Focusing Review

# Analysis of Chemical Equilibria in Aqueous Solution Related with Separation Development Using Capillary Zone Electrophoresis

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**Abstract**

Capillary zone electrophoresis has been utilized for the analysis of chemical equilibria in aqueous solution. Electrophoretic mobility of analyte changes along with the addition of interacting reagent in the migrating solution, and precise measurement of the electrophoretic mobility allows analyzing equilibrium reactions. The capillary electrophoretic method including mathematical analyses was validated through complex formation reactions, and it was applied to the investigation of ion association reactions. Various types of interactions have been clarified; contributions of hydrophobicity, probability, and aromatic−aromatic interaction were quantitatively evaluated. Ion association reaction in aqueous solution also elucidated the stepwise reactions of liquid−liquid distribution of ion associates. The technique was further developed to the binding analysis of ions and ion associates to nonionic surfactant micelles.

*Keywords: chemical equilibria, intermolecular interaction, capillary zone electrophoresis, electrophoretic mobility, ion association reaction, binding reaction*

## 1. Introduction

Capillary zone electrophoresis (CZE) has become a popular technique for the analysis of ionic substances. The separation mechanism is based on the differences in electrophoretic mobility of analyte ions, as well as on the electroosmotic flow. Although the separation efficiency in CZE is amazingly high, various types of interacting reagents are often used to control the electrophoretic mobility of analytes, when the resolution among analytes is not sufficient. The interacting reagents used are so−called "modifiers," and various types of inter−molecular interactions have been utilized. Investigation of the modifiers has contributed to establish new analytical systems. Since the nature of molecular interactions utilized in the CZE separation is essentially the chemical equilibria in solution, we can analyze the chemical equilibria by measuring the change in electrophoretic mobility of analytes, *vice versa*.

Journal of Chromatography A has issued a special volume entitled "Estimation of Physicochemical Properties by Chromatographic and Electrophoretic Techniques," this year (2004) [1]. In that issue, various chromatographic techniques were introduced to analyze the properties including liquid−liquid partition, vapor pressure, diffusion coefficients, acid−base, complex−forming, critical micelle concentration, and binding constants. Capillary zone electrophoresis has its predominant characteristics as mentioned above, and the advantages utilized have been introduced for the equilibrium analysis of acid−base, metal complex, and binding affinity of biomolecules [2]. In the CZE analysis of the chemical equilibria, the general procedure includes that the electrophoretic mobility of analyte is measured with a series of migrating solutions containing different concentrations of the interacting reagent, and that the changes in the apparent electrophoretic mobility is mathematically analyzed to give equilibrium constants.

This paper reviews the potential of the CZE techniques for equilibrium analysis in aqueous solution achieved by the present author's group. The method was validated by the complex formation analysis of crown ethers, and was further utilized for the analysis of weak ion−ion interactions in aqueous solution: several interesting factors were elucidated. The ion association reaction in aqueous solution led to understand the stepwise reactions in liquid−liquid distribution of ion−pair extraction. Binding reactions to nonionic surfactant micelle are also interpreted in this review.

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## 2. Utilization of capillary zone electrophoresis for equilibrium analysis in homogeneous solution and its validation

### 2. 1 Change in electrophoretic mobility of analyte by the interaction with modifier

Utilization of modifier additives, including surfactant, has been noticed at the early stage of the CZE separations on the control of electrophoretic mobility of analytes [3, 4]. An interacting reagent, a modifier, is added in the migrating solution at the concentration to be much greater than that of the analytes of interest, so that the concentration of free modifier is only slightly changed by the reaction with the analytes. As shown in Figure 1 (a), analytes in the sample solution, $A^-$ in this case, interact with the modifier, L, during the electrophoretic separation, and such interactions lead to form the associate $LA^-$ accompanying the change in the apparent molecular mass, volume and/or charge of the analyte. Apparent changes in those physicochemical properties are reflected on the apparent electrophoretic mobility of the analyte, $\mu_{ep}$'. The degree of the interaction is different among the analytes; such differences give different apparent electrophoretic mobility values. Consequently, the resolution among the analytes can be improved. Change in the mobility of analyte is due to the interaction with the interacting reagent, and we can analyze the interaction and equilibrium reaction through the measurement of electrophoretic mobility. A series of different concentrations of modifier give corresponding apparent electrophoretic mobility values, as shown in Figure 1 (b), and the set is offered to analyze the equilibrium through mathematical analysis.

Equilibrium analyses through electrophoretic mobility have succeeded in bio–molecules. Protein–sugar interactions [5], antigen–antibody interaction [6, 7], binding of ligand to protein [8], binding of carbohydrates to peptides [9], lectin–sugar interaction [10], and binding of Vancomycin to dipeptides [11] and to precursors [12] have been analyzed. Higher order equilibria, such as aggregation formation [13] and cyclodextrin binding [14], were successfully analyzed. Theoretical approaches [15] and examination of experimental parameters [16, 17] have been developed to optimize the resolution among chiral compounds and isomers.

### 2. 2 Prominent features of the CZE on equilibrium analysis

Capillary zone electrophoresis is based on the migration of ionic substances under a constant electric field, and therefore, we can utilize its characteristics with many advantages. It can be noticed at first that the CZE includes electrophoretic separation and that any pure analytes are not necessary. When a sample solution contains several analytes, simultaneous analysis can be performed in a series of the measurements. Coexisting substances, which do not affect electrophoretic mobility of analyte and/or equilibrium reaction, are also allowed to exist in the migrating solution. Measurement of migration time of analytes from sample injection point to a detector is an essential piece of information given by the mobility measurement in CZE, and therefore, various types of detection methods are available; photometric, fluorometric, and conductometric detections can be chosen depending on the physical properties of the objective analytes. Other detection methods under development are also applicable to the measurement of electrophoretic mobility. Depending on the detection system, the CZE method allows the concentration of analyte to be reduced to a very low concentration ( ~$10^{-6}$ M). When the analyte of interest give even tiny

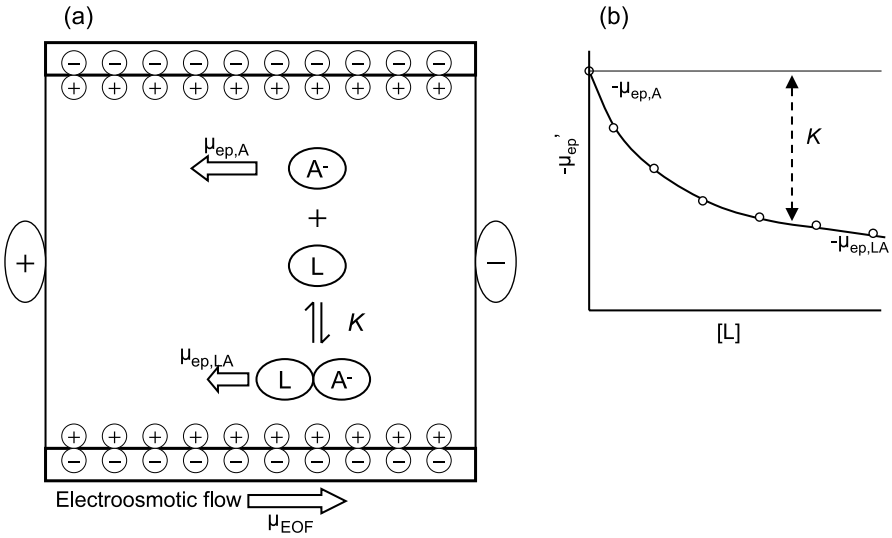



**Figure 1.** Schematic illustration for mobility change in capillary zone electrophoresis by equilibrium reaction during electrophoretic migration. An anion ($A^-$) is used as analyte ion and neutral substance (L) as an interacting reagent present in the migrating solution. (a) Electrophoretic mobility of anion ($\mu_{ep,A}$) is apparently reduced to $\mu_{ep,LA}$ by the equilibrium reaction, where $K$ is an equilibrium constant. (b) Degree of formation of LA by the equilibrium is reflected on the apparent electrophoretic mobility of $A^-$ ($-\mu_{ep,A}$').

signal, we can use its migration time for the equilibrium analysis. Low concentration of analytes can exclude the formation of insoluble precipitates, which is often troublesome on general analysis.

### 2. 3 Validation of the analysis through the complex formation of crown ethers with alkali metal ions

Although the capillary zone electrophoretic method can determine various types of equilibrium constants through measurements of electrophoretic mobility, as mentioned above, the method should be validated to establish the reliability. Crown ethers are famous cyclic polyether and they bind alkali and alkaline earth metal ions in its "cavity hole," depending on the size. The reactivity of 18–crown–6 with alkali metal ions in various solvents is well established in details [18], and the complex formation reaction was adopted as a model one to validate the equilibrium analysis by CZE; the reaction and its equilibrium constants are presented in Eqs. (1) and (2).

$$M^+ + L \rightleftharpoons ML^+ \quad (1)$$

$$K_{ML} = \frac{[ML^+]}{[M^+]\,[L]} \quad (2)$$

where $M^+$, L, and $ML^+$ are metal ion, crown ether and the complex, respectively, and $K_{ML}$ is an equilibrium constant. Crown ethers, such as dibenzo–18–crown–6 (DB 18 C 6) and benzo–18–crown–6 (B 18 C 6), are essentially neutral, and they become cationic through the incorporation of alkali and alkaline earth metal ions [19, 20]. The crown ethers were used as analytes, and some alkali metal salt as interactive reagent is present in the migrating solution. Along with the increase in the concentration of alkali metal ion, the crown ether becomes a cationic complex and the apparent electrophoretic mobility of the crown ether increases as is shown in Figure 2. The complex formation constants are determined through the mobility change by analyzing it mathematically.

While some mathematical approaches have been proposed to analyze the equilibrium reactions, handling with the electrophoretic

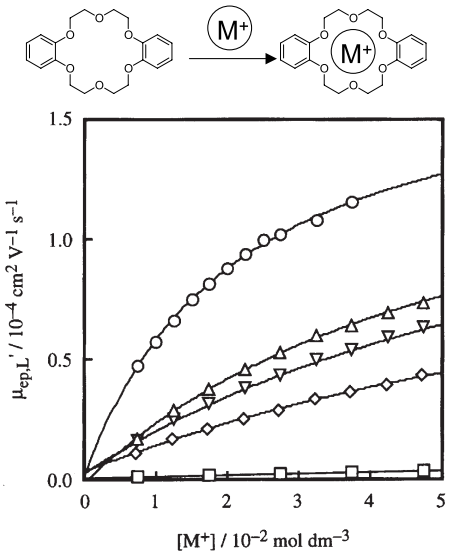



**Figure 2.** Changes in electrophoretic mobility of dibenzo–18–crown–6 by complex formation reaction with alkali metal ions. $M^+$ : □, $Li^+$ ; △, $Na^+$ ; ○, $K^+$ ; ▽, $Rb^+$ ; ◇, $Cs^+$. Positive electrophoretic mobility is observed for essentially neutral crown ethers by the reaction with alkali metal ion. Non–linear least–squares analysis gave complex formation constants in aqueous solution, as are listed in Table 1. See details in Ref. 20.

mobility is more convenient than with the migration time; *i.e.,* migration time includes experimental parameters such as capillary length and applied voltage. The migration time also contains the contribution of electroosmotic flow. Several linear analyses have been proposed and have succeeded on various types of equilibrium analysis [21, 22]. The linear analyses, however, are applicable to such reactions as to give sufficient change in the mobility; some experimentally obtained data is used as constants, and in such cases, they must be obtained very correctly. Thus, such linear analyses possess their unavoidable limitations, as long as one handles experimental data as constants. Non–linear analysis methods are more reliable for handling small changes in electrophoretic mobility and weak interactions. Schwarz *et al.* used a fitted function

**Table 1.** Complex formation constants (log $K_{ML}$) of crown ethers

| Crown ether | $M^+$ | | | | |
|---|---|---|---|---|---|
| (L) | $Li^+$ | $Na^+$ | $K^+$ | $Rb^+$ | $Cs^+$ |
| DB 18 C 6[1)] | (–0.3 ± 0.2) | 1.18 ± 0.02 | 1.67 ± 0.02 | 1.14 ± 0.03 | 0.96 ± 0.06 |
| DB 18 C 6[2)] | 0 | 1.16 | 1.67 | 1.08 | 0.83 |
| DB 18 C 6[3)] | — | 4.4 | 5.0 | 4.2 | — |
| 18 C 6[4)] | — | 0.8 | 2.03 | 1.56 | 0.99 |
| B 18 C 6[5)] | — | 1.38 | 1.744 | 1.15 | (0.88) |

1) Determined through CZE. Error: 3 σ 2) Reported values determined by spectrophotometry. 3) Values in methanol as a medium. 4) Reported values for 18–crown–6. 5) Reported values for Benzo–18–crown–6.

for the analysis of aggregate formation [13], and we adopted a non−linear least−squares analysis [23]. Havel and Janos developed a Fortran 77−based CELET program to determine equilibrium constants [24]. Prominent features in those non−linear analyses are: all experimental data are handled equivalently, and the electrophoretic mobility of species can be also optimized through the analysis.

The complex formation constants determined by using non−linear least−squares analysis are summarized in Table 1 [20, 25]; they agreed very well with the reported values. In addition to the reliability of the analysis, the CZE method has offered its superiority. Solubility of DB 18 C 6 in water is as low as 1.3 x $10^{-5}$ M, and it was sometimes troublesome on precise equilibrium analysis. The CZE analysis, however, the concentration of DB 18 C 6 is allowed to be less than $10^{-5}$ M, and we can get rid of the solubility problem owing to the photometric detection sensitivity.

When less UV−absorptive 18−crown−6 was subjected, the crown ether was added in the migrating solution as a modifier, and the electrophoretic mobility of alkali metal ions was monitored using indirect photometric reagent [26]. Fair results were obtained through the mobility analysis, and the equilibrium constants obtained agreed well with the reported values.

### 2. 4 Analysis of acid−base property of Phenolphthalein

Acid−base reaction is a popular equilibrium to analyze it by CZE. We only change the pH of the migrating solution, and the mobility measurement gives acid dissociation constants (p$K_a$). Many studies have been reported on acid dissociation reaction by using CZE analysis [27]. We noticed the separation features in CZE besides the mobility change, and applied the analysis to an unstable substance in an aqueous solution.

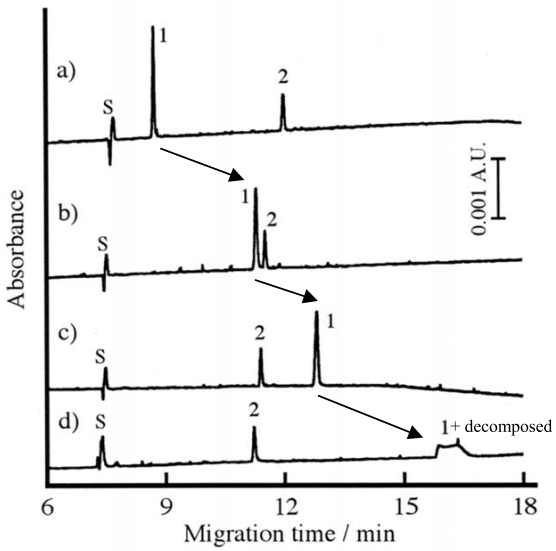



**Figure 3.** Electropherograms for Phenolphthalein and Methyl Orange at different pH conditions. pH: a) 8.64; b) 9.42; c) 9.81; d) 11.60. Signals: 1, Phenolphthalein; 2, Methyl Orange (mobility standard); S, ethanol (EOF marker). See detail conditions in Ref. 30.

Phenolphthalein is one of the most famous acid−base indicators, but its property has not been known in details, even textbooks still contain confused data. It is because Phenolphthalein is unstable in aqueous alkaline media and gradually decomposes [28, 29]. Compared with the classical photometric method, the CZE method has a prominent advantage that gradually decomposing species is separated from equilibrium species of interest during electrophoretic separation (Figure 3). Thus, the two steps of acid dissociation reaction of phenolphthalein were firstly clarified in detail; values for p$K_{a1}$ and p$K_{a2}$ are 8.84 and 9.40, respectively [30]. Comparison of the results with other pH indicators validated the values.

## 3. Equilibrium analysis of ion association reaction in aqueous solution

### 3. 1 Capillary zone electrophoresis as a powerful tool to investigate ion association reactions

Interactions between organic ions have widely been used in the research field of analytical chemistry by coupling it with distribution to hydrophobic media, including ion−pair solvent extraction, ion−pair reversed−phase high performance liquid chromatography, *etc.* [31]. The interaction between organic ions in aqueous solution has been discussed through the hydrophobicity of ions, as well as the electrostatic interactions. While electrostatic interaction works strongly between small ions in the solvents with low dielectric constants, the hydrophobic one works with relatively large ions in the solvents with high dielectric constants such as water. It goes without saying that electrostatic interaction has been established through conductivity measurements. However, hydrophobic (organic) ion associates easily precipitate in aqueous solution, and we did not have available tool to utilize the reaction in aqueous phase and to analyze the equilibrium, until the potential of capillary zone electrophoresis was thoroughly noticed.

Capillary zone electrophoresis has prominent feature that the electrophoretic separation is performed in homogeneous solution. Terabe and Isemura used cationic polyelectrolytes to resolve anionic analytes by utilizing ion−ion interactions [32, 33]. Quaternary ammonium ions have also been used to improve the resolution among isomers by utilizing the ion association reaction in aqueous solution [34]. Important questions may arise how the selectivity emerges and what kind of interactions work on ion−ion interactions in aqueous media. Ion−pair solvent extraction could give the ion association constant in aqueous solution, but the reliability was too inferior [35]. Capillary zone electrophoresis can handle the concentration of ion associate as low as $10^{-5}$ M level or less, and ion association reactions in an aqueous solution can be directly analyzed through the change in electrophoretic mobility of analytes. Figure 4

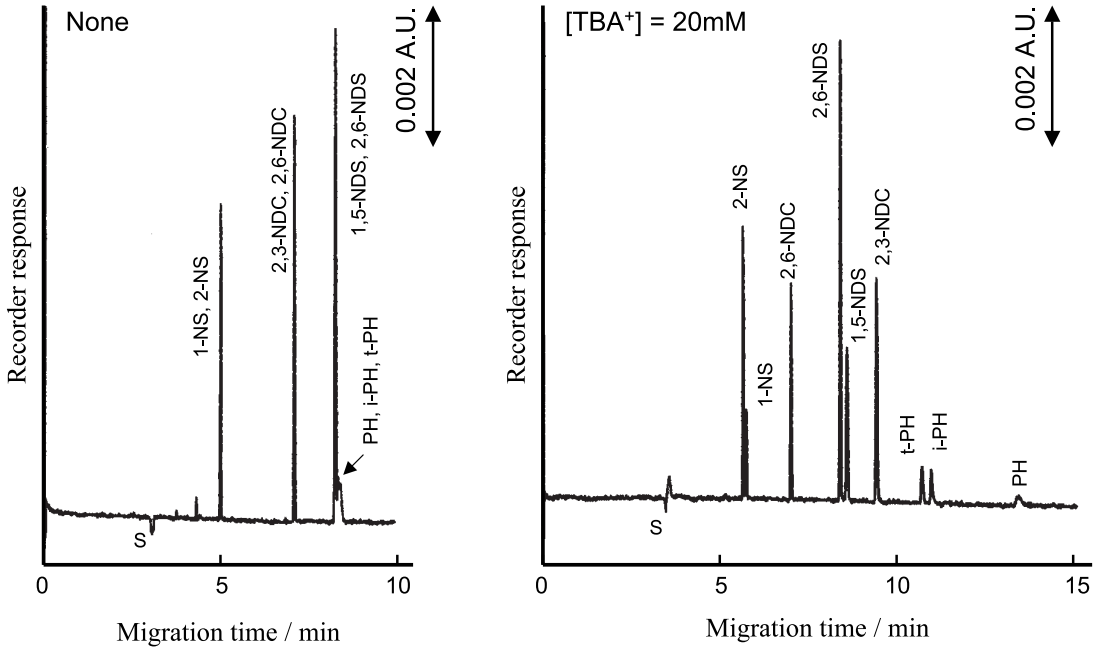



**Figure 4.** Electropherograms for aromatic anions in the absence and presence of ion association reagent. In the presence of $TBA^+$, the resolutions among isomers are developed. See details in Ref. 23.

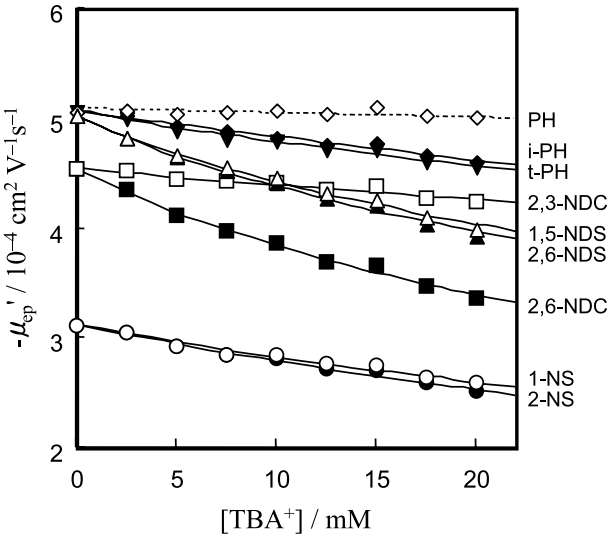



**Figure 5.** Changes in electrophoretic mobility of aromatic anions with increasing concentrations in $TBA^+$. See details in Ref. 23.

shows a typical separation improvement for 11 aromatic anions by using tetrabutylammonium ion ($TBA^+$) as an ion association reagent [23]. Electrophoretic mobility of the analyte anions decreased with increasing concentration of $TBA^+$ in the migrating solution (Figure 5). We first determined the ion association constants using linear analysis [36], and the reliability and the accuracy were much developed by using non−linear least−squares analysis [23]. The interactions discovered are summarized in the following section. The equilibrium reaction and its constant are represented in Eqs. (3) and (4), respectively, unless noticed.

$$C^+ + A^- \rightleftharpoons C^+A^- \quad (3)$$

$$K_{ass} = \frac{[C^+A^-]}{[C^+]\,[A^-]} \quad (4)$$

where $C^+$, $A^-$, and $C^+A^-$ are pairing cation, pairing anion, and 1:1 ion associate, and $K_{ass}$ is ion association constant.

### 3. 2 Factors contributing to the ion associability in aqueous solution

Electrostatic interaction in solution is a classical one, and the interaction is well known and established in details. Smaller ions attract with each other, and the interaction is weakened by solvation. The electrostatic interaction works weakly in high dielectric−constant media such as water, because those solvents solvate and their inter−ionic distance is lengthened to be than their crystal ionic radii, as seen in Stokes' radii. Electrostatic interaction between aromatic anions and primary to quaternary ammonium ions was examined by capillary zone electrophoresis [37]. An evident trend in ion associability was observed: from electrostatic interactions with primary ammonium ions to hydrophobic interactions with quaternary ammonium ions. In most aromatic anions, minimum ion association constants were obtained with secondary ammonium ions. Reversal of migration order among anionic isomers was also observed, along with the change in ion associability. The phenomenon is very useful for designing separation systems; we can choose the interacting reagent and its interactions for developing separation selectivity.

Ion association constants ($K_{ass}$) of aromatic anions were determined with different sizes of quaternary ammonium ions to establish the concept on hydrophobic interactions [23, 38, 39]. A linear free energy relationship (LFER) was examined with the increase in the size of quaternary ammonium ions; the carbon number of alkyl chains in quaternary ammonium ion was used as an index of hydrophobicity. Logarithmic value of $K_{ass}$ increased linearly with increasing bulkiness/carbon number of quaternary ammonium ion (Figure 6). The mean slope, 0.06, represents the contribution of hydrophobicity per one carbon atom of quaternary ammonium ions in aqueous solution. The contribution, 0.06 in logarithmic unit, is found to be about one−tenth of that obtained in liquid − liquid distribution system of ion associates, 0.6 [31].

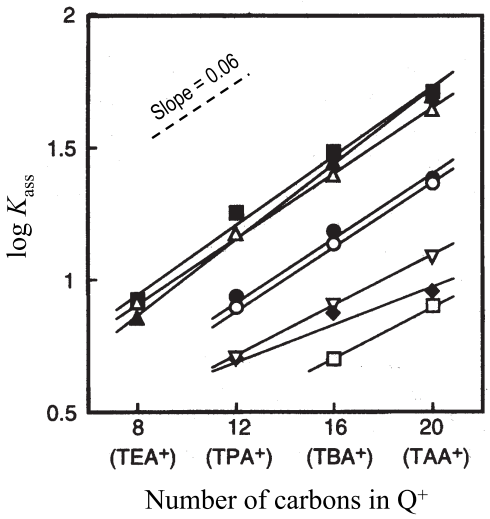



**Figure 6.** Linear free energy relationship between ion association constants and the carbon number of quaternary ammonium ions ($Q^+$) as an index of hydrophobic parameter. Symbols correspond to individual aromatic anions. The mean slope, 0.06, is attributed to the contribution of hydrophobicity in ion association reaction in an aqueous medium (Ref. 23).

The $K_{ass}$ values are compared based on the number of charge. Divalent anions are found to have large $K_{ass}$ values than monovalent ones by the order of about 0.3 in logarithmic units, which means that divalent anions are twice as associable as monovalent anions (log 2 = 0.3) [40].

Divalent quaternary ammonium ions are also designed to utilize multipoint interactions, and the distance between two ammonium sites is controlled with the chain length of methylene group [41]. Ion association constants were determined and ion associability was discussed on the basis of intramolecular ion−ion distance. Ion associability was proved to be contributed from fitted distance between intramolecular ionic sites and hydrophobicity of the methylene group.

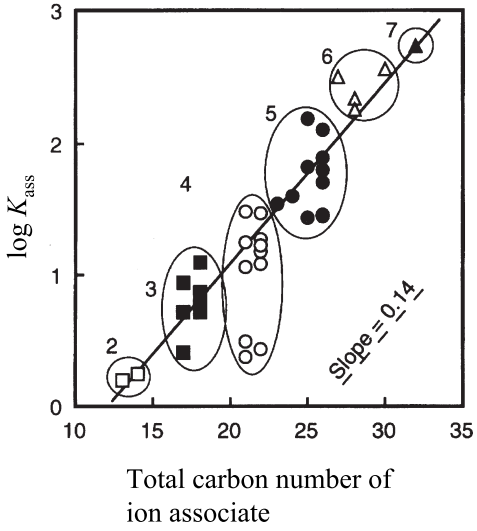
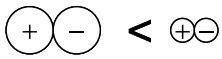
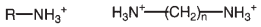
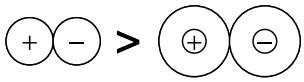
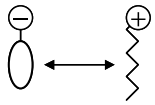
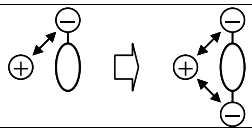
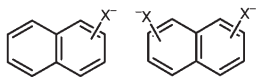
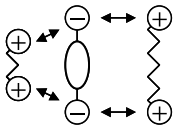
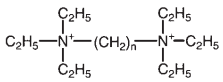
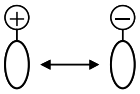
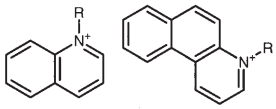
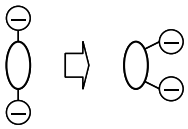
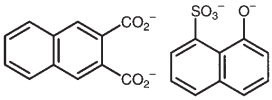



**Figure 7.** Linear free energy relationship between ion association constants and carbon number of ion associates formed between aromatic cations and anions. The mean slope, 0.14, contains the contribution of hydrophobicity, and the contribution of aromatic − aromatic interaction is deduced to be 0.08 for log $K_{ass}$ (Ref. 42).

Aromatic−aromatic interaction also plays an important role in ion associability in aqueous solution [42]. Ion association constants between aromatic ions are determined and plotted against the number of carbon atom on the ion associate, as is shown in Figure 7. A typical interaction was observed with anthracene ring − pyrene ring; the ion association constant between 1−propylbenzo[f]quinolinium ion and 1−pyrenecarboxylate ion reached to $10^{2.74}$, while the ion associate became composed of a monovalent cation and an anion. Higher ion associability provides that low concentration of ion association reagent can realize sufficient resolution among analytes. Such operating conditions reduce the electric current and Joule heat, and stabilize the migration time. The linear free energy relationship provided the increase in $K_{ass}$ values by $10^{0.14}$ per one aromatic carbon. Considering that the hydrophobicity of carbon atom is contributing to the ion associability by $10^{0.06}$, one can estimate the contribution of aromatic−aromatic interaction to ion associability to be $10^{0.08}$. Aromatic−aromatic interaction was also revealed with divalent viologen cations; hydrophobicity, multipoint interaction, and aromatic−aromatic interaction are synergistically utilized with the viologen cations [43].

Factors clarified for ion association reactions in aqueous solution are summarized in Table 2. The present author reviewed the investigation of ion association reaction through CZE in detail [44, 45].

**Table 2.** Intermolecular ion–ion interactions found to work in an aqueous solution

| Interactions | Detail | Schematic representation of ion associability | Typical reagents |
|---|---|---|---|
| Electrostatic | Classically known interaction |  |  |
| Solvation | Hydrated ion: Stokes' radius |  | |
| Hydrophobic | Works between hydrophobic ions<br>$\Delta\log K_{ass}$ = 0.06 per one methylene moiety |  |  R = $CH_3$-, $C_2H_5$-, $C_3H_7$-, $C_4H_9$-, $C_5H_{11}$- |
| Probability | Divalent ions are twice as associable as monovalent ones.<br>$\Delta\log K_{ass}$ = 0.3 (log 2) |  |  |
| Multipoint | Recognition of intramolecular ionic distance |  |  |
| Aromatic - aromatic | Stacking of π electrons |  |  |
| Charge density and polarity | Positively contributes to electrostatic ion associates<br>Negatively contributes to hydrophobic ion associates |  |  |

### 3. 3 Stepwise aspects of ion association reaction in aqueous solution on liquid–liquid extraction and its CZE analysis

Ion–pair extraction has widely been used in analytical and separation sciences. The stepwise reaction of ion–pair extraction is conveniently expressed with formation of electrically neutral ion–associate in aqueous solution and distribution of the ion–associate to water–immiscible organic phase, as is illustrated in Figure 8. The extraction reaction is represented by Eq. (5):

$$K_{ex} = \frac{[C^+A^-]_{(o)}}{[C^+]\,[A^-]} = K_{ass} \times K_{D,IP} \qquad (5)$$

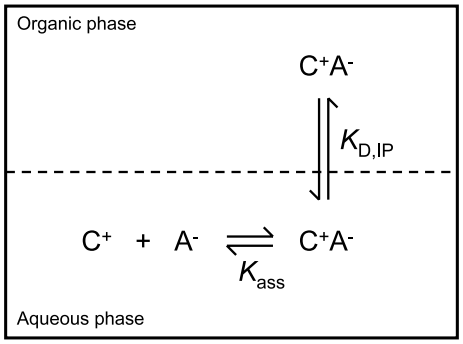



**Figure 8.** Ion–pair extraction of simple 1:1 ion associate into organic phase. The extraction constant, $K_{ex}$, comes from the product of $K_{ass}$ and $K_{D,IP}$.

where $C^+$, $A^-$, and $C^+A^-$ are pairing cation, pairing anion, and 1:1 ion associate, and $K_{ex}$, $K_{ass}$, and $K_{D,IP}$ are extraction constant, ion association constant, and distribution coefficient of ion associate between aqueous and organic phases. Subscript (o) denotes that the species exists in the organic phase. It can be easily understood from Eq. (5) that the extraction constant simply consists from the product of $K_{ass}$ and $K_{D,IP}$. Once the ion association reaction in aqueous solution is clarified by mobility measurements in CZE, the extraction constant can easily be divided into two stepwise reactions of $K_{ass}$ and $K_{D,IP}$. We have determined stepwise reactions of $K_{ass}$ and $K_{D,IP}$ for ion associates of tetrabutylammonium ion – phenolate and benzoate ions by using $K_{ex}$ values determined by solvent extraction and $K_{ass}$ values determined by CZE [40, 46, 47]. The equilibrium constants are summarized in Table 3. Both $K_{ass}$ and $K_{D,IP}$ values increase with increasing molecular volume, and consequently the extraction constant, $K_{ex}$, increases. However, the magnitude in the increase is larger in $K_{D,IP}$ than $K_{ass}$, and the distribution process is found to contribute effectively. The results well agree to the fact that contribution of hydrophobicity in $K_{ass}$ is 1/10 of that in $K_{ex}$.

Alkali metal ions can be extracted into organic phase by using dibenzo–18–crown–6 (DB 18 C 6) and picrate ion ($Pic^-$), as illustrated in Figure 9. The entire extraction reaction ($K_{ex}$) involves four stepwise reactions ($K_{D,L}$, $K_{ML}$, $K_{MLX}$, and $K_{D,MLX}$). The extraction constant is expressed as in Eq. (6).

**Table 3.** Equilibrium constants concerning liquid–liquid distribution of ion associates between an aqueous and a chloroform phase*

| *Anion* | log $K_{ass}$ | log $K_{D,IP}$ | log $K_{ex}$** |
|---|---|---|---|
| **Phenolate ions** | | | |
| Phenolate | 0.40 | –0.70 | –0.30 |
| o–Nitrophenolate | 0.64 | 1.53 | 2.17 |
| m– Nitrophenolate | 0.60 | 1.09 | 1.69 |
| p– Nitrophenolate | 0.80 | 1.72 | 2.52 |
| 2, 4– Dinitrophenolate | 0.80 | 3.21 | 4.01 |
| 2, 6– Dinitrophenolate | 0.68 | 3.30 | 3.98 |
| 2, 4, 6–Trinitrophenolate | 0.67 | 4.86 | 5.53 |
| **Benzoate ions** | | | |
| Benzoate | 0.69 | –0.01 | 0.68 |
| p–Nitrobenzoate | 0.55 | 1.12 | 1.67 |
| **Benzenesulfonate ions** | | | |
| Benzenesulfonate | 0.69 | 1.03 | 1.72 |
| p–Nitrobenzenesulfonate | 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 |

* Tetrabutylammonium ion was used as a pairing cation.
** Reference values.

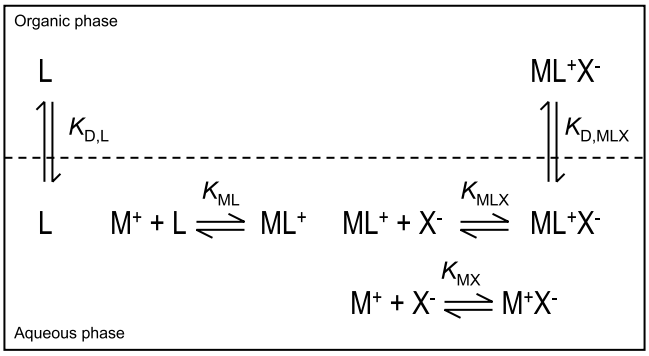



**Figure 9.** Ion–pair extraction of alkali metal ions ($M^+$) with DB 18 C 6 (L) and hydrophobic anion ($X^-$). The overall extraction constant, $K_{ex}$, consists of $K_{D,L}$, $K_{ML}$, $K_{MLX}$, and $K_{D,MLX}$ (Ref. 20).

$$K_{ex} = \frac{K_{ML} \times K_{MLX} \times K_{D,MLX}}{K_{D,L}} \quad (6)$$

Complexation analysis provides the $K_{ML}$ value, and values of $K_{ex}$ and $K_{D,L}$ can be determined through solvent extraction study. However, it was impossible to resolve $K_{MLX}$ and $K_{D,MLX}$ values experimentally. We have succeeded in the direct analysis of ion–pair formation reaction between alkali metal–crown ether complexes and pairing anions in aqueous solution ($K_{MLX}$) using the CZE method, the successive equilibrium constants have been determined for the first time [20]; they are summarized in Table 4. Extraction selectivity for potassium ion against sodium ion was also evaluated by comparing the stepwise reactions. It can be seen in Table 4 that distribution of ion associate, M–DB 18 C $6^+$ – $Pic^-$, between aqueous and benzene phases is predominant on extraction selectivity. The selectivity study was further carried out with benzo–18–crown –6 [48] and 18–crown–6 [49].

## 4. Binding reaction to nonionic surfactant micelle

### 4. 1 Binding analysis of aromatic anions to nonionic surfactant micelle

Since the development of Micellar Electrokinetic Chromatography by Terabe *et al.* [3,4], surfactant micelles have been utilized in capillary electrophoresis as a pseudo–stationary phase. Non–ionic surfactant micelles were found to modify the resolution of ionic substances by Matsubara *et al.* [50]. We performed binding analysis of aromatic anions to the nonionic surfactant micelle [51]; the binding reaction is illustrated in Figure 10. The electrophoretic mobility of aromatic anions decrease with increasing concentration of nonionic surfactant, resolution among positional isomers was developed. Binding constants to nonionic surfactant micelle were determined using the mobility change and aggregation number of the surfactant. The binding constants were validated through fluorometry. Because the binding reaction includes distribution to hydrophobic media, less charged ions are more likely to be bound to the micelle. Different types of surfactant micelles are examined based on hydrophilicity – lipophilicity balance (HLB) and aggrega-

**Table 4.** Equilibrium constants related to ion–association extraction of alkali metal ions

| Crown ether | Pairing anion | Constant | $M^+$ | | | | | |
|---|---|---|---|---|---|---|---|---|
| (L) | ($X^-$) | | $Li^+$ | $Na^+$ | $K^+$ | $Rb^+$ | $Cs^+$ | |
| DB 18 C 6 | picrate | log $K_{ex}$ [1)] | — | 2.21 | 4.65 | 3.75 | 3.07 | |
| DB 18 C 6 | | log $K_{D,L}$ [3)] | | | | | | 2.9 |
| DB 18 C 6 | | log $K_{ML}$ [4)] | –0.3 [2)] | 1.24 ± 0.02 | 1.67 ± 0.02 | 1.07 ± 0.02 | 0.94 ± 0.02 | |
| DB 18 C 6 | picrate | log $K_{MLX}$ [4)] | — | 2.23 ± 0.29 | 1.95 ± 0.10 | — | — | |
| DB 18 C 6 | picrate | log $K_{D,MLX}$ [5)] | — | 1.7 | 3.9 | — | — | |

1) Ion–association extraction constants with picrate ion to benzene. 2) Estimated values. 3) Reported value. 4) Determined through CZE. Error: 3 σ 5) Distribution coefficient of ion associate, $ML^+X^-$, calculated by $K_{D,MLX} = (K_{ex} \times K_{D,L}) / (K_{ML} \times K_{MLX})$.

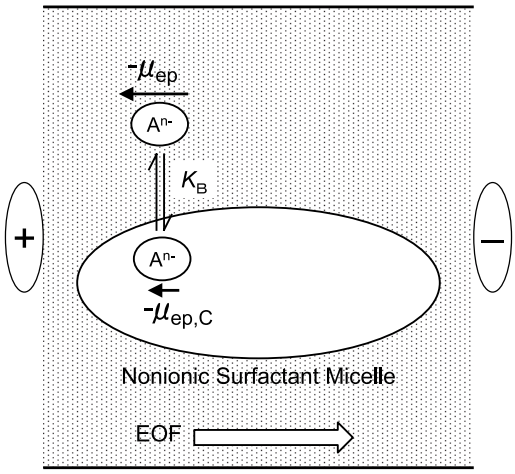



**Figure 10.** Binding reaction of anionic species to nonionic surfactant micelle in connection with the change in electrophoretic mobility of the analyte anions (Ref. 51).

tion number. More hydrophobic micelles bind aromatic anions strongly.

### 4.2 Binding analysis of ion associates to nonionic surfactant micelle

Binding of ion associates to nonionic surfactant micelle (Figure 11) was also explored [52]. As can be seen in the scheme, the reaction is realized in the presence of both ion association reagent and nonionic surfactant micelle, and three equilibria take part in the entire system. Ion association reaction ($K_{ass}$) and binding reaction of anion ($K_B$) were separately examined in the presence of either ion association reagent or nonionic surfactant micelle, and the equilibrium constants obtained were used for the determination of binding constant of ion associates ($K_{B,IA}$).

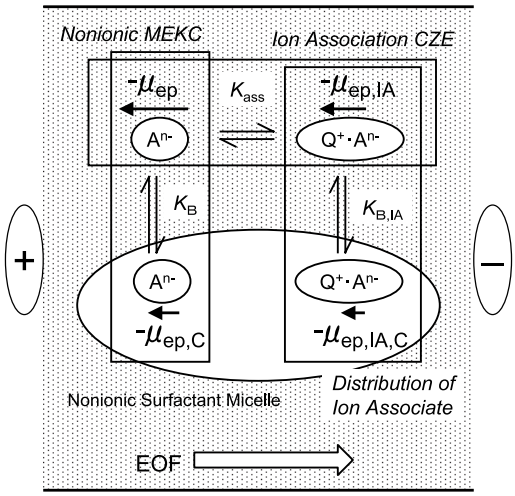



**Figure 11.** Schematic representations for ion–association nonionic micellar electrokinetic chromatography. Ion association reaction in aqueous solution, binding of anion to nonionic surfactant micelle, and binding of ion associate to nonionic surfactant micelle work cooperatively (Ref. 52).

Since the charge of aromatic anion is reduced by ion association reaction, $K_{B,IA}$ as ion associate gave larger values than $K_B$ for each aromatic anion. The results can be explained from distribution phenomena that less charged species are more likely distributed to hydrophobic media. In the comparison among aromatic anions, the $K_{B,IA}$ values for such ion associates as are formed from monovalent anions and pairing cations are larger than those formed from divalent anions and pairing cations. The results also agree with the distribution phenomena that electrically neutral or less charged species are more likely bound to hydrophobic media than more charged ones, while divalent anions are more associable with pairing cations than monovalent anions. The ion–association – micelle distribution CZE is also proved to be useful to develop separation systems.

### 4.3 Binding analysis of phenols to nonionic surfactant micelle

When binding of nonionic substances, such as phenol, to nonionic surfactant micelle is subjected to analyze, direct measurement of the binding reaction is impossible by the electrophoretic method. It is because both charges of the neutral analyte and the bounded species are zero and we cannot observe any change in electrophoretic mobility in a usual measurement. In such case, change in acid dissociation constant is utilized for the equilibrium analysis, as is shown in Figure 12. Apparent acid dissociation constant, $K_a$', should become larger in the presence of NIS micelles, because neutral species would be more easily bound to the micelle than the anionic species [53]. Binding constant of phenols, $K_{B,HA}$, has the relationship with $K_a$ in aqueous solution, $K_a$' in the presence of micelle, and binding constant of anions ($K_{B,A}$), as is written in Eq. (7).

$$K_{B,HA} = \frac{K_a}{K_a'} (1 + K_{B,A}) - 1 \qquad (7)$$

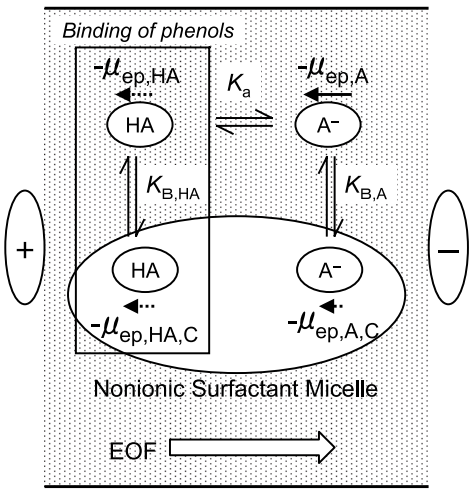



**Figure 12.** Schematic representations for binding of phenol derivatives to nonionic surfactant micelles (Ref. 53).

When $K_a$' value is determined experimentally at certain conditions, we can determine $K_{B,HA}$ values by using Eq. (7) and the known values of $K_a$ and $K_{B,A}$. From the comparison of the equilibrium constants, $K_{B,HA}$ values are larger than the corresponding $K_{B,A}$ values in the order of 0.22 – 0.96 in logarithmic units. The hydrophobic concept of micelle is also applied in this case: less charged neutral species is more likely bound to the micelle. It can be also noticed from the comparison that $K_{B,HA}$ values are slightly larger than $K_{B,IA}$ values, which means that ion associates are not completely neutral as in the protonated species, but are in the state where the positive and negative charges remain in the ion associates.

## 5. Conclusion

The fundamental criterion in affinity capillary electrophoresis is the control of electrophoretic mobility of analytes by using interacting reagent in the migrating solution. We have performed various types of equilibrium analysis through the electrophoretic mobility in capillary zone electrophoresis by utilizing its prominent features. Various types of molecular interactions have been clarified in ion association reactions, in addition to the classical electrostatic interaction. Exploration of ion association reaction in aqueous solution allowed us to investigate the stepwise reactions in two −phase distribution phenomena including liquid−liquid distribution of ion associates. Nonionic surfactant micelle as hydrophobic matrix was elucidated through binding analysis of anions, ion associates, and nonionic substances, as well as its utilization as separation modifier. When one is aware that molecular interactions are widely used in separations and separation instruments, the equilibrium can further be analyzed through physicochemical properties easily.

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## References

[1] Poole, C. F. Ed.; *Estimation of Physicochemical Properties by Chromatographic and Electrophoretic Techniques; J. Chromatogr. A,* **2004**, Volume 1037.

[2] Janos, P. *J. Chromatogr. A,* **1999**, *834,* 3−20.

[3] Terabe, S.; Otsuka, K.; Ichikawa, K.; Tsuchiya, A.; Ando, T. *Anal. Chem.,* **1984**, *56,* 111−113.

[4] Terabe. S.; Otsuka, K.; Ando, T. *Anal. Chem.,* **1985**, *57,* 834−841.

[5] Honda, S.; Taga, A.; Suzuki, K.; Suzuki, S.; Kakehi, K. *J. Chromatogr.,* **1992**, *597,* 377−382.

[6] Shimura, K.; Karger, B. L. *Anal. Chem.,* **1994**, *66,* 9−15.

[7] Mammen, M.; Gomez, F. A.; Whitesides, G. M. *Anal. Chem.,* **1995**, *67,* 3526−3535.

[8] Gomez, F. A.; Avila, L. Z.; Chu, Y.−H.; Whitesides, G. *Anal. Chem.,* **1994**, *66,* 1785−1791.

[9] Heegaard, N. H. H.; Robey, F. A. *Anal. Chem.,* **1992**, *64,* 2479−2482.

[10] Kuhn, R.; Frei, R.; Christen, M. *Anal. Biochem.,* **1994**, *218,* 131−135.

[11] Carpenter, J. L.; Camilleri, P.; Dhanak, D.; Goodall, D. *J. Chem. Soc., Chem. Commun.,* **1992**, 804−806.

[12] Liu, J.; Volk, K. J.; Lee, M. S.; Pucci, M.; Handwerger, S. *Anal. Chem.,* **1994**, *66,* 2412.

[13] Schwarz, M. A.; Neubert, R. H. H.; Ruttinger H. H. *J. Chromatogr. A,* **1996**, *745,* 135−143.

[14] Bowser, M. T.; Chen, D. D. Y. Anal. Chem., **1998**, *70,* 3261 −3270.

[15] Wren, S. A. C.; Rowe, R. C. *J. Chromatogr.,* **1992**, *603,* 235−241.

[16] Nielen, M. W. F. *J. Chromatogr.,* **1991**, *542,* 173−183.

[17] Ingelse, B. A.; Claessens, H. C.; Wal, S.; Duchateau, A. L. L.; Everaerts, F. M. *J. Chromatogr. A,* **1996**, *745,* 61−71.

[18] Izatt, R. M.; Bradshaw, J. S.; Nielsen, S. A.; Lamb, J. D.; Christensen, J. J.; Sen, D. *Chem. Rev.,* **1985**, *85,* 271−339.

[19] Okada, T. *J. Chromatogr. A,* **1995**, *695,* 309−317.

[20] Takayanagi, T.; Iwachido, T.; Motomizu, S. *Bull. Chem. Soc. Jpn.,* **1998**, *71,* 1373−1379.

[21] Rundlett, K. L.; Armstrong, D. W. *J. Chromatogr. A,* **1996**, *721,* 173−186.

[22] Rundlett, K. L.; Armstrong, D. W. *Electrophoresis,* **2001**, *22,* 1419−1427.

[23] Takayanagi, T.; Wada, E.; Motomizu, S. *Analyst* **1997**, *122,* 57−62.

[24] Havel, J.; Janos, P. *J. Chromatogr. A,* **1997**, *786,* 321−331.

[25] Manege, L. C.; Takayanagi, T.; Oshima, M.; Iwachido, T.; Motomizu, S. *Bull. Chem. Soc. Jpn.,* **1999**, *72,* 1301−1306.

[26] Takayanagi, T.; Motomizu, S. *Chem. Lett.,* **1999**, 523−524.

[27] Bruzzoniti, M. C.; Mentasti, E.; Sarzanini, C. *J. Chromatogr. B,* **1998**, *717,* 3−25.

[28] Ozeki, T.; Morikawa, H.; Kihara, H. *Bunseki Kagaku,* **1993**, *42,* 887−894.

[29] Tamura, Z.; Abe, S.; Ito, K.; Maeda, M. *Anal. Sci.,* **1996**, *12,* 927−930.

[30] Takayanagi, T.; Motomizu, S. *Chem. Lett.,* **2001**, 14−15.

[31] Motomizu, S. *Bunseki Kagaku,* **1999**, *48,* 151−181.

[32] Terabe, S.; Isemura, T. *Anal. Chem.,* **1990**, *62,* 650–652.

[33] Terabe, S.; Isemura, T. *J. Chromatogr.,* **1990**, *515,* 667–676.

[34] Nashabeh, W.; Rassi, Z. E. *J. Chromatogr.,* **1991**, *536,* 31–42.

[35] Motomizu, S.; Toei, K.; Iwachido, T. *Bull. Chem. Soc. Jpn.,* **1969**, *42,* 1006–1010.

[36] Takayanagi, T.; Motomizu, S. *Chem. Lett.,* **1995**, 593–594.

[37] Takayanagi, T.; Wada, E.; Oshima, M.; Motomizu, S. *Bull. Chem. Soc. Jpn.,* **1999**, *72,* 1785–1791.

[38] Takayanagi, T.; Tanaka, H.; Motomizu, S. *Anal. Sci.,* **1997**, *13,* 11–18.

[39] Takayanagi, T.; Ban, N.; Takao, H.; Nakamura, N.; Oshima, M.; Motomizu, S. *Anal. Sci.,* **1999**, *15,* 593–596.

[40] Takayanagi, T.; Wada, E.; Motomizu, S. *Bunseki Kagaku,* **1997**, *46,* 467–475.

[41] Takayanagi, T.; Wada, E.; Motomizu, S. *Analyst,* **1997**, *122,* 1387–1391.

[42] Takayanagi, T.; Ban, N.; Wada, E.; Oshima, M.; and Motomizu, S. *Bull. Chem. Soc. Jpn.,* **2000**, *73,* 669–673.

[43] Wada, E.; Takayanagi, T.; Motomizu, S.; *Analyst,* **1998**, *123,* 493–495.

[44] Takayanagi, T. *Chemistry and Chemical Industry,* **2002**, *55,* 117–120.

[45] Takayanagi, T. *Anal. Sci.,* **2004**, *20,* 255–265

[46] Motomizu, S.; Takayanagi, T.; Wada, E. *Anal. Sci.,* **1997**, *13, Supplement,* 239–242.

[47] Motomizu, S.; Takayanagi, T. *J. Chromatogr. A,* **1999**, *853,* 63–69.

[48] Manege, L. C.; Takayanagi, T.; Oshima, M.; Motomizu, S. *Analyst,* **2000**, *125,* 699–703.

[49] Manege, L. C.; Takayanagi, T.; Oshima, M.; Motomizu, S. *Analyst,* **2000**, *125,* 1928–1932.

[50] Matsubara, N.; Terabe, S. *J. Chromatogr. A,* **1994**, 680, 311–315.

[51] Takayanagi, T.; Motomizu, S. *J. Chromatogr. A,* **1999**, *853,* 55–61.

[52] Takayanagi, T.; Fushimi, K.; Motomizu, S. *Bull. Chem. Soc. Jpn.,* **2000**, *73,* 2505–2511.

[53] Takayanagi, T.; Motomizu, S. *Bull. Chem. Soc. Jpn,.* **2001**, *74,* 2083–2089.