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Synthesis of Cationic Diazacrown Ethers and Their Successful Application as Selectivity Modifiers in the Capillary Electrophoresis Separation of Aromatic Anions Involving Positional Isomers

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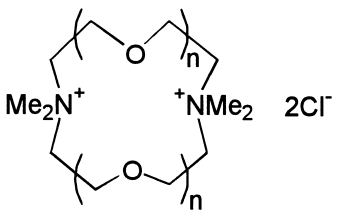
Two types of positively charged diazacrown ether derivatives with different ring sizes were investigated concerning their ability as selectivity modifiers in capillary electrophoresis. The addition of the cationic diazacrowns to a migrating solution led to the successful separation of the positional isomers of naphthalenedisulfonate, naphthalenedicarboxylate, and phthalate, such that a total of eight aromatic anions were completely resolved in less than 5 minutes. The separation mechanism was studied by examining the effects of the ionic strength and the pH of the running buffer, by determining the ion-association constants between the analytes and the additives, and by elucidating their molecular structures. As a result, a combination of ion-exchange, hydrophobic interaction, and molecular geometry was found to play an important role in the observed separation behavior.

"Crown ether" is a general term given to a series of macrocyclic polyoxyethylene compounds, which have received much attention during the last three decades in almost all fields of chemistry.[1] In separation science, crown ethers are generally used as one of the important additives in chromatography as an aid in the separation of alkali, alkaline earth, and other transition metal cations, since the pioneering study by Blasius et al.[2] For example, Lamb et al. utilized crown ether-based ion exchange chromatography for separating not only cations,[3,4] but also anionic species involving inorganic anions,[5,6] nucleosides, and nucleotides[7] based on the host-guest interaction with them.

On the other hand, it is well-known that capillary electrophoresis (CE) is a useful and powerful technique, especially for analyzing an ionic species in terms of its short analytical time and large peak efficiency, although the applications of crown ether as an additive have been quite limited, almost exclusively for the separation of cationic species,[8–12] in comparison with chromatography. Recently, Lamb[13] and Shih[14] applied 18-crown-6 and cryptand to the separation of inorganic anions by CE, in which, however, crown ether mainly acts as an electroosmotic flow (EOF) modifier rather than as an additive for directly improving the separation of anions. Although a bifunctional cryptand also exhibited high effectiveness for the separation of anionic species, the inherent protonation at the bridge-head nitrogen atoms strongly hampered its uses under pH 7. With regard to the separation of organic anions, a combination of crown ether and alkali metal ion[15] permitted the successful separation of analytes, while the resolution was less effective than that using other cationic additives, e.g., mono/divalent quaternary ammonium ions,[16,17] viologen cations,[18] macrocyclic polyamines,[19] and polyelectrolytes.[20–25]



1 : n = 1
2 : n = 2

Fig. 1. Cationic diazacrown ether derivatives **1** and **2**.

As a part of our study aiming to develop a new CE additive for efficiently separating anionic species, we very recently reported that a cationic macrocycle **2** (Fig. 1), which is a 18-crown-6 analogue containing two quaternary nitrogen atoms on the framework, serves as a highly efficient additive for separating the positional isomers of aromatic anions, such as naphthalenedisulfonate, naphthalenedicarboxylate, and phthalate, which were used as model analytes.[26] This foregoing result strongly prompted us to prepare an analogous 12-membered derivative **1** and to investigate the ability as a CE additive by examining the separation behavior of the same analyte anions used in our previous work. As a result, the use of the

cationic diazacrown **1** as an additive in a migrating solution was found to enable us to separate a total of eight aromatic anions in an even shorter analytical time (< 5 min), as compared with **2**. Furthermore, it was also found, through a detailed investigation of the ion-association phenomena between the diazacrowns and the analytes, that diazacrown **1** exhibits greater selectivity toward the analyte anions than **2**. In this paper, we report on the synthesis of the cationic diazacrowns **1** and **2**, the successful separation of the positional isomers of aromatic anions, and the separation mechanism.

Experimental

General Information for the Synthesis. The melting points were determined on a Yanagimoto MP-J3 and are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL EX-400 using D_2O (Wako Chemicals, Osaka, Japan) as the solvent and sodium 3-(trimethysilyl)-propionate-2,2,3,3-d_4 (Aldrich, Milwaukee, WI, USA) as an internal standard. Fast atom bombardment (FAB) and field desorption (FD) mass spectra were obtained on a JEOL JMS-SX102A, and elemental analyses were performed on a Yanagimoto MT-5. Reagent-grade silver(I) chloride was purchased from Wako Chemicals (Osaka, Japan) and used as received. Water was deionized and then distilled twice by using a Yamato WA 73 automatic still. Ethanol of reagent grade was purchased from Kishida Chemicals (Osaka, Japan) and used after distillation. *N*,*N*,*N*′,*N*′-Tetramethyl-1,7-dioxa-4,10-diazacyclododecane diiodide (**3**) and *N*,*N*,*N*′,*N*′-tetramethyl-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane diiodide (**4**) were prepared according to the described procedure.[27]

Preparation of *N*,*N*,*N*′,*N*′-Tetramethyl-1,7-dioxa-4,10-diazacyclododecane Dichloride (1). To a suspension of silver(I) chloride (0.96 g, 6.7 mmol) in water (12 mL), **3** (1.01 g, 2.08 mmol) was added in one portion at 60 °C. The reaction mixture was stirred at 60 °C for 2 hours, and then at room temperature for 7 days. After filtration through Celite, the silver salts on the filter were washed with water (40 mL), and the combined filtrate was evaporated to dryness. The residue was treated again with silver(I) chloride as mentioned above to completely exchange the counter ions from iodide to chloride. The resulting solid was triturated twice with ethanol (30 mL × 2) to give **1** (527.3 mg, 83.7%) as a colorless powder, mp > 300 °C (dec.). ^{1}H NMR (D_2O) δ 3.21 (s, 12 H, CH_3), 3.71 (m, 8H, OCH_2CH_2N), 3.99 (m, 8H, CH_2N). MS (FAB) *m*/*z* 116 ($[M-2Cl]^{2+}$). Anal. Calcd for $C_{12}H_{28}Cl_2N_2O_4 \cdot 0.1C_2H_5OH \cdot 0.2H_2O$: C, 47.04; H, 9.38; N, 8.99; Cl, 22.76%. Found: C, 47.08; H, 9.20; N, 8.79; Cl, 22.70%.

Preparation of *N*,*N*,*N*′,*N*′-Tetramethyl-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane Dichloride (2). According to a previous report,[26] compound **2** was prepared from compound **4** in quantitative yield. Colorless powder, mp > 300 °C (dec.). ^{1}H NMR (D_2O) δ 3.20 (s, 12H, CH_3), 3.68 (t, J = 4.9 Hz, 8H, OCH_2CH_2N), 3.73 (s, 8H, OCH_2CH_2O), 3.98 (m, 8H, CH_2N). MS (FD) *m*/*z* 357 (M^+-Cl, 5.6%), 355 (M^+-Cl, 12.0%), 305 (M^+-CH_3-2Cl, 100%). Anal. Calcd for $C_{16}H_{36}Cl_2N_2O_4$: C, 49.10; H, 9.27; N, 7.16; Cl, 18.12%. Found: C, 48.97; H, 9.10; N, 7.01; Cl, 18.11%.

Capillary Electrophoresis. CE experiments were performed on an HP^{3D} capillary system (Hewlett-Packard, Waldbronn, Germany) equipped with a UV-visible detector. A fused-silica capillary and a CElect N coating capillary were purchased from GL-Science (Tokyo, Japan) and SUPELCO (Bellefonte, PA, USA), respectively. The total length of the capillary was cut to 60 cm (51.5 cm from inlet to detector, 50 μm i.d.). All of the analyte anions examined were purchased from Tokyo Kasei Kogyo (Tokyo, Japan) and were sodium salts, except for carboxylic compounds. The divalent aromatic anions were naphthalene-1,5-disulfonate (1,5-NDS), naphthalene-2,6-disulfonate (2,6-NDS), naphthalene-2,7-disulfonate (2,7-NDS), naphthalene-2,3-dicarboxylate (2,3-NDC), naphthalene-2,6-dicarboxylate (2,6-NDC), phthalate (PH), isophthalate (*i*-PH), and terephthalate (*t*-PH) ions. Carboxylic compounds were used after neutralization with 2 equimolar amounts of NaOH. Methanol was purchased from Wako Chemicals (Osaka, Japan) and was used as a marker of EOF.

An aqueous solution of 10 mM sodium dihydrogenphosphate (1 M = 1 mol dm^{-3}) was prepared as a running buffer, and the pH was adjusted with 0.1 M NaOH. After appropriate amounts of 0.1 M diazacrowns were added to the buffer, the resulting solutions were passed through a 0.2 μm cellulose acetate filter before use. Sample solutions of 0.01 mM NDS, NDC, and PH isomers were injected into the capillary from the cathodic end under pressure for 3 sec, and a voltage of −25 kV was applied for the separation.

A UV detector was placed at the anodic end, and the analyte anions were detected at 230 nm. All CE experiments were performed at 25 °C, and the apparent mobilities of the analyte samples were evaluated using the standard procedure.

Theoretical Calculations. All calculations were carried out on a Silicon Graphics O2 workstation. The initial structures of the diazacrowns and analyte anions were generated by a 10000-step Monte-Carlo multiple minimum simulation using a MM2* force field[28] and the GB/SA solvation model for H_2O[29] implemented in Macro Model version 5.5.[30] For a simplification, counter ions of diazacrowns and anions were not included in the simulation. These global minimum structures were then submitted to the mixed-mode procedure of stochastic molecular dynamics and a Monte-Carlo simulation using the MM2* force field and the GB/SA solvation model for H_2O. Sampling was carried out every 1 ps for a period of 1.2 ns after the initial 300 ps equilibration, and a total of 1200 conformers were stored and used to evaluate the interatomic distances of the charged sites.

Result and Discussion

Synthesis. As synthetic precursors of diazacrowns **1** and **2**, *N*,*N*,*N*′,*N*′-tetramethyl-1,7-dioxa-4,10-diazacyclododecane diiodide (**3**) and *N*,*N*,*N*′,*N*′-tetramethyl-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane diiodide (**4**) were prepared according to Jurczak's report.[27] The subsequent anion exchange of **3** and **4** was successfully accomplished with silver(I) chloride to afford the desired **1** and **2** in good-to-quantitative yields, respectively; this anion exchange was necessary for suppressing the UV-interference induced by the iodide ion when performing CE analyses using UV-detection system. It was very difficult to check the end point of the anion exchange reaction because almost no changes in the ^{1}H-NMR spectra were observed before and after the reactions. However, complete exchange of the counter ions from iodide to chloride was fully confirmed based on the spectral and microanalytical data. Only pure samples of diazacrown **1** and **2**, which absolutely satisfied elemental analysis, were used in the following CE experiments.

Choice of Capillary Column. Electroosmotic mobility (μ_{eof}) and effective electrophoretic mobility (μ_{eff}) are important parameters for determining the ionic association constants be-

tween the cationic diazacrowns and the analyte anions. An untreated silica capillary generally used in the normal CE was, however, unsuitable in the present study because the μ_{eof} values of 3.0×10^{-4} cm^2/Vs for **1** and of 3.6×10^{-4} cm^2/Vs for **2** at low concentration of the diazacrowns (1 mM) were almost the oppositely same as those of the analyte anions. As a result, it took $>$ 60 minutes to detect some of them, and such the prolonged analysis time caused not only a poor reproducibility of the migration time of analytes, but also a poor peak efficiency. In contrast, a hydrophilic polymer-coating capillary (CElect N) could overcome these problems based on the small EOF. The direction of EOF was reversed from the anodic to the cathodic direction with increasing the concentration of diazacrowns ($>$ ca. 4 mM), and all of the analyte anions were detected within about 6 minutes by an UV detector equipped at the anodic side. In the absence of diazacrowns, μ_{eof} retained a value of 2.7×10^{-4} cm^2/Vs; this value was gradually decreased to -1.9×10^{-4} cm^2/Vs for **1** and to -1.3×10^{-4} cm^2/Vs for **2** in the presence of 15 mM diazacrowns in the running buffer. Due to the peak efficiencies and the reproducibility of the migration times, a coated capillary was much better than an untreated silica capillary; therefore, the former one was chosen in this study.

Separation of Positional Isomers. Figure 2 shows the migration behaviors of eight aromatic anions in the absence and presence of diazacrowns in a running buffer. Positional isomers of the aromatic anions, which have the same molecular weight, were not resolved in the absence of diazacrowns (Fig. 2a). In contrast, the resolution among them was drastically changed in the presence of diazacrowns as counter cations, and complete separation of the anions was achieved in less than 5 minutes at concentrations of **1** of 0.5 mM and of **2** of 2 mM, as shown in Figs. 2b and 2c. The μ_{eff} values for the anions increased with increasing the concentration of the cationic diazacrowns (Fig. 3);[31] **1** exhibited larger changes in μ_{eff} than **2**. The observed changes in the mobility clearly indicate that the ion-associated species between the divalent cations and the analyte anions would be formed in an aqueous medium, as discussed in the following sections.

Influence of Coexisting NaCl. The coexistence of inorganic electrolytes in the running buffer is known to decrease the ion-exchange effect, thereby allowing sample anions to migrate more rapidly.[24] Indeed, coexisting salt, such as NaCl, led to a faster migration of the analytes; the resolution was remarkably decreased with increasing the ionic strength of the buffer, as shown in Fig. 4. As a consequence, the migration order of the analyte anions in the presence of 100 mM NaCl became almost the same as that in the absence of diazacrowns, indicating that ion association between cationic diazacrowns and the analyte anions is suppressed by the coexistence of excessive NaCl. In particular, the large variations in μ_{eff} for the NDS isomers strongly suggest that these isomers undergo a greater ion-exchange effect than the isomers of PH and NDC.

Effect of Buffer pH. The buffer pH is also an important factor to understand the association phenomena of the diazacrown ethers with the aromatic anions. Figure 5 illustrates how the pH values of the running buffer influenced the resolution between *i*-PH and *t*-PH in the absence and presence of diazacrowns. The resolutions (R_s) were calculated from the relevant

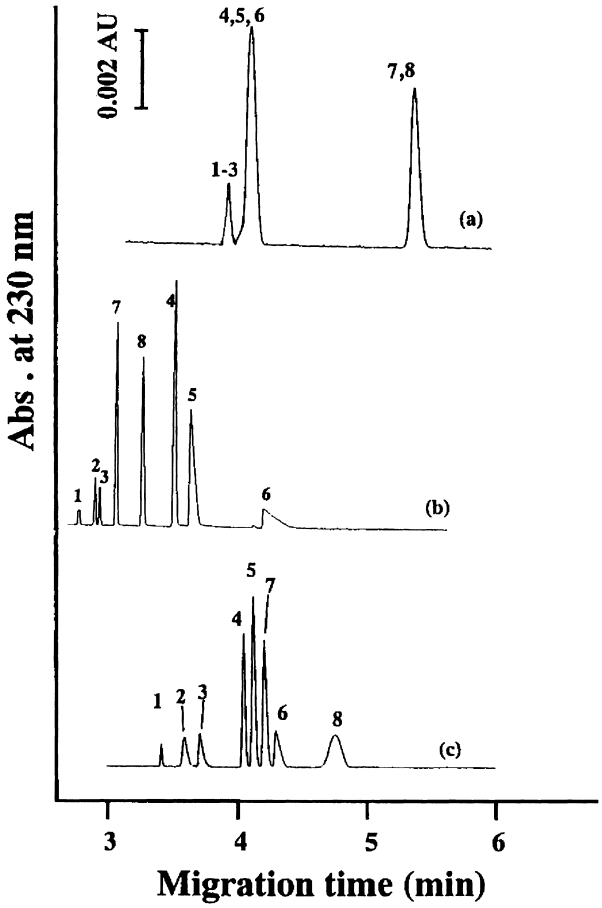


Fig. 2. Electropherograms of eight aromatic anions in the absence and presence of the diazacrowns: (a) no additive; (b) **1** (0.5 mM); (c) **2** (2 mM). Currents: (a) 11 μA; (b) 12 μA; (c) 18 μA. Analyte peaks (in order of elution): (1) PH; (2) *t*-PH; (3) i-PH; (4) 2,6-NDS; (5) 2,7-NDS; (6) 1,5-NDS; (7) 2,3-NDC; (8) 2,6-NDC. Separation conditions: capillary length, 50 μm i.d. × 60 cm (51.5 cm to detector); mobile phase, phosphate buffer (10 mM, pH 7); voltage, −25 kV; UV detection at 230 nm.

electropherograms using Eq. 1.[32]

$$R_s = \frac{1}{4} \cdot \frac{\Delta\mu_{eff}}{\mu_{eff,ave}} \sqrt{N}. \quad (1)$$

In this equation, $\Delta\mu_{eff}$ is the difference in the effective electrophoretic mobilities of two analytes, $\mu_{eff,ave}$ is the averaged effective mobility, and N is the number of theoretical plates.

In the range of buffer pH from 2.5 to 7.0, the EOF mobilities gradually increased independently of the presence of the diazacrowns. Even in the absence of diazacrowns, the *i*-PH and *t*-PH isomers were slightly separated at around pH 4.5, mainly due to the difference in their electrophoretic velocities arising from the difference in the acid dissociation constants (pK_1 = 3.50 and pK_2 = 4.50 for *i*-PH,[33] and pK_1 = 3.54 and pK_2 = 4.46 for *t*-PH[34]). However, no satisfactory resolution was accomplished for the isomers. In contrast, when the cationic diazacrowns of 2 mM were added to the running buffer, the resolution was remarkably improved until pH 4.5, and was little changed above pH 5 independently of the type of diazacrowns,

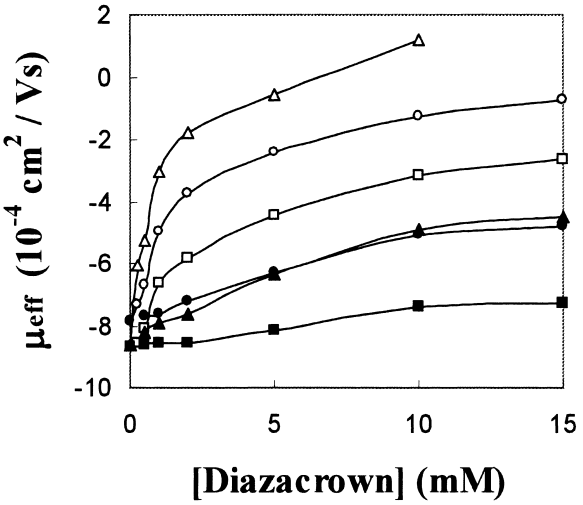


Fig. 3. Effect of the concentration of the diazacrowns on the mobility of the analytes. Currents: 11–24 μA. Analytes: (□) PH for **1**; (■) PH for **2**; (○) 2,6-NDC for **1**; (●) 2,6-NDC for **2**; (△) 1,5-NDS for **1**; (▲) 1,5-NDS for **2**. Other experimental conditions are the same as those in Fig. 2.

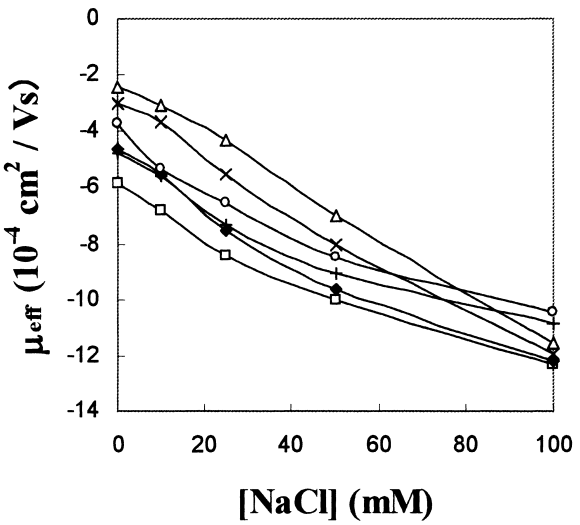


Fig. 4. Influence of ionic strength on the mobility of the analytes in the presence of 2 mM diazacrown **1**. Plot identities: (□) PH; (◆) *i*-PH; (+) 2,3-NDC; (○) 2,6-NDC; (△) 1,5-NDS; (×) 2,6-NDS. Other experimental conditions are the same as those in Fig. 2.

as shown in Fig. 5. These results clearly indicate that complete ionization of the functional groups in the analytes plays a significant role for the intermolecular interaction with diazacrowns, and thus for the separation of the positional isomers.

Ion Association Constants. To quantify the observed migration behavior, the ion-association constants (K_{ass}) between the cationic diazacrowns and the analyte anions were determined using the μ_{eff} values of the anions according to the method of Kuhn et al.,[35] who successfully applied the well-established Benesi–Hildebrand method[36] to a CE system. K_{ass} for 1:1 association between the diazacrowns and the analytes is expressed as

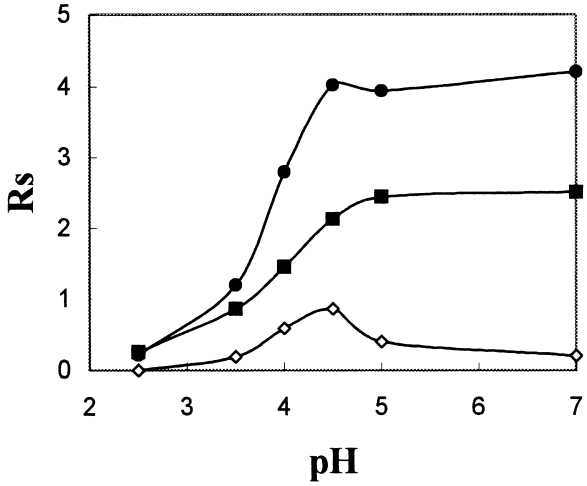


Fig. 5. Influence of buffer pH on the resolution between *i*-PH and *t*-PH. Plot identities: resolutions in the absence of diazacrowns (◇) and in the presence of because **1** of 2 mM (●) and of **2** of 2 mM (■). Separation conditions are the same as those in Fig. 2 except for the running buffer. The buffer solutions of pH 2.5, 3.5, and 7 were prepared from 10 mM sodium dihydrogenphosphate, and those of pH 4, 4.5, and 5 were from 10 mM sodium acetate.

$$\frac{1}{\mu_{eff} - \mu_{ep}} = \frac{1}{\mu_{CA} - \mu_{ep}}\left(\frac{1}{K_{ass}}\cdot\frac{1}{[C]} + 1\right), \qquad (2)$$

where μ_{CA} is the electrophoretic mobility of a solute completely bound to a cationic diazacrown, μ_{ep} is the mobility of the analyte in the absence of the diazacrowns, and $[C]$ is the molar concentration of the cationic diazacrown ethers. According to this method, the migration behavior of the analytes was studied by varying the concentration of diazacrowns from 0 to 10 mM. A linear regression of the Benesi–Hildebrand plots yielded correlation coefficients (r^2) of 0.9882–0.9986 for **1** and of 0.9888–0.9992 for **2**, indicating that 1:1 association between the diazacrowns and the analyte anions would be the dominant species in solution. This result is in good agreement with that using X-ray crystallography, which described the molecular structure of 1,5-NDS and diazacrown **2** associated in a 1:1 ratio.[37] As summarized in Table 1, the K_{ass} constants increased in the order of PH < 2,3-NDC < *t*-PH < *i*-PH < 2,6-NDC < 2,6-NDS < 2,7-NDS < 1,5-NDS for both **1** and **2**. It should be noted that the K_{ass} constants observed for **1** were twice or more as large as those for **2**, and that the constants strongly depend on the functionalities of the analyte anions, such that the aromatic anions containing sulfonate groups revealed a larger K_{ass} for both the diazacrowns than those involving carboxylate groups.

Separation Mechanism. In general, the ion associability between an analyte and an additive in CE is affected by their molecular structures, by the ionic and hydrophobic interaction between them, and by the concentration and pH of the migration solution.[16–18] The present CE experiments were performed under the same buffer condition, and therefore the observed separation behavior would be explained from the following mechanistic points of view.

Firstly, ion-exchange selectivity is assumed to contribute to

Table 1. Ion Association Constants (K_{ass}) between the Diazacrown Ethers and the Analytes, Calculated O^-–O^- Distances in the Anions, and Their Differences from the Interatomic Distances of the Charged Sites in the Diazacrown Ethers

Analyte	K_{ass}/M^{-1}		O^-–O^- Distance/Å[a)]	Difference/Å	
	1	**2**[a)]		**1**	**2**[a)]
PH	55.1 ± 0.25	20.3 ± 0.08	3.79 ± 0.81	−2.01 ± 0.70	−2.35 ± 0.88
i-PH	166 ± 0.13	68.8 ± 0.10	6.28 ± 0.73	+0.48 ± 0.62	−0.04 ± 0.81
t-PH	136 ± 0.16	61.6 ± 0.10	7.25 ± 0.18	+1.45 ± 0.07	+0.63 ± 0.38
2,3-NDC	60.2 ± 0.14	30.6 ± 0.09	3.80 ± 0.79	−2.00 ± 0.68	−2.52 ± 0.86
2,6-NDC	169 ± 0.04	80.3 ± 0.08	9.39 ± 0.25	+3.59 ± 0.14	+3.07 ± 0.42
1,5-NDS	991 ± 0.03	126.5 ± 0.07	8.33 ± 0.47	+2.53 ± 0.36	+2.01 ± 0.58
2,6-NDS	300 ± 0.10	89.7 ± 0.04	9.53 ± 0.29	−3.73 ± 0.18	+3.21 ± 0.45
2,7-NDS	425 ± 0.04	95.1 ± 0.04	8.82 ± 0.67	−3.02 ± 0.56	+2.50 ± 0.75

a) Ref. 26.

the rough separation of the aromatic analytes based on the functional groups in the anions. As proved by the experimental results, that the buffer pH and the coexisting NaCl largely affected the separation behavior of the analytes (Figs. 4 and 5), the intermolecular interaction between the diazacrowns and the analyte anions would be mainly induced by the electrostatic attraction between them. This is also easily understandable by the fact that the electrophoretic mobilities of the anions were remarkably decreased by the use of **1** and **2** as additives in the running buffer (Fig. 3). As a result, it is very likely that diazacrowns recognize the functional groups of the analytes to roughly separate them, such that the analyte anions containing carboxylate groups are eluted first, follow by the NDS isomers involving sulfonate groups, which undergo a larger ion-exchange effect.

Secondly, such the roughly-partitioned analytes should be further separated by a hydrophobic interaction with the diazacrowns, as discussed by Cheng's[38] and Takayanagi's[16–18] research groups. However, the difference in the hydrophobicity of **1** and **2** was very difficult to evaluate due to the fact that they are almost freely soluble in water or methanol/water. Hence, less soluble **3** and **4** were used to compare the hydrophobicity inherent in the crown frameworks, and their solubility in water was determined to be 0.04 and 110 mg/ml, respectively. Consequently, it is reasonable to presume that the large difference in K_{ass} observed for **1** and **2** (Table 1) would result from the difference in their hydrophobicity. In other circumstances, this consideration is parallel to the experimental result that a series of the PH isomers showed a smaller K_{ass} for both **1** and **2** than those of the other analytes. Actually, it is most likely, on the whole, that the difference in the hydrophobicity of the analyte, itself, together with their molecular size, allowed the PH isomers to be eluted faster than the NDC isomers which have the same carboxylate groups (Fig. 2).

Finally, the distance between the charge sites of these sample anions also contributes to the fine separation of the positional isomers. Takayanagi et al.[17] pointed out that ion association would be facilitated when the interatomic distance of charged sites in anion is ca. 2.0 Å longer than that in the cation, through a study of the separation behavior of divalent aromatic anions by capillary zone electrophoresis using divalent quaternary ammonium ions. To elucidate the molecular structures of the diazacrowns and the analytes, molecular-dynamics calculations were carried out using the MM2* force field[28] and the GB/SA solvation model for H_2O[29] implemented in Macro Model version 5.5.[30] The calculated distances between the charged sites are summarized in Table 1. Our results are in good agreement with the conclusion of Takayanagi et al.,[17] although the favorable O^-–O^- distance in anion was ca. 2.0–2.5 Å longer, in our case, than the N^+–N^+ distances of **1** (6.32 ± 0.34 Å) and **2** (5.80 ± 0.10 Å) based on the largest association constant with 1,5-NDS. Qualitatively, the greater were the differences from the optimum distance of ca. 8.3 Å in the anions, the smaller were the association constants with the diazacrowns (Table 1). Although this was not true for some of the analytes, it is reasonable to presume, on the whole, that a close relationship exists between the interatomic distance of the charged sites and the observed association constants. As a consequence, it is quite likely that the analyte anions, which were roughly separated by the ionic and hydrophobic interactions with diazacrowns, were further resolved into the positional isomers by the difference in the separation distance of the charged sites.

To summarize, the separation behavior observed in the present study was rationalized by considering the multiple contributions from the above mechanisms, i.e., the types of functional group, hydrophobicity, molecular size, and the separation distance of the charged sites. Consequently, it is reasonable to conclude that a combination of these effects plays an important role in determining the degree of ion associability, and thus in defining the migration order of aromatic anions.

Conclusion

The present study clearly demonstrates that both the cationic diazacrowns **1** and **2** serve as highly efficient CE additives for improving the separation of the aromatic anions involving the positional isomers. In particular, we should place great emphasis on the experimental fact that the complete separation of the positional isomers was accomplished in a short analytical time by using very small amounts of the cationic diazacrowns as selectivity modifiers (< 0.5 mM for **1** and < 2 mM for **2**). On this basis, the diazacrowns will be also applied to a separation study of inorganic anions to establish their scope and limitations, which should be useful for development strategies toward new CE additives.

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