} for the aromatic anions with various Q^+ are summarized in Tables 1, 2 and 3, respectively. The values obtained with the CElect-N capillary are also given.

The calculated results for μ_{ep} were found to be almost identical with the experimental results, as shown in Table 1. This indicates that the value of μ_{ep} need not be obtained experimentally. From Table 2, it was found that μ_{epIA} was less than half of μ_{ep}. This agrees with the fact that the apparent charge of divalent anions decreased to −1 on association with Q^+ and that of monovalent anions with Q^+ should be non-charged. Further, the values of μ_{epIA} indicate that the apparent molecular mass of the anions increases on ion association with Q^+. In the case of 1-NS and 2-NS, the values of K_{ass} were also calculated by assuming μ_{epIA} to be zero. The results for the K_{ass} values of 1-NS and 2-NS by the two calculations were similar. The results for μ_{ep} values obtained with the coated capillary were slightly larger than those obtained with the silica capillary, because the EOF in the coated capillary was very small and the correction of μ_{ep} affected by the EOF could not be complete.

Although the μ_{ep} and μ_{epIA} values obtained by using the two kinds of capillary were slightly different from each other, the K_{ass} values obtained were almost identical. Changes in the electrophoretic mobility were too small to determine the ion association constants when TMA^+ was used as a pairing ion and phthalate ion was used. When the mobility of PH along with an increase in Q^+ was used as a standard, however, the corrected mobility of 1,5-NDS and 2,6-NDS gave ion association constants of $10^{0.6 \pm 0.1}$ and $10^{0.5 \pm 0.1}$, respectively. The values are smaller than those with other Q^+, which also indicates less associability of TMA^+.

The lines shown in Fig. 4(*b*) are the results obtained by the calculation; they show that the experimental data and calculated results agree favourably with each other. For the other cations, except for TMA^+, the experimental data and calculated results also agreed well with each other. The ion association constants

Table 1 Comparison of electrophoretic mobilities of anions, $-\mu_{ep}$, between experimental and calculated results

	$-\mu_{ep}$*/10^{-4} cm^2 V^{-1} s^{-1}								
Q^+	1-NS	2-NS	1,5-NDS	2,6-NDS	2,3,-NDC	2,6-NDC	PH	i-PH	t-PH
TMA^+	—†	—	—	—	—	—	—	—	—
	(3.04)	(3.04)	(4.95)	(4.95)	(4.47)	(4.47)	(4.99)	(4.99)	(4.99)
TEA^+	—	—	4.96 ± 0.06	4.95 ± 0.06	—	4.47 ± 0.03	—	—	—
	(3.05)	(3.05)	(4.97)	(4.97)	(4.48)	(4.48)	(5.01)	(5.01)	(5.01)
TPA^+	3.04 ± 0.06	3.05 ± 0.08	4.93 ± 0.08	4.93 ± 0.08	—	4.46 ± 0.08	—	5.00 ± 0.10	4.99 ± 0.08
	(3.01)	(3.01)	(4.90)	(4.90)	(4.42)	(4.42)	(4.94)	(4.94)	(4.94)
TBA^+	3.12 ± 0.08	3.12 ± 0.07	5.01 ± 0.08	5.02 ± 0.09	4.54 ± 0.07	4.55 ± 0.01	—	5.07 ± 0.06	5.05 ± 0.08
	(3.12)	(3.12)	(5.02)	(5.02)	(4.54)	(4.54)	(5.06)	(5.06)	(5.06)
TBA^+‡	3.61 ± 0.14	3.62 ± 0.15	5.80 ± 0.20	5.81 ± 0.22	5.24 ± 0.23	5.26 ± 0.23	—	5.80 ± 0.27	5.77 ± 0.21
	(3.44)	(3.44)	(5.64)	(5.64)	(5.12)	(5.12)	(5.71)	(5.71)	(5.68)
TAA^+	3.06 ± 0.08	3.07 ± 0.06	5.01 ± 0.09	5.02 ± 0.06	4.52 ± 0.04	4.52 ± 0.04	—	5.05 ± 0.02	5.05 ± 0.03
	(3.09)	(3.09)	(5.03)	(5.03)	(4.53)	(4.53)	(5.06)	(5.06)	(5.06)
$HTMA^+$	3.17 ± 0.06	3.17 ± 0.06	5.10 ± 0.02	5.10 ± 0.02	4.62 ± 0.03	4.62 ± 0.04	—	5.15 ± 0.04	5.14 ± 0.03
	(3.15)	(3.15)	(5.10)	(5.10)	(4.61)	(4.61)	(5.13)	(5.13)	(5.13)
$OTMA^+$	3.20 ± 0.05	3.20 ± 0.06	5.16 ± 0.10	5.16 ± 0.08	4.66 ± 0.09	4.67 ± 0.07	—	5.24 ± 0.04	5.22 ± 0.06
	(3.21)	(3.21)	(5.18)	(5.18)	(4.68)	(4.68)	(5.22)	(5.22)	(5.22)

* Error: 3σ. The values in parentheses are the experimental results. † Not obtained. ‡ Values obtained in a coated capillary.

obtained in this work were close to those obtained in the previous work, which were $10^{1.3}$–$10^{1.4}$ for the ion association between TBA^+ and anions such as 1,5-NDS, 2,6-NDS, and 2,6-NDC.[18] The fact that the ion association constant of 2-NS is larger than that of 1-NS seems to be related to the difference in the charge density of the functional group. The acid dissociation constant (pK_a) of 1-naphthol (9.34) is smaller than that of 2-naphthol (9.51), and that of 1-naphthoic acid (3.70) is smaller than that of 2-naphthoic acid (4.17), which suggests that the negative charge of the functional group on the 2-position is larger than that on the 1-position. The more basic the functional group is, the more associable the anion should be with pairing ions.

Contribution of Methylene Moiety to Ion Associability

The ion association constants obtained by the least-squares method were plotted against the number of carbon atoms in Q^+ (Fig. 6). For each aromatic anion, K_{ass} increased with an increase in the carbon number or the size of Q^+, that is, the logarithmic values of the ion association constants increased almost linearly with increase in the bulkiness of Q^+, and their mean slope was about 0.06, which represents the increase in log K_{ass} per methylene group. This indicates that a linear free energy relationship can be valid in an aqueous solution, as well as in the case of liquid–liquid distribution system of ion associates. Fig. 6 also indicates that the ion association in aqueous media is closely related to the hydrophobicity of the pairing cations. The contribution of the methylene moiety to log K_{ass}, 0.06 (mean value), was one tenth of that in the liquid–liquid distribution system of ion associates, 0.6.[11] In the liquid–

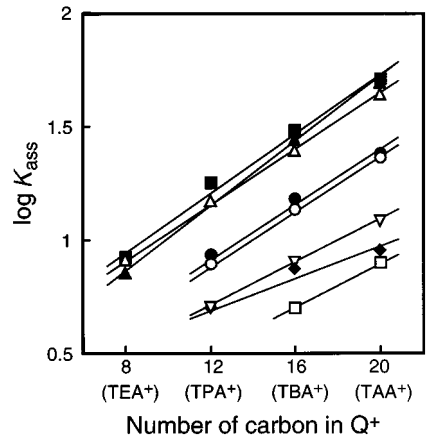

Fig. 6 Relationships between the ion association constants and the carbon number of quaternary ammonium ions (Q^+). ○, 1-NS; ●, 2-NS; △, 1,5-NDS; ▲, 2,6-NDS; □, 2,3-NDC; ■, 2,6-NDC; ◆, i-PH; and ▽, t-PH.

Table 2 Electrophoretic mobility of ion associates, $-\mu_{epIA}$: calculated results

	$-\mu_{epIA}{}^*/10^{-4}$ cm^2 V^{-1} s^{-1}								
Q^+	1-NS	2-NS	1,5-NDS	2,6-NDS	2,3-NDC	2,6-NDC	PH	i-PH	t-PH
TMA^+	—[†]	—	—	—	—	—	—	—	—
TEA^+	—	—	1.45 ± 0.61	1.79 ± 0.74	—	1.81 ± 0.37	—	—	—
TPA^+	0.10 ± 0.83	0.00 ± 1.03	1.78 ± 0.49	1.78 ± 0.49	—	1.48 ± 0.41	—	1.58 ± 1.58	1.33 ± 1.43
TBA^+	0.66 ± 0.56	0.63 ± 0.46	2.07 ± 0.33	2.08 ± 0.32	1.46 ± 1.19	1.50 ± 0.35	—	1.59 ± 0.73	1.52 ± 0.82
$TBA^{+‡}$	0.33 ± 1.00	0.41 ± 0.92	2.34 ± 0.62	2.34 ± 0.60	1.46 ± 2.86	1.56 ± 0.60	—	1.49 ± 2.39	1.46 ± 2.26
TAA^+	0.77 ± 1.25	0.67 ± 0.88	2.43 ± 0.70	2.39 ± 0.46	1.69 ± 1.54	1.81 ± 0.23	—	1.79 ± 0.59	1.72 ± 0.73
$HTMA^+$	0.49 ± 0.69	0.49 ± 0.69	2.26 ± 0.17	2.26 ± 0.15	1.96 ± 0.53	1.91 ± 0.30	—	1.42 ± 1.28	1.33 ± 0.77
$OTMA^+$	0.69 ± 0.57	0.50 ± 0.37	2.11 ± 0.58	2.15 ± 0.48	2.00 ± 2.12	1.71 ± 0.45	—	1.63 ± 0.90	1.79 ± 1.20

[*] Error: 3σ. [†] Not obtained. [‡] Values obtained in a coated capillary.

Table 3 Ion association constants, K_{ass}, obtained by the least-squares method

	Log $K_{ass}{}^*$								
Q^+	1-NS[†]	2-NS[†]	1,5-NDS	2,6-NDS	2,3-NDC	2,6-NDC	PH	i-PH	t-PH
TMA^+	—[‡]	—	0.6 ± 0.1[§]	0.5 ± 0.1[§]	—	0.6 ± 0.1[§]	—	—	—
TEA^+	—	—	0.91 ± 0.10	0.85 ± 0.13	—	0.92 ± 0.08	—	—	—
TPA^+	0.89 ± 0.17 (0.77 ± 0.16)	0.93 ± 0.21 (0.81 ± 0.19)	1.17 ± 0.08	1.17 ± 0.08	—	1.25 ± 0.08	—	0.70 ± 0.29	0.70 ± 0.22
TBA^+	1.13 ± 0.14 (0.98 ± 0.15)	1.18 ± 0.10 (1.06 ± 0.10)	1.39 ± 0.07	1.44 ± 0.07	0.70 ± 0.23	1.48 ± 0.07	—	0.87 ± 0.11	0.90 ± 0.12
$TBA^{+¶}$	1.12 ± 0.19 (1.07 ± 0.17)	1.20 ± 0.18 (1.14 ± 0.16)	1.45 ± 0.12	1.49 ± 0.13	0.79 ± 0.15	1.49 ± 0.12	—	0.89 ± 0.37	0.91 ± 0.29
TAA^+	1.36 ± 0.42 (1.21 ± 0.40)	1.38 ± 0.20 (1.26 ± 0.22)	1.64 ± 0.16	1.69 ± 0.10	0.90 ± 0.35	1.71 ± 0.06	—	0.95 ± 0.09	1.08 ± 0.11
$HTMA^+$	0.91 ± 0.14 (0.83 ± 0.14)	0.91 ± 0.14 (0.83 ± 0.14)	1.18 ± 0.03	1.15 ± 0.03	0.78 ± 0.16	1.18 ± 0.06	—	0.64 ± 0.19	0.70 ± 0.10
$OTMA^+$	0.91 ± 0.13 (0.79 ± 0.14)	0.91 ± 0.07 (0.83 ± 0.08)	1.20 ± 0.11	1.22 ± 0.09	0.61 ± 0.75	1.20 ± 0.09	—	0.77 ± 0.14	0.84 ± 0.20

[*] Error: 3σ. [†] Values in parentheses were the results when μ_{epIA} was assumed to be zero. [‡] Not obtained. [§] These constants were obtained by assuming that PH did not form ion associate with TMA^+ and using the increase in the electrophoretic mobility of pH with increase in the electrophoretic mobility of PH with increase in the concentration of Q^+ as the effect of temperature and ionic strength on the mobility of ions. [¶] Values obtained in a coated capillary.

liquid distribution system, the reaction and its equilibrium constant are represented by the equations

$$Q^+ + A^- \rightleftharpoons Q^+ \cdot A_{(o)} \quad (7)$$

$$K_{ex} = \frac{[Q^+ \cdot A^-]_{(o)}}{[Q^+][A^-]} = K_{ass} K_{D,ip} \quad (8)$$

where the subscript (o) denotes the species in the organic phase, K_{ex} is the extraction constant of a ion associate, $Q^+ \cdot A^-$, and $K_{D,ip}$ is the distribution coefficient of the ion associate between the aqueous and organic phase. The present results indicate that the contribution of a methylene moiety to the distribution process is a more dominant factor in the extraction equilibrium than in the ion association process in aqueous media.

Conclusion

This study has demonstrated the usefulness of ion association reactions in aqueous solution for the improvement of CE separations. Ion association constants were determined by measuring the electrophoretic mobility changes of anionic species. Ion associability was shown to be affected by the hydrophobicity of the pairing ion. The contribution of the hydrophobicity to ion association constants was relatively small, 0.05–0.07 for $\triangle \log K_{ass}$ per methylene moiety, compared with those in the solvent extraction of ion associates. Ion association combined with CE enabled us to separate anion isomers effectively.

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