Ion Association Reaction between Divalent Anionic Azo Dyes and Hydrophobic Quaternary Ammonium Ions in Aqueous Solution as Studied by Capillary Zone Electrophoresis

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Ion association properties of four kinds of anionic divalent azo dyes were investigated in an aqueous solution containing hydrophobic quaternary ammonium ions. The dyes used in this study possessed a hydroxy and a sulfonate group, which existed as a divalent anion in an alkaline solution. In capillary electrophoresis measurements, the apparent electrophoretic mobility of the dyes decreased along with an increase in the concentration of quaternary ammonium ion (Q^+) in a migrating solution. Ion association constants of the dyes with Q^+, K_{ass}, were determined by a simulation method of the mobility change using a non-linear least square method, when 1 : 1 ion associates formed. The increase in log K_{ass} was about 0.07 log unit per one methylene group; that is, the hydrophobicity of a pairing ion played an important role in the ion association reaction in aqueous solutions, as well as ion association in solvent extraction systems. Nitro groups of the dyes slightly affected the ion association constants, whereas the position of the sulfonate group in the azo dyes showed a significant change in ion association constants.

Keywords Ion association, aqueous solution, capillary electrophoresis, azo dye anion, quaternary ammonium ion

Separation of ionic species by capillary zone electrophoresis (CZE) is based on a unique mechanism, which utilizes the difference in the electrophoretic mobility, as well as electroosmotic flow. The separation of the compounds possessing similar electrophoretic mobilities has been improved using several separation modes based on the difference in acid dissociation, distribution to the micelle additives (Micellar Electrokinetic Chromatography, MEKC)[1], binding of guest molecule to the host one such as cyclodextrin[2] and chiral selector.[3] This ion association reaction has also been utilized in combination with MEKC[4] and in homogeneous aqueous solutions.[5] When a micelle-forming reagent and an ion association reagent both are added to the migrating solution, the ion association reaction is coupled with a distribution reaction[4], whereas the ion association reaction is dominant in the absence of any micelle additive.[5] The mode of CZE based only on ion association in an aqueous solution has been demonstrated in the separation of metal chelate anions with quaternary ammonium ions by Iki *et al.*[5] and by the authors[6], and the separation of aromatic anions with polymer cations has also been reported.[7] The improvement of separation based on the ion association in aqueous solutions and its utilization are of great interest. Therefore, the ion association in CZE should be clarified quantitatively.

In CE, analyses of binding reactions have been carried out in molecular associations such as protein–sugar interaction[8], benzenesulfonamide–carbonic anhydrase[9] and aromatic compound–SDS micelle.[10] However, they have never been carried out in ion association reactions in aqueous solutions, until the authors examined the analysis of ion association properties based on the change in electrophoretic mobility of anions.[11]

An ion association reaction has often been utilized by coupling it with solvent extraction, which has been used mainly for the separation and the concentration of ionic analytes. Several studies on the extractability of ion associates have already been reported by Motomizu and his colleague[12–16], Taguchi and his colleagues[17,18], and Yotsuyanagi and his colleague.[19] The extraction constant includes two steps: the formation of ion associate in the aqueous solution and the distribution of the formed ion associate. The mechanism will be applied to the ion association MEKC, which has already been studied by Nishi *et al.*[4] In solvent extraction, the equilibrium constants corresponding to the two steps, association and distribution, have never been determined separately because of the lack of methods to determine ion association constants.

In this study, ion association behaviors of anions with quaternary ammonium ions were examined in an aqueous solution, where newly synthesized anionic dyes were adopted as analyte anions. The philosophy for designing new anionic dyes is as follows: (1) they are divalent because the ion associability of monovalent anions is very weak, (2) a hydroxy and an azo group are contained because they are useful for a good chromogen, and (3) nitro groups are contained because the comparison of their contribution to the ion associability in aqueous solutions with that in liquid-liquid extraction is very interesting. Therefore, (polynitrophenylazo)-naphthol sulfonate was designed and synthesized for the present purpose. Ion association constants (K_{ass}) of the dyes with quaternary ammonium ions were determined by a non-linear least square method which gave more precise values than other slope calculations. The authors could discuss the ion associability of the azo dyes by comparing it with the value obtained in aromatic divalent anions.[11]

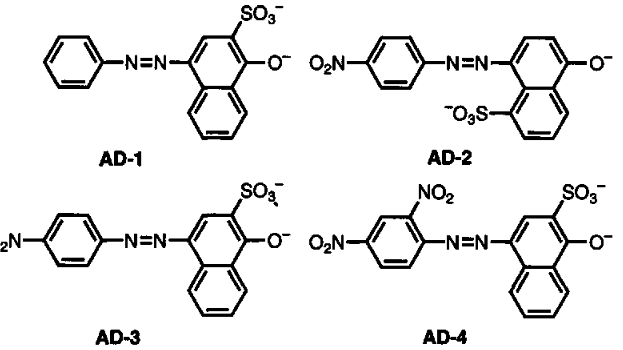

Fig. 1 Divalent anionic azo dyes synthesized and examined in this work.

Experimental

Apparatus

An Applied Biosystems 270A Capillary Electrophoresis System with a visible-range photometric detector was used. A fused silica capillary, purchased from GL Sciences, was used after being cut to the required length. A detection window was made by burning polyimide coating. The length of the capillary was 72 cm total with a 50 cm effective length for the detector; the inner diameter was 50 μm. The capillary was held in a thermostated room kept at 35°C during the measurement. Electropherograms were recorded by a Hitachi D-2000 Chromato-Integrator. A Hewlett Packard 8452A Diode Array Spectrophotometer was used for spectral measurements. A Corning Ion Analyzer 250 was used for pH measurements.

Reagent

Quaternary ammonium salts ($Q^+ \cdot X^-$). Tetramethylammonium bromide ($TMA^+ \cdot Br^-$), tetraethylammonium bromide ($TEA^+ \cdot Br^-$), tetrapropylammonium bromide ($TPA^+ \cdot Br^-$), tetrabutylammonium bromide ($TBA^+ \cdot Br^-$), tetraamylammonium bromide ($TAA^+ \cdot Br^-$), and stearyltrimethylammonium chloride ($STMA^+ \cdot Cl^-$) were purchased from Tokyo Kasei Kogyo Co., Ltd., and were used after being dried under vacuum. Cat-Floc, poly-(diaryldimethylammonium) chloride, was purchased from Calgon Co., Ltd. Sodium tetraborate, borax, was purchased from Wako Pure Chemical Industries, Ltd. and used without further purification.

Divalent anionic azo dyes, shown in Fig. 1 (AD-1 to AD-4, abbreviated as AD^{2-}), were synthesized by a diazotization reaction, followed by a coupling reaction. For example, AD-3 was synthesized in the following manner. *p*-Nitroaniline (0.42 g; 3×10^{-3} mol) was dissolved in 10 ml of water containing 1 ml of hydrochloric acid, and then 0.4 g of sodium nitrite (6×10^{-3} mol) was added to it stepwise for 2 h at 5°C. The reaction mixture was then poured into 25 ml of water, and urea was added to the solution until the gas stopped evolving. Then, 0.8 g of sodium 1-naphthol-5-sulfonate (3.3×10^{-3} mol) was added to the solution. After the reaction mixture was stirred for 4 h at ice temperature, it was filtered with a No. 5C filter paper. Crude brown product obtained was recrystalized from ethanol: 0.5 g of purified product was obtained (43% yield), which was used in the following experiments after being dried in a vacuum at 110°C. Other dyes were also synthesized in a similar manner. The purity of the reagents was examined by a molar ratio method using the solvent extraction with tetraamylammonium ion; the purity of the recrystallized products was more than 95%. The results of the separation and detection (230 nm) of the recrystallized products by CZE showed only one signal in all four azo dyes, which indicates that the purity of the reagents is enough for the present work.

Other reagents used were of a guaranteed reagent grade. Water purified by deionization and distillation was used throughout the experiment.

Procedure for capillary zone electrophoresis measurement

Sample solutions were prepared by dissolving the azo dyes into water to give 2×10^{-5} mol dm^{-3} solutions. The sample solutions also contained 3%(v/v) of ethanol, which was used to monitor the electroosmotic flow. The pH of migrating solutions was adjusted to 9.2 with 1×10^{-2} mol dm^{-3} of sodium tetraborate. Certain amounts of quaternary ammonium salt were added in the migrating solution to give less than 2.5×10^{-2} mol dm^{-3} solutions. Sample solutions were injected with a vacuum system for 3 s (injection volume: about 9 nl) from the anodic end of the capillary. A voltage of 25 kV was applied, and the analytes, dye anions, were separated and detected photometrically at 550 nm. Electrophoretic mobility of the analytes, as well as an electroosmotic mobility, was measured in an ordinary manner.[20] More than three measurements were carried out to obtain each of the mean electrophoretic mobility values.

Results and Discussion

Optical and chemical properties of novel azo dyes

Absorption spectra over a visible range were ex-

Table 1 Some properties of the novel anionic azo dyes

Compound	pK_a^{a}	Protonated form		Deprotonated form	
		λ_{max}/nm	ε^b	λ_{max}/nm	ε^b
AD-1	7.17	500	1.2×10^4	492	1.6×10^4
AD-2	8.33	504	2.3×10^4	548	2.1×10^4
AD-3	7.51	490	3.0×10^4	560	2.7×10^4
AD-4	7.51	480	3.0×10^4	608	3.8×10^4

a. Acid dissociation constant of hydroxy groups. b. At λ_{max}, $dm^3\ mol^{-1}\ cm^{-1}$.

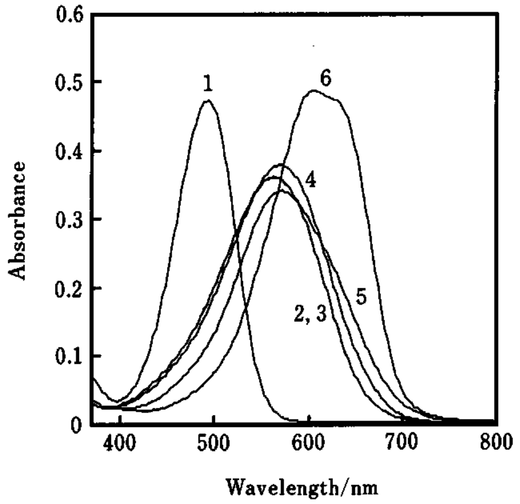

Fig. 2 Absorption spectra of AD-3 in the presence and in the absence of quaternary ammonium ions. [AD-3]=1.5×10^{-5} mol dm^{-3}. pH: curve 1, 4.0; curves 2-6, 9.0. [$Q^+\cdot X^-$]: curves 1 and 2, none; curve 3, [$TBA^+\cdot Br^-$]=5×10^{-5} mol dm^{-3}; curve 4, [$TBA^+\cdot Br^-$]=1×10^{-2} mol dm^{-3}; curve 5, [Cat-Floc]= 2.4×10^{-4} eq. dm^{-3}; curve 6, [$STMA^+\cdot Cl^-$]=5×10^{-5} mol dm^{-3}. Curves 2 and 3 are overlapped.

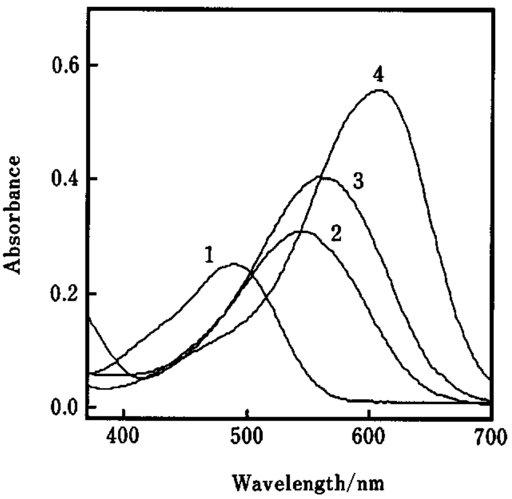

Fig. 3 Absorption spectra of anionic azo dyes. [AD^{2-}]= 4×10^{-5} mol dm^{-3}, pH 9.2 (borax). Curve 1, AD-1; curve 2, AD-2; curve 3, AD-3; curve 4, AD-4.

amined; Fig. 2 shows the spectra of AD-3. The spectrum showed a red shift from 490 nm to 560 nm of the maximum absorption wavelength due to the dissociation of a proton of the dye. It further shifted to 606 nm by adding $STMA^+\cdot Cl^-$, and the absorption intensity was enhanced. The absorbance at 606 nm increased linearly with an increase in the concentration of $STMA^+\cdot Cl^-$ at the concentration ranges up to 1 : 1 molar ratio. This indicates that ion association between AD-3 and $STMA^+$ can occur quantitatively, and that the color change reaction will be utilized for the determination of cationic surfactants. The ion association in an aqueous solution was also observed by adding Cat-Floc, a water-soluble cationic polymer, although the change in the spectrum was slight. The addition of $TBA^+\cdot Br^-$ also showed a slight change in the spectrum even at the concentration of 1×10^{-2} mol dm^{-3}. The selectivity in the ion association with various kinds of quaternary ammonium ions is of great interest to design various analytical systems based on ion association in an aqueous solution.

Acid dissociation constants, pK_a, for the azo dyes were determined spectrophotometrically in a usual manner; these are summarized in Table 1, along with absorption maximum wavelengths and molar absorptivities of the protonated and deprotonated forms of the azo dyes. Table 1 shows that the dyes at the concentrations of about 10^{-5} mol dm^{-3} exist as divalent anions in borax buffer solutions (pH 9.2). The absorption spectra of the deprotonated form of the reagents are shown in Fig. 3. In the further experiments of CZE measurements, wavelength of 550 nm was chosen to detect four dyes.

Separation of azo dyes by capillary zone electrophoresis

Capillary zone electrophoresis is promising for the analysis of ion association reaction with its high potential for the separation efficiency based on the charge, the molecular weight and the hydration of analytes, though the reproducibility of electrophoretic mobility is essential.

The effect of applied voltage on the mobility was examined; the results obtained are shown in Fig. 4.

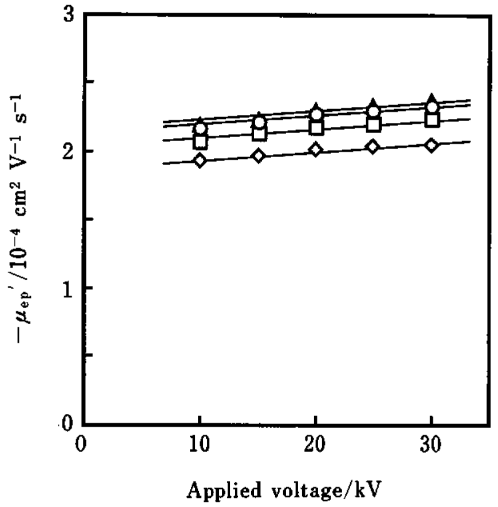

Fig. 4 Effect of applied voltage on electrophoretic mobility of azo dyes. Sample: four kinds of 2×10^{-5} mol dm^{-3} AD^{2-}. Migrating solution: 0.01 mol dm^{-3} borax (pH 9.2)+0.02 mol dm^{-3} $TBA^+\cdot Br^-$. CZE conditions: 35°C, 550 nm. Injection period: 3 s (about 9 nl). ○, AD-1; △, AD-2; □, AD-3; ◇, AD-4.

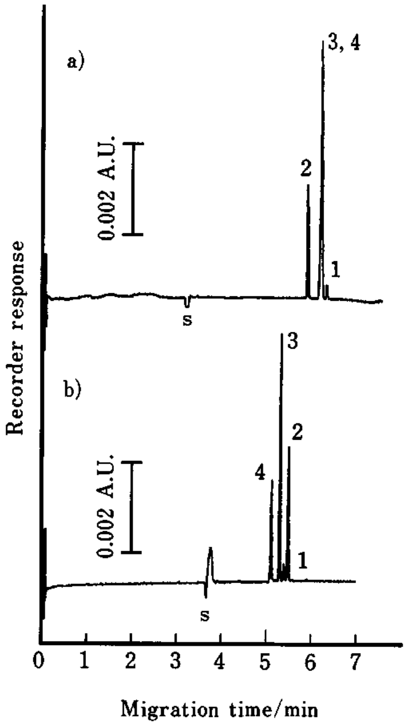

Fig. 5 Typical Electropherograms of azo dyes. Sample: four kinds of 2×10^{-5} mol dm^{-3} AD^{2-}. Migrating solution: (a), 0.01 mol dm^{-3} borax (pH 9.2); (b) 0.01 mol dm^{-3} borax (pH 9.2)+0.025 mol dm^{-3} $TBA^+\cdot Br^-$. CZE conditions: 25 kV, 35°C, 550 nm. Injection period: 3 s (about 9 nl). 1, AD-1; 2, AD-2; 3, AD-3; 4, AD-4; S, solvent (ethanol).

Though the concentrations of electrolytes were relatively large under such conditions, changes in mobility of the azo dyes were small. This indicates that the Joule heat generated in the capillary can be well released and the temperature in the capillary can be kept almost constant, which is very important to determine ion association constants more accurately.

A typical electropherogram of four kinds of the anions is shown in Fig. 5(a); this was obtained by using a 0.01 mol dm^{-3} borax buffer as a migrating solution. All the azo dyes were detected within 7 min. However, each migration time of the dyes was very close, as expected from the similar molecular structure of the dyes. To investigate the ion association property of the dyes, a symmetrical $Q^+\cdot Br^-$ was added to the migrating solution by varying the concentrations from 0 to 2.5×10^{-2} mol dm^{-3}. The electropherogram obtained in the presence of 2.5×10^{-2} mol dm^{-3} of $TBA^+\cdot Br^-$ is shown in Fig. 5(b). From Fig. 5(b), it can be seen that the separation of the dye anions was improved. A decrease in migration time for the dye anions indicates that the electrophoretic mobility of the anions was decreased and that these dyes formed an ion associate with Q^+ in the migrating solution during the electrophoresis. A decrease in the rate of EOF was attributed to the increase in ionic strength and the adsorption of some parts of Q^+ on the internal wall of the capillary.

Determination of ion association constants of azo dyes with quaternary ammonium ions

Apparent electrophoretic mobility, μ_{ep}', of the azo dyes was obtained in a usual manner[5] using the following equation:

$$\mu_{ep}' = \frac{L_T \times L_D}{V}\left(\frac{1}{t} - \frac{1}{t_{EOF}}\right) \quad (1)$$

where L_T, L_D, V, t, and t_{EOF} are the total length of the capillary, the capillary length from anodic end to the detector, an applied voltage, the migration time of an analyte anion, and the residence time of the neutral substance, ethanol, respectively. The plots of μ_{ep}' against $[Q^+]$ are shown in Fig. 6(a) - (d).

When tetraamylammonium ion was used, peaks of analytes became very broad and reliable electrophoretic mobility could not be obtained. This is because the precipitates of ion associates occurred in the migrating solutions. In other quaternary ammonium ions, however, it was found that a more bulky Q^+ gave a more significant decrease in mobility of the azo dyes.

The change in the apparent electrophoretic mobility, which was affected by the concentration and the bulkiness of Q^+, was analyzed in the following manner. A (1 : 1) ion associate should be formed between Q^+ and AD^{2-}, as shown in the reaction (2) and equilibrium constant (3), which correspond to the reaction between Q^+ and aromatic anions in our previous work.[11]

$$Q^+ + AD^{2-} \rightleftarrows Q^+\cdot AD^{2-} \quad (2)$$

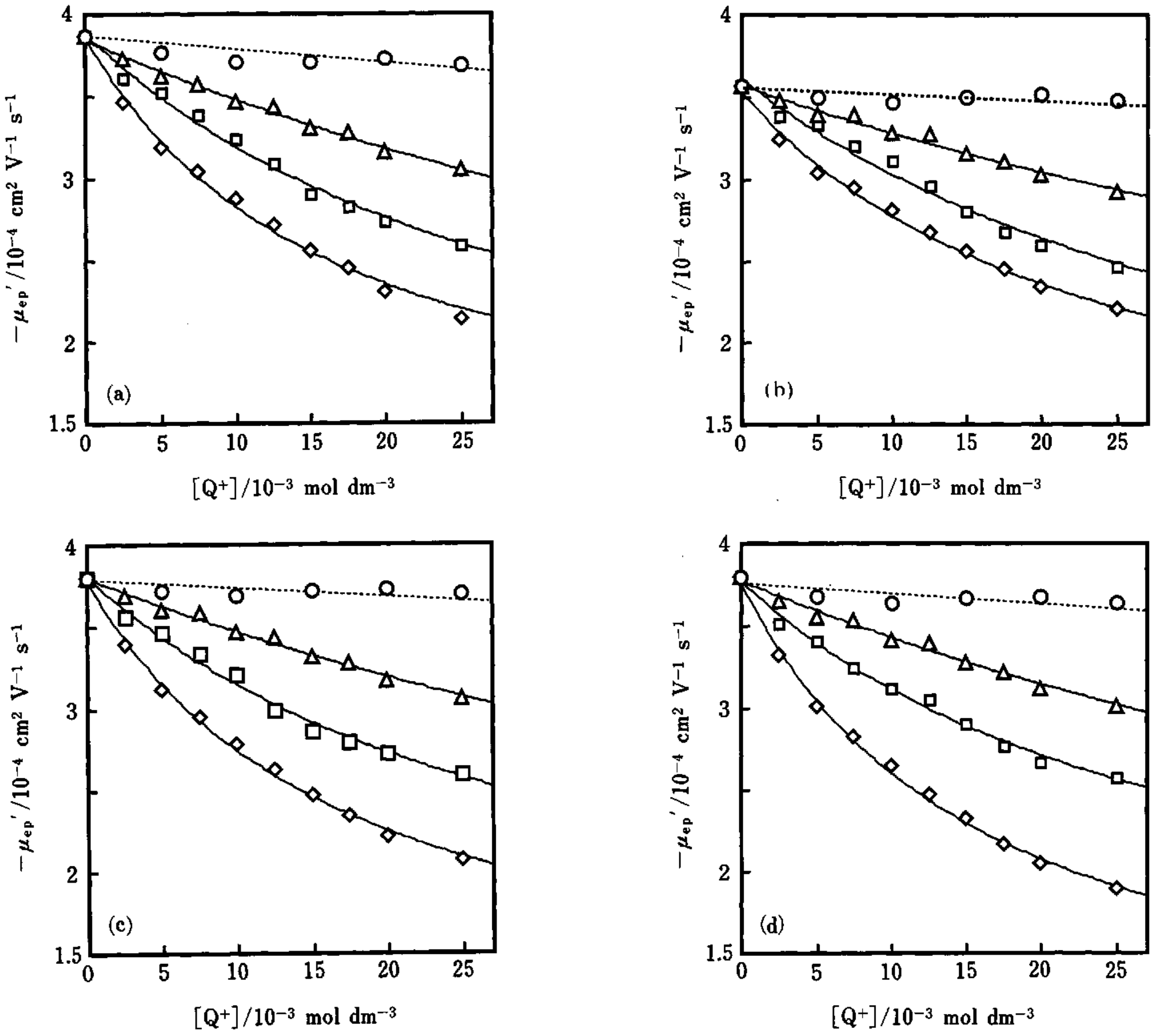

Fig. 6 Effect of the bulkiness of quaternary ammonium ions on electrophoretic mobility of the azo dyes. The curves, except in the case of TMA$^+$, were simulation results which used the obtained values; they agreed well with the experimental results. Migrating solution: 0.01 mol dm^{-3} borax (pH 9.2)+0 – 0.025 mol dm^{-3} $Q^+ \cdot Br^-$. The sample solution and CZE conditions are same as in Fig. 5. (a), AD-1; (b), AD-2; (c), AD-3; (d), AD-4. Q^+: (○), TMA$^+$; (△), TEA$^+$; (□), TPA$^+$; (◇), TBA$^+$.

$$K_{ass} = \frac{[Q^+ \cdot AD^{2-}]}{[Q^+][AD^{2-}]} \qquad (3)$$

where K_{ass} is an ion association constant. The apparent mobility of anion, μ_{ep}', can be represented by using the mobility of the anion, μ_{ep}, and that of the ion associate, μ_{epIA}, which is represented as Eq. (4) using the mass balance relationship of the dyes and Eq. (3).

$$-\mu_{ep}' = -\mu_{ep} \cdot \frac{1}{1 + K_{ass}[Q^+]} - \mu_{epIA} \cdot \frac{K_{ass}[Q^+]}{1 + K_{ass}[Q^+]} \qquad (4)$$

Equation (4) can be converted to Eq. (5), as the same derivation as in our previous work.[11]

$$-\mu_{ep}' = -\mu_{epIA} + \frac{-\mu_{ep} + \mu_{ep}'}{[Q^+]} \cdot \frac{1}{K_{ass}} \qquad (5)$$

The analysis of the ion association using Eq. (5) is different from the analysis proposed by Honda *et al.*[8] and Rundlett *et al.*[10] We also applied the analysis method proposed by Rundlett *et al.* to the analysis of ion association in this study. The following two equations can be derived from Eq. (4):

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

$$\frac{[Q^+]}{(-\mu_{ep}' + \mu_{ep})} = \frac{1}{(-\mu_{epIA} + \mu_{ep})} \cdot [Q^+] + \frac{1}{(-\mu_{epIA} + \mu_{ep})K_{ass}} \qquad (7)$$

Table 2 Ion association constants of azo dyes obtained by various analysis methods

Q^+	log K_{ass}[a]					
	AD-1	AD-2	AD-3	AD-4	1,5-NDS[b]	2,3-NDC[c]
TMA^{+d}	—[e]	—	—	—		
TEA^{+d}	1.15±0.07	1.02±0.10	1.14±0.09	1.19±0.10		
TPA^{+d}	1.47±0.09	1.33±0.12	1.47±0.10	1.50±0.08		
TBA^{+d}	1.75±0.07	1.60±0.07	1.74±0.06	1.79±0.06		
TBA^{+f}	1.85	1.86	1.84	1.90	1.4±0.1[g]	—[g]
TBA^{+h}	1.85	1.86	1.84	1.90		
TBA^{+i}	1.76	1.75	1.77	1.84		

a. Error: 3σ. b. 1,5-Naphthalenedisulfonate. c. 2,3-Naphthalenedicarboxylate. d. The calculation is based on a least square method. e. Change in mobility was too small to determine the values. f. The calculation is based on Eq. (5). g. The value is taken from ref. 11; it was obtained from Eq. (5), as described in the text. Constants for 2,3-NDC were not obtained in the method. h. The calculation is based on Eq. (6). i. The calculation is based on Eq. (7).

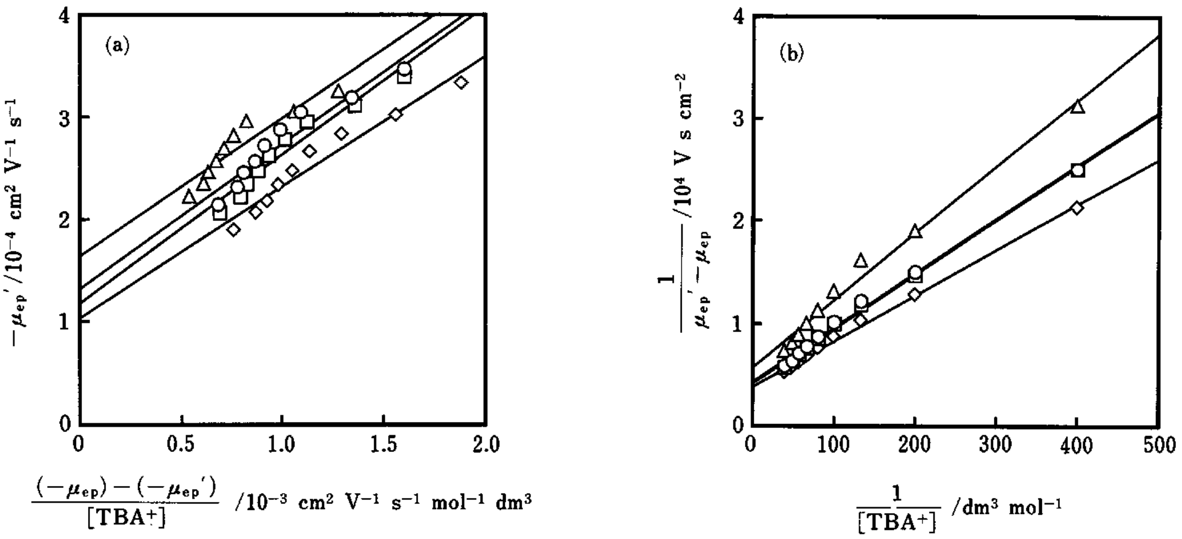

Fig. 7 Plots of the calculations to obtain K_{ass} values. The calculation method is based on: (a), Eq. (5); (b), Eq. (6). Values of K_{ass} are obtained from (1/slope) in (a) and from (intercept/slope) in (b). ○, AD-1; △, AD-2; □, AD-3; ◇, AD-4.

A method using Eq. (5) was also applied to the analysis of K_{ass} between TBA^+ and AD^{2-} in the present work. Ion association constants can be obtained by plotting $(-\mu_{ep}')$ against the term, $(-\mu_{ep}+\mu_{ep}')/[Q^+]$ as shown in Fig. 7(a); the slope of the plots corresponds to $(1/K_{ass})$, which gave K_{ass} values from $10^{1.84}$ to $10^{1.90}$, as summarized in Table 2. The linearity of the slopes are not good in low TBA^+ concentrations. This is because the mobility of anions in the absence of Q^+, μ_{ep}, was used as a standard of the analyte anion in each plot, which therefore must be determined very precisely. Further, a significant change in mobility was required to obtain ion association constants using the plot of Eq. (5). In fact, the values could not be obtained in those analyses when the cation was less bulky than TBA^+.

Ion association constants were also determined using Eq. (6) from the value of (intercept/slope) by plotting $1/(-\mu_{ep}'+\mu_{ep})$ *vs.* $1/[Q^+]$. Plots based on Eq. (6) are shown in Fig. 7(b). The values of K_{ass} were in the range from $10^{1.84}$ to $10^{1.90}$ (Table 2). The values, however, did not reflect the significant decrease in the mobility of AD-2, as seen in the pherograms and results in Fig. 6. Plots based on Eq. (7) also gave similar results to those obtained using Eq. (6). From these analysis method, it was found that no methods can give precise ion association constants when mobility changes are relatively small.

Therefore, a curve-fitting method based on Eq. (4) using a non-linear least square method was applied to the determination of the ion association constants. In this method, a series of the concentrations of Q^+ and the values of μ_{ep}' under a certain condition were input in Eq. (4), and the values of μ_{ep}, μ_{epIA}, and K_{ass} were optimized in the equation to simulate the experimental

Table 3 Electrophoretic mobility of azo dyes, $-\mu_{ep}$, obtained by calculating in the least square method

Q^+	$-\mu_{ep}{}^a/10^{-4}$ cm^2 V^{-1} s^{-1}			
	AD-1	AD-2	AD-3	AD-4
TMA$^+$	—^{b}	—	—	—
TEA$^+$	3.85±0.07	3.57±0.08	3.80±0.08	3.78±0.08
TPA$^+$	3.88±0.14	3.61±0.16	3.80±0.14	3.77±0.11
TBA$^+$	3.85±0.15	3.53±0.12	3.77±0.12	3.77±0.13
(Exp)^c	(3.86)	(3.57)	(3.79)	(3.79)

a. Error: 3σ.
b. Change in mobility was too small to determine the values.
c. Experimental values with TBA$^+$.

Table 4 Electrophoretic mobility of ion associates, $-\mu_{epIA}$, obtained by calculating in the least square method

Q^+	$-\mu_{epIA}{}^a/10^{-4}$ cm^2 V^{-1} s^{-1}			
	AD-1	AD-2	AD-3	AD-4
TMA$^+$	—^{b}	—	—	—
TEA$^+$	0.83±0.36	0.57±0.55	1.00±0.47	1.08±0.44
TPA$^+$	0.87±0.40	0.39±0.59	0.91±0.40	1.03±0.31
TBA$^+$	1.00±0.27	0.91±0.26	0.85±0.22	0.70±0.21

a. Error: 3σ.
b. Change in mobility was too small to determine the values.

data of μ_{ep}' as accurately as possible. By using the values obtained by the calculation, the relationships between μ_{ep}' and $[Q^+]$ were simulated. These are shown by the solid lines in Fig. 6(a) - (d), which were in good agreement with the experimental data. The values of K_{ass}, μ_{ep}, and μ_{epIA} obtained by the proposed method are summarized in Tables 2, 3 and 4, respectively. Ion association constants of azo dyes with TBA$^+$ obtained in the proposed method were almost identical to the values obtained according to Eq. (5) and were within small deviations. Moreover, the fact that the change in μ_{ep}' was smaller in the case of AD-2 due to the addition of TBA$^+$ was reflected in the K_{ass} values. The proposed method is found more reliable for the determination of K_{ass} values. When the least bulky quaternary ammonium ion studied in this work, TMA$^+$, was used, the change in mobility was too small to determine the values, which indicates that the ion association constants of the azo dyes with TMA$^+$ are very small. Table 2 shows that the more bulkier or the more hydrophobic the pairing cation is, the larger the ion association constant is. This means that the hydrophobicity of the pairing ions plays an important role in ion associability in an aqueous solution.

In the case of AD-2, the experimental condition of pH was not so much far from its pK_a value, (pH−pK_a=0.9), and the effect of the protonation of AD-2 (protonated form of AD^{2-}: HAD$^-$) was considered in the following equation (8).

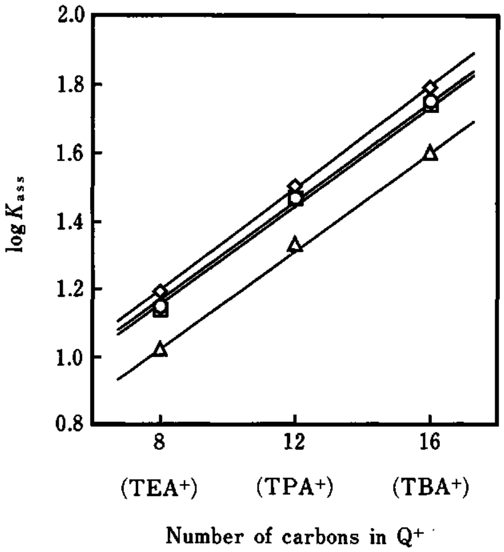

Fig. 8 Relationships between the ion association constants and carbon number of Q$^+$. ○, AD-1; △, AD-2; □, AD-3; ◇, AD-4.

$$-\mu_{ep}'=\frac{-\mu_{ep}[\mathrm{HAD^-}]/2-\mu_{ep}[\mathrm{AD^{2-}}]-\mu_{epIA}[\mathrm{Q^+\cdot AD^{2-}}]}{[\mathrm{HAD^-}]+[\mathrm{AD^{2-}}]+[\mathrm{Q^+\cdot AD^{2-}}]} \tag{8}$$

The logarithmic K_{ass} value obtained with TBA$^+$ as a pairing ion was 1.61±0.08 with Eq. (8), which was almost identical to the result obtained with Eq. (4).

The ion association constants obtained with TBA$^+$ were larger than the values of naphthalenedisulfonate compounds ($10^{1.3}$ to $10^{1.4}$).[11] This result indicates that an increased molecular volume will make some contributions to the ion associability; that is, the difference in ion associability between the present azo dyes and the previously reported aromatic anions seems to be caused by the difference in hydrophobicity.

The ion association constants obtained were plotted against the number of carbon atoms in Q$^+$ (Fig. 8). In each anionic azo dye, ion association constants increased along with an increase in carbon number or the bulkiness of Q$^+$; that is, the logarithmic values of K_{ass} increased almost linearly along with the increase in the bulkiness of Q$^+$, and their mean slope was about 0.07, which corresponds to the increase in log K_{ass} per one methylene group. This indicates that a linear free energy relationship can be valid in an aqueous solution, as well as in the case of a liquid-liquid distribution system of ion associates. However, the mean increase in log K_{ass}, 0.07, was very small, about one-eighth of the mean increase obtained in ion-association extraction system.[16] From this result, a methylene group affects more significantly the transference of an ion associate from an aqueous to an organic phase.

The position of a sulfonate group on the azo dye also gave a significant effect in ion associability, which can be seen by comparing AD-2 with other three dyes. This is probably because the sulfonate group in AD-2 is protected by a phenylazo group and a bulky cation can not approach to the sulfonate group easily. The number of nitro groups affected ion associability slightly, though the nitro groups can contribute to the increase in the extractability of ion associates very effectively.[16] The nitro groups are remote from ion–ion interaction points, and therefore, they do not affect the ion associability. This indicates that the nitro group works effectively when the ion associates transfer from an aqueous to an organic phase.

In conclusion, ion association in an aqueous solution has been studied by using novel divalent anionic azo dyes possessing nitro groups with hydrophobic quaternary ammonium ions. The effect of hydrophobicity of pairing cations, as well as the nitro group, was found to be less than that in solvent extraction of ion associates. Ion association was found to be utilized for improving the separation of ions when coupled with CZE in aqueous media.

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