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Short communication

Electrophoretic mobility study on ion–ion interactions in an aqueous solution

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Abstract

Ion–ion interactions between anions and their pairing ions in aqueous solutions were studied through the measurements of electrophoretic mobilities of analyte ions in capillary zone electrophoresis, where the electrophoretic method for the analysis of ion association reaction is shown to be more useful than the conductometric method widely used in the analysis of the reactions. The electrophoretic mobility of monovalent inorganic anions was almost identical even when the concentrations of alkali metal ions and quaternary ammonium ions in the migrating solution were varied up to 15 mM. On the other hand, the electrophoretic mobility of organic anions, such as monovalent and divalent anions, decreased with increasing concentrations of quaternary ammonium ions. Changes in the electrophoretic mobilities were analyzed by a non-linear least-squares method giving ion association constants. The results indicate that the proposed method is applicable to the analysis of such reactions to give the mobility change. The ion association constants obtained in an aqueous solution were related to the extraction constants of the ion associates, and the contributions of the association process and the distribution process were clarified. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Electrophoretic mobility; Ion–ion interactions; Association constants; Inorganic anions; Phenolic compounds; Organic acids

1. Introduction

Recently, capillary zone electrophoresis (CZE) has been widely used for the separation and determination of ionic substances. In CZE, many kinds of modifying reagents in the migrating solutions have been effectively used to improve the separation of the analytes. In addition to the improvement in separation, CZE is a powerful tool for various kinds of interaction analyses in aqueous solutions. The interactions so far studied are a ligand–protein

interaction [1], a protein–sugar interaction [2], a binding of a fluorescence labeled molecule to antibody [3], a binding of neutral compounds to sodium dodecyl sulfate micelles [4], and a binding of 2-phenoxypropionic acid enantiomers to β -cyclodextrin [4].

Ion–ion interactions, such as an ion association reactions, have been analyzed mainly by a conductometric method, which is based on the Debye–Hückel activity theory and Onsager conductance equation [5]. Although the conductometric method contains some theoretical assumptions, many ion association constants have been determined by this method [6,7]. Recently, a novel method using electrophoretic mobility, which is easily measured by

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CZE, has been proposed for the analysis of the ion association reactions in an aqueous solution by the present authors [8–10].

In this paper, the authors determined ion association constants of organic anions, as well as inorganic anions, with quaternary ammonium cations as a pairing ion, and clarified some factors contributing to ion associability. From the ion association constants obtained with some monovalent organic anions, the contribution of the ion associability to the extractability of ion associates was also clarified.

2. Experimental

2.1. Apparatus

As a capillary electrophoresis system, an Applied Biosystems (Foster City, CA, USA) Model 270A-HT, equipped with a UV detector was used. A Hitachi (Tokyo, Japan) Chromato-Integrator D-2500 was used for recording the electropherograms. A fused-silica capillary, purchased from GL Sciences (Tokyo, Japan), was cut to the required length and was kept in a temperature-controlled compartment of the system. The size of the capillary was 72 cm (50 cm effective length from the sample injection point to the detector) \times 50 μ m I.D.

2.2. Reagents

As migrating buffer components, NaOH, NaH₂PO₄–Na₂HPO₄, and Na₂B₄O₇ (borax) (Wako, Osaka, Japan) were used. Tetrabutylammonium ion (chloride salt: TBA⁺·Cl[−]; Tokyo Kasei Kogyo, Tokyo, Japan) was used as a pairing ion in the ion association reaction with the organic anions. Sodium and potassium chloride were used as electrolytes for adjusting the ionic strength of the migrating solution.

The solutions of analyte anions were prepared by dissolving alkali metal salts of K₄[Fe(CN)₆], K₃[Fe(CN)₆], K₂CrO₄, Na₂MoO₄, Na₂WO₄, NaReO₄, NaBr, NaI, NaNO₂, NaNO₃, KSCN and NaBrO₃ in water. Some aromatic anions were also examined as analytes; they were phenol (BO), benzoic acid (BC), sodium benzenesulfonate (BS), 1-naphthol (1-NO), 2-naphthol (2-NO), 1-naphthoic acid (1-NC), 2-naphthoic acid (2-NC), sodium 1-

naphthalenesulfonate (1-NS), sodium 2-naphthalenesulfonate (2-NS) and some nitrophenol derivatives (NP) (Tokyo Kasei Kogyo). These acids were dissolved in water after neutralisation with equivalent amounts of NaOH.

2.3. Procedure

A migrating solution containing pH buffer components, an ion association reagent and an electrolyte (NaCl) for adjusting an ionic strength was placed in both an anodic and a cathodic reservoir, and then filled into the capillary by a reduced pressure system. A sample solution containing some analyte anions was injected from the inlet end for 3 s by a hydrodynamic injection. A voltage of 15 kV was then applied and electrophoresis was started. The analyte anions were detected spectrophotometrically at the outlet end of the capillary. Ethanol (3% v/v) was contained in the sample solution as a marker of the electroosmotic flow (EOF). The electrophoretic mobility of each analyte anion ($-\mu'_{ep}$), was calculated from the migration time of the analyte and that of EOF in the usual manner.

3. Results and discussions

3.1. Analysis of ion association reactions by a conductometric and an electrophoretic method

The conductometric method often used for the analysis of ion–ion interactions in solutions is a well-established one, and several analytical methods, e.g. Davies [11], MacInnes–Shedlovsky [12], Shedlovsky [13,14], and Fuoss–Edelson [15], have been proposed. These methods utilized the decrease in molar conductivity with the increasing concentrations of a given electrolyte in a solution, where the differences between experimentally obtained values of molar conductivity and theoretical values derived on the basis of the Debye–Hückel and Onsager equations are used to determine equilibrium constants, such as those of complex formation and ion association. Most of the conductivity methods can be only used to analyze a single electrolyte solution at relatively high concentrations ($>10^{-3}$ M).

On the other hand, an electrophoretic method,

recently proposed by the present authors, has proved to be applicable to the accurate analysis of the ion association in solutions. A certain analyte ion at relatively low concentrations can interact with its pairing ion, an ion association reagent, present at relatively high concentrations in the migrating solutions during the electrophoresis. In the absence of the ion association reagent, the analyte ion migrates with their inherent electrophoretic mobility, while in the presence of the ion association reagent, the apparent mobility of the analyte ion changes by the degree of the interaction, which can induce the changes in apparent charges and apparent mass of the analyte. Therefore, by analyzing the mobility changes, the ion association constants can be determined.

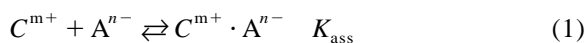
Moreover, the CZE method used in this work proves the merit of its application to multi-reaction species at a single series of measurements if each analyte can be separated in the migrating solution. In the conductometric method, only one reaction species can be analyzed in a single series of measurements. The CE systems commonly available possess good absorptometric detectors, and a slight change in absorbances can be detected; $<10^{-4}$ absorbance unit can be easily detectable, which allows the analysis of the analytes at low concentrations ($<10^{-6}$ M). Because of the applicability to the low concentrations, the proposed CZE method is useful for slightly soluble ion associates. Another merit of the proposed method is that the ionic strength of migrating solutions can be adjusted by adding non-interacting electrolytes in migrating solutions.

3.2. Analysis of ion association reaction by using non-linear least-squares method

Some analysis methods on the basis of CZE measurements for solute–solute interactions in solutions were proposed by using some linear analysis methods. The authors developed another method using non-linear least-squares analysis, and applied it to ion association reaction with bulky and hydrophobic ions [9,10]. By using the proposed analysis method, relatively weak interactions can be analyzed, even when the changes in mobility was very slight [16]. Separations of ionic isomers utilizing multi-point interaction [17], stacking of aromatic rings [18]

and strongly electrostatic interaction [19] were also carried out based on the proposed method.

In CZE, the apparent electrophoretic mobility of a certain analyte anion (A^{n-}), $-\mu'_{ep}$, decreases with an increase in the concentration of an ion association reagent (C^{m+}) added to the migrating solution. The decrease in $-\mu'_{ep}$ can be attributed to the formation of an ion associate as shown in the reaction (1), and the corresponding equilibrium constant can be written as Eq. (2). The apparent electrophoretic mobility of A^{n-} , $-\mu'_{ep}$, is written in Eq. (3) from the definition.



$$K_{\text{ass}} = \frac{[C^{m+} \cdot A^{n-}]}{[C^{m+}][A^{n-}]} \quad (2)$$

$$-\mu'_{ep} = \frac{[A^{n-}]}{[A^{n-}] + [C^{m+} \cdot A^{n-}]} (-\mu_{ep,A}) + \frac{[C^{m+} \cdot A^{n-}]}{[A^{n-}] + [C^{m+} \cdot A^{n-}]} (-\mu_{ep,CA}) \quad (3)$$

Eq. (4) can be derived from Eq. (3) by using the mass balance of A^{n-} and the equilibrium constant (2).

$$-\mu'_{ep} = \frac{1}{1 + K_{\text{ass}}[C^{m+}]} (-\mu_{ep,A}) + \frac{K_{\text{ass}}[C^{m+}]}{1 + K_{\text{ass}}[C^{m+}]} (-\mu_{ep,CA}) \quad (4)$$

where $-\mu_{ep,A}$ and $-\mu_{ep,CA}$ are the electrophoretic mobilities of the analyte anion and that of the corresponding 1:1 ion associate, respectively, and K_{ass} is the ion association constant of the equilibrium (1). A series of pairs of known concentrations of C^{m+} in the migrating solution and experimentally obtained values of $-\mu'_{ep}$ were put into Eq. (4), and the values of K_{ass} , $-\mu_{ep,A}$, and $-\mu_{ep,CA}$ were optimized till the errors between the calculated and the experimental values of $-\mu'_{ep,A}$ are the smallest. When the ion associate is formed between an equally charged cation and anion, the apparent charge of the ion associate is zero; that is, the value of $-\mu_{ep,CA}$ can be assumed to be zero.

3.3. Ion associability of inorganic anions with alkali metal ions

Ion association constants of several inorganic anions with alkali metal ions have already been studied using conductometric measurements [6,7]. The ion associability of these ion associates has been discussed mainly on the basis of electrostatic interaction theories, such as Bjerrum theory [20]. In this study, several ion associates were also examined through the mobility measurements. Changes in the mobility with an increase in the concentrations of Na^+ as a pairing ion are shown in Fig. 1. It was found that the electrophoretic mobility of most of the monovalent and divalent anions examined in this work did not change significantly even when Na^+ was present in large amounts, while $[\text{Fe}(\text{CN})_6]^{4-}$ and $[\text{Fe}(\text{CN})_6]^{3-}$ showed ion associability with Na^+ , and their $(-\mu'_{\text{ep}})$ values decreased with an increase

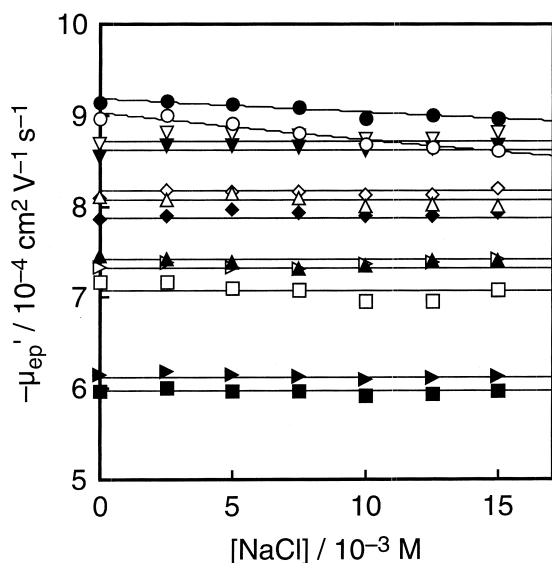


Fig. 1. Mobility change of inorganic anions by increasing NaCl concentrations in the migrating solution. Migrating solution: $1 \cdot 10^{-2}$ M phosphate buffer (pH 7.0, $[\text{Na}^+] = 1.9 \cdot 10^{-2}$ M) + $0 - 1.5 \cdot 10^{-2}$ M NaCl. Sample solution: $1 \cdot 10^{-5}$ M anions. CE conditions: applied voltage, 15 kV; detection wavelength, 195 nm; capillary compartment temperature, 35°C; injection volume, 3 s (about 9 nl). Capillary: polymer coated capillary (CElect-N, Supelco). Electroosmotic flow was monitored with 3% ethanol aqueous solution. Analyte anions: \circ , $[\text{Fe}(\text{CN})_6]^{4-}$; \bullet , $[\text{Fe}(\text{CN})_6]^{3-}$; \triangle , CrO_4^{2-} ; \blacktriangle , MoO_4^{2-} ; \square , WO_4^{2-} ; \blacksquare , ReO_4^- ; ∇ , Br^- ; \blacktriangledown , I^- ; \diamond , NO_2^- ; \blacklozenge , NO_3^- ; \triangleright , SCN^- ; \blacktriangleright , BrO_3^- .

in Na^+ concentrations. The multivalent, tervalent and trivalent anions, however, are weakly associable with Na^+ , and their K_{ass} values are 1.08 and 0.70 for $[\text{Fe}(\text{CN})_6]^{4-}$ and $[\text{Fe}(\text{CN})_6]^{3-}$, respectively. These results suggest that the small change in the ionic strength of the migrating buffer does not affect the electrophoretic mobility of ions when no ion association reactions occur.

3.4. Ion associability of aromatic anions with tetrabutylammonium ion (TBA^+)

Ion associability of nine kinds of aromatic anions was examined with TBA^+ at a constant ionic strength ($I=0.025$). Changes in the electrophoretic mobility of organic anions with increasing concentrations of TBA^+ in the presence of NaCl in the migrating solution are shown in Fig. 2. The degree of the decreases in the electrophoretic mobility of each aromatic anion was almost identical whichever of Na^+ and K^+ was used to by adjust the ionic strength.

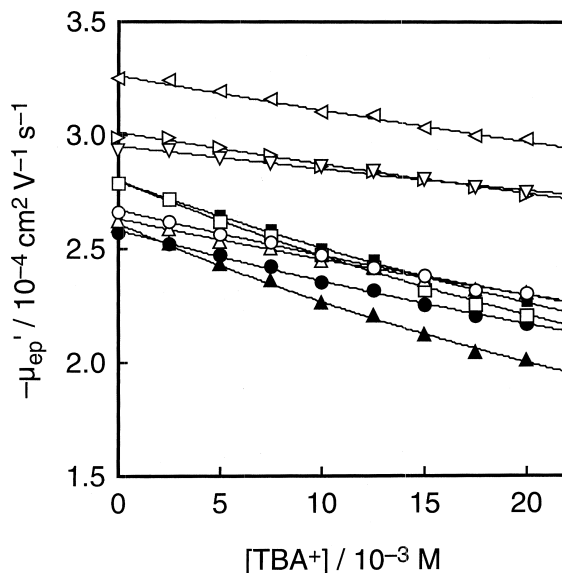


Fig. 2. Change in electrophoretic mobility of aromatic anions with an increase in TBA^+ concentrations. Migrating buffer: $5 \cdot 10^{-3}$ M NaOH + NaCl + $(0-20) \cdot 10^{-3}$ M $\text{TBA}^+ \cdot \text{Cl}^-$; ionic strength was adjusted to 0.025 with NaCl. Sample solution: $1 \cdot 10^{-5}$ M anions. CE conditions: applied voltage, 15 kV; detection wavelength, 210 nm; capillary compartment temperature, 25°C; injection volume, 3 s (about 9 nl). Analyte anions: ∇ , BO; \triangleright , BC; \triangleleft , BS; \circ , 1-NO; \triangle , 1-NC; \square , 1-NS; \bullet , 2-NO; \blacktriangle , 2-NC; \blacksquare , 2-NS.

The changes in the mobility were analyzed in the same manner described earlier, and the ion association constants were determined; the K_{ass} values obtained are summarized in Table 1. The values are almost equal to those of our previous work, which were obtained without adjusting the ionic strength [16]. In other words, the aromatic anions examined in this work did not show any ion associability with Na^+ and K^+ , and were not affected by the components of the migrating buffer, phosphate (pH 7.0) or borate (pH 9.2). The results in Table 1 indicate that TBA^+ can interact with the aromatic anions much more than alkali metal ions, also that the ion associability of the anions possessing a naphthalene ring is more than those possessing a benzene ring, which means that the hydrophobicity or bulkiness of pairing ions enhances the ion associability in aqueous medium.

3.5. Ion associability of nitro-substituted phenolate anions with TBA^+

When an improvement of the extractability of ion associates is required in liquid–liquid distribution, the introduction of nitro groups into pairing ions has been often performed; for example, a picrate ion has been often used as a pairing anion. In this work, the effect of the nitro groups on the ion associability was examined with some aromatic anions in order to clarify the contribution of the ion association in an aqueous solution to the extractability of ion associates. The aromatic anions examined were the deprotonated anions of phenol (P), *o*-nitrophenol (*o*-NP), *m*-nitrophenol (*m*-NP), *p*-nitrophenol (*p*-NP), 2,4-dinitrophenol (2,4-DNP), 2,6-dinitrophenol (2,6-DNP), and picric acid (2,4,6-TNP). For comparison, the derivatives of a benzenecarboxylate and

a benzenesulfonate ion were also examined; they were the deprotonated anions of benzoic acid (BC), *p*-nitrobenzoic acid (*p*-NBC), benzenesulfonic acid (BS), and *p*-nitrobenzenesulfonic acid (*p*-NBS). The electrophoretic mobility was measured in the presence of TBA^+ as an ion association reagent. The changes in the mobility were analyzed as described earlier, and the ion association constants were determined; the K_{ass} values obtained are summarized in Table 2. The ion associability of the aromatic anions with TBA^+ is not much affected by factors such as the position or number of nitro groups, and the type of anionic group, while the extractability of the ion associates is affected by these factors. The nitro groups are very useful when coupled with the liquid–liquid distribution. The contribution of the nitro groups can be explained as follows: the introduction of nitro groups, which is an electron-withdrawing group, into the aromatic ring is able to disperse the anionic charge over the anionic reagent, which decreases the hydrophilicity around the anionic groups, and increases the distribution coefficient of the ion associates.

3.6. Contribution of ion associability in an aqueous solution to the extractability of ion associates

The ion association reaction has been widely used in the research field of analytical chemistry, coupled with liquid–liquid distribution reactions. Stepwise reactions concerning the liquid–liquid distribution are the ion association reaction in an aqueous solution and the distribution reaction of the ion associate formed in the aqueous phase to an organic phase. Such reactions are written as follows:

Table 1
Ion association constants obtained at constant ionic strength and at varied ionic strength

Pairing cation (ionic strength, adjusted with MCl)	$\log K_{\text{ass}}^{\text{a}}$								
	BO	BC	BS	1-NO	2-NO	1-NC	2-NC	1-NS	2-NS
TBA^+ ($I=0.025$, NaCl)	0.55 ± 0.12	0.69 ± 0.09	0.69 ± 0.09	0.92 ± 0.06	0.98 ± 0.04	0.87 ± 0.08	1.19 ± 0.06	1.08 ± 0.05	1.13 ± 0.05
TBA^+ ($I=0.025$, KCl)	0.70 ± 0.09	0.82 ± 0.08	0.81 ± 0.08	0.95 ± 0.05	1.03 ± 0.04	0.95 ± 0.05	1.22 ± 0.04	1.11 ± 0.04	1.16 ± 0.03
TBA^+ ($I=0.005\text{--}0.025$) ^b	0.3 ± 0.2	0.5 ± 0.3	0.5 ± 0.2	0.91 ± 0.11	0.98 ± 0.11	0.87 ± 0.12	1.17 ± 0.06	1.06 ± 0.11	1.13 ± 0.06

^a Error: 3σ .

^b Results of previous study (Ref. [16]).

Table 2
Equilibrium constants concerning liquid–liquid distribution of ion associates between an aqueous and a chloroform phase^a

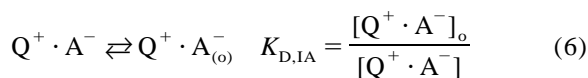
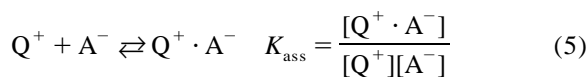
Anion	Log $K_{\text{ass}}^{\text{b}}$	Log $K_{\text{D,IA}}^{\text{c}}$	Log K_{ex}^{d}
<i>Phenolate ions</i>			
P	0.40	−0.70	−0.30
<i>o</i> -NP	0.64	1.53	2.17
<i>m</i> -NP	0.60	1.09	1.69
<i>p</i> -NP	0.80	1.72	2.52
2,4-DNP	0.80	3.21	4.01
2,6-DNP	0.68	3.30	3.98
2,4,6-TNP	0.67	4.86	5.53
<i>Benzoate ions</i>			
BC	0.69	−0.01	0.68
<i>p</i> -NBC	0.55	1.12	1.67
<i>Benzenesulfonate ions</i>			
BS	0.69	1.03	1.72
<i>p</i> -NBS	0.64	2.38	3.02
Naphthalene-2-carboxylate (2-NC)	1.19	1.02	2.21
Naphthalene-2-sulfonate (2-NS)	1.13	2.12	3.25

^a Tetrabutylammonium ion was used as a pairing cation.

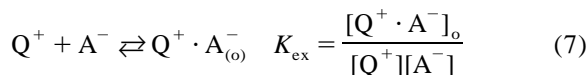
^b Values are determined by the electrophoretic method.

^c Values are obtained from K_{ass} and K_{ex} values.

^d Cited values from Ref. [21]; solvent, chloroform.



where Q^+ , A^- and $\text{Q}^+ \cdot \text{A}^-$ are quaternary ammonium ion, organic anion and the ion associate, respectively, K_{ass} and $K_{\text{D,IA}}$ are the ion association constant and the distribution coefficient of the ion associate, respectively, and the subscript (o) denotes the species in the organic phase. The overall equilibrium reaction can be written as follows:



where K_{ex} is an extraction constant. From the reactions (5)–(7), a relationship, $K_{\text{ex}} = K_{\text{ass}} \times K_{\text{D,IA}}$, can be derived. The K_{ass} values of nitrophenolate anions can be determined in the present study, and therefore the liquid–liquid distribution coefficient of the ion associate ($K_{\text{D,IA}}$) can be calculated from the

extraction constant K_{ex} obtained previously [21]; the values of $K_{\text{D,IA}}$ are summarized in Table 2. From the values shown in Table 2, it is noticed that the ion association reaction in the aqueous phase contributed more to the extraction of phenolate (P) and benzoate (BC) anions with TBA^+ than the distribution coefficients, and the contribution of $K_{\text{D,IA}}$ to the extractability of the ion associates increases when the anionic group is changed from $-\text{O}^-$ to $-\text{CO}_2^-$ and $-\text{SO}_3^-$. The contribution of $K_{\text{D,IA}}$ to the extractability also increases by changing the benzene ring to a naphthalene ring. Also, the electron dispersion of an anionic charge and the hydrophobicity of the pairing ions can work favorably towards the improvement of the distribution of the ion associates.

The contribution of a methylene group to the ion associability in an aqueous solution is about 0.06 logarithmic unit of K_{ass} [9,10], while the contribution of the methylene group to the extractability is 0.6 logarithmic unit of K_{ex} [21]. These results suggest that the methylene group contributes largely to the improvement of the distribution reaction of the ion associates, similarly to the nitro group.

4. Conclusions

This study demonstrated a novel usage of CZE in physicochemical studies in an aqueous media, and the proposed analysis method using electrophoretic mobility was used for the reaction analysis in solutions. The study focused on the ion–ion interaction and ion associability. One of the main advantages of the proposed method is that it is applicable to slightly soluble ion associates; analytes can be detected at concentrations of 10^{-5} M or less. By using the ion association constants obtained in aqueous solutions, the ion association extraction equilibria were systematically clarified for the first time in the present study.

Studies on the analysis of ion association equilibria in aqueous solutions utilizing the electrophoretic mobility will be continued and be applied to more complicated ion associates, in order to clarify the importance of ion associability in an aqueous media, such as a biological tissue and an aquatic environment.

Acknowledgements

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References

- [1] A.F. Gomez, Z.L. Avilla, Y-H. Chu, M.G. Whitesides, *Anal. Chem.* 66 (1994) 1785.
- [2] S. Honda, A. Taga, K. Suzuki, K. Kakehi, *J. Chromatogr.* 597 (1992) 377.
- [3] K. Shimura, L.B. Karger, *Anal. Chem.* 66 (1994) 9.
- [4] K.L. Rundlett, D.W. Armstrong, *J. Chromatogr. A* 721 (1996) 173.
- [5] H.S. Harned, B.B. Owen, in: *The Physical Chemistry of Electrolyte Solutions*, 3rd ed, Reinhold, New York, 1964, pp. 70–283.
- [6] C.W. Davies, *Ion Association*, Butterworth, London, 1962.
- [7] G.H. Nancollas, *Interactions in Electrolyte Solutions*, Elsevier, Amsterdam, 1966.
- [8] T. Takayanagi, S. Motomizu, *Chem. Lett.* (1995) 593.
- [9] T. Takayanagi, E. Wada, S. Motomizu, *Analyst* 122 (1997) 57.
- [10] T. Takayanagi, T. Tanaka, S. Motomizu, *Anal. Sci.* 13 (1997) 11.
- [11] C.W. Davies, *Trans. Faraday Soc.* 23 (1927) 351.
- [12] D.A. MacInnes, T. Shedlovsky, *J. Am. Chem. Soc.* 54 (1932) 1429.
- [13] T. Shedlovsky, *J. Franklin Inst.* 225 (1938) 739.
- [14] R.M. Fuoss, T. Shedlovsky, *J. Am. Chem. Soc.* 71 (1949) 1496.
- [15] R.M. Fuoss, D. Edelson, *J. Am. Chem. Soc.* 73 (1951) 269.
- [16] T. Takayanagi, E. Wada, S. Motomizu, *Bunseki Kagaku* 46 (1997) 467.
- [17] T. Takayanagi, E. Wada, S. Motomizu, *Analyst* 122 (1997) 1387.
- [18] E. Wada, T. Takayanagi, S. Motomizu, *Analyst* 123 (1998) 493.
- [19] T. Takayanagi, Y. Ohba, H. Haruki, E. Wada, S. Motomizu, *Anal. Sci.* 14 (1998) 311.
- [20] N. Bjerrum, *Det Kgl. Danske Videnskab. Selskab. Math.-Fys. Medd.* 7 (1926) 1.
- [21] S. Motomizu, *Bunseki Kagaku* 38 (1989) 147.