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Short communication

# Cationic diazacrown ether for the separation of positional isomers by capillary electrophoresis

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## Abstract

The use of a positively charged diazacrown ether, *N,N,N',N'*-tetramethyl-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane dichloride (**1**), in capillary electrophoresis is reported, for the first time. The addition of the cationic diazacrown **1** to the running buffer permitted the successful separation of positional isomers of aromatic anions such as naphthalenedisulfonate, naphthalenedicarboxylate and phthalate. At a concentration of **1** of 2 mM, all the analyte anions were completely separated in less than 5 min. The ion association constants of the solute anions with diazacrown **1** were evaluated and the findings are discussed. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** Positional isomers; Buffer composition; Crown ethers; Diazacrown ethers; Organic acids

## 1. Introduction

It is well known that capillary electrophoresis (CE) is a powerful and attractive separation tool, especially for ionic molecules, in terms of its short analysis time and large peak efficiency. In order to improve the separation efficiency, many compounds have been used as additives. Crown ethers have been extensively used as additives in CE, as an aid in the separation of alkali, alkaline-earth and other transition metal cations [1–3]. Although some special crown ethers have also been applied to the chiral separation of organic compounds [4,5], most have been employed for the general separation of metal and ammonium cations. Complex formation is based on the ion–dipole interaction between the charge on

the cationic species and the unshared pair of electrons in the crown cavity, and therefore is strongly dependent on size [6]. For this reason, crown ethers have not been used for the separation of anionic species using CE, although Timerbaev et al. [7] and Lamb et al. [8] recently used macrocyclic polyamines and crown ether as alkali metal chelates, which act as pairing ions for anionic analytes.

The aim of the present study was to expand the application of crown ether relatives to the separation of anionic samples. We thus synthesised *N,N,N',N'*-tetramethyl-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane dichloride (**1**), which is an analogue of 18-crown-6 and which contains two positively charged nitrogen atoms on the framework as shown in Fig. 1. For the first application of this cationic diazacrown **1** to CE, positional isomers of aromatic anions such as naphthalenedisulfonate, naphthalenedicarboxylate and phthalate were selected as model anions. By

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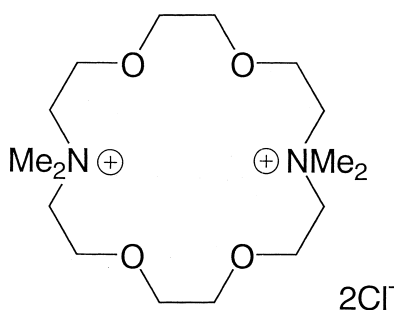


Fig. 1. Structure of cationic diazacrown **1**.

using the diazacrown **1** as an additive in the running buffer, all eight of the aromatic anions were completely separated in less than 5 min. In this paper, we report the successful separation of the positional isomers as well as the ion association constants between **1** and the analyte anions.

## 2. Experimental

### 2.1. Synthesis

#### 2.1.1. Equipment and chemicals

Melting points were determined on a Yanagimoto MP-J3 and are uncorrected.  $^1\text{H-NMR}$  spectra were recorded on a Joel EX-400 using  $^2\text{H}_2\text{O}$  as the solvent (Wako, Osaka, Japan) and sodium [ $^2\text{H}_4$ ]3-(trimethylsilyl)propionate-2,2,3,3- $\text{d}_4$  (Aldrich, Milwaukee, WI, USA) as an internal standard. 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 and used without further purification. Distilled water was obtained by using a Yamato WA73 automatic still, and ethanol and diethyl ether (Kishida, Osaka, Japan) were freshly distilled prior to use. *N,N,N',N'*-Tetramethyl-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane diiodide (**2**), which is a synthetic precursor of **1**, was prepared according to the literature procedure [9].

#### 2.1.2. Synthesis of *N,N,N',N'*-tetramethyl-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane dichloride (**1**)

To a suspension of silver(I) chloride (1.30 g, 9.07 mmol) in water (15 ml), **2** (1.61 g, 2.80 mmol) was

added in one portion at  $60^\circ\text{C}$ . After stirring for 6 days at room temperature, the mixture was filtered 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 reprecipitated from ethanol–diethyl ether (3:5, v/v) to give **1** as a colourless powder (1.09 g, quant.), m.p.  $>300^\circ\text{C}$  (dec.).  $^1\text{H-NMR}$  ( $^2\text{H}_2\text{O}$ , 400 MHz)  $\delta$  3.20 (s, 12H,  $\text{CH}_3$ ), 3.68 (t,  $J=4.9$  Hz, 8H,  $\text{OCH}_2\text{CH}_2\text{N}$ ), 3.73 (s, 8H,  $\text{OCH}_2\text{CH}_2\text{O}$ ), 3.98 (m, 8H,  $\text{CH}_2\text{N}$ ). MS (FD)  $m/z$  357 ( $[\text{M}-\text{Cl}]^+$ ; 5.6%), 355 ( $[\text{M}-\text{Cl}]^+$ ; 12.0%), 305 ( $[\text{M}-\text{CH}_3-2\text{Cl}]^+$ ; 100%), 160 ( $[\text{M}-2\text{Cl}]^{2+}$ ; 6.5%). Anal. Calcd. for  $\text{C}_{16}\text{H}_{36}\text{Cl}_2\text{N}_2\text{O}_4$ : 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.

### 2.2. Capillary electrophoresis

#### 2.2.1. Apparatus and chemicals

CE experiments were performed on an HP<sup>3D</sup> 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  $\mu\text{m}$  I.D. All of the analyte anions examined were purchased from Tokyo Kasei Kogyo (Tokyo, Japan), and were sodium salts, except for the compounds, which contained carboxyl groups. 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 neutralisation with NaOH. Methanol was purchased from Wako and was used as a marker of electroosmotic flow (EOF).

#### 2.2.2. Measurements

An aqueous solution of 10 mM  $\text{NaH}_2\text{PO}_4$  was prepared as a running buffer, and the pH was adjusted with 0.1 M NaOH. After appropriate amounts of 0.1 M diazacrown **1** were added to the buffer, the resulting solutions were passed through

0.2- $\mu\text{m}$  cellulose acetate filters before use. Sample solutions of 0.01 mM NDS, the NDC and PH isomers were injected into the capillary from the cathodic end under pressure for 3 s. A voltage of  $-25$  kV was then applied for the separations, and the analyte anions were detected at 230 nm. All CE experiments were performed at  $25^\circ\text{C}$ , and the analytes were evaluated using standard procedures.

### 3. Results and discussion

#### 3.1. EOF measurement

In order to evaluate the effective electrophoretic mobilities of the analyte anions, the velocities of EOF were determined in the presence of diazacrown **1**. In the present study, we used a hydrophilic polymer-coating capillary (CElect N), because the capillary shows a lower electroosmotic mobility, the direction of which is opposite to the electrophoretic migration of anions. The mobility of the EOF was  $2.7 \cdot 10^{-4} \text{ cm}^2/\text{V s}$  in the absence of diazacrown **1**, and the mobility became  $-1.3 \cdot 10^{-4} \text{ cm}^2/\text{V s}$ , when 15 mM diazacrown **1** was added. Since the EOF is small or the direction of EOF is reversed by the presence of diazacrown **1**, all the analyte anions were detected within about 8 min in the coating capillary.

#### 3.2. Separation of positional isomers

The migration behaviour of eight aromatic anions was investigated by increasing the concentration of **1** in the running buffer, and some typical electropherograms are shown in Fig. 2. The positional isomers of the divalent anions which had the same molecular mass were not resolved in the absence of diazacrown **1** (Fig. 2a), whereas the separation behaviour of the positional isomers changed dramatically in the presence of **1**. Especially at a concentration of **1** of 2 mM, all analytes were completely separated within 5 min, as shown in Fig. 2b. However, further addition of **1** into the running buffer resulted in a decrease in the resolution of *i*-PH and *t*-PH, although the resolution of NDS and NDC were improved (Fig. 2c).

In order to clarify the observed separation behaviour, the association constants between **1** and the

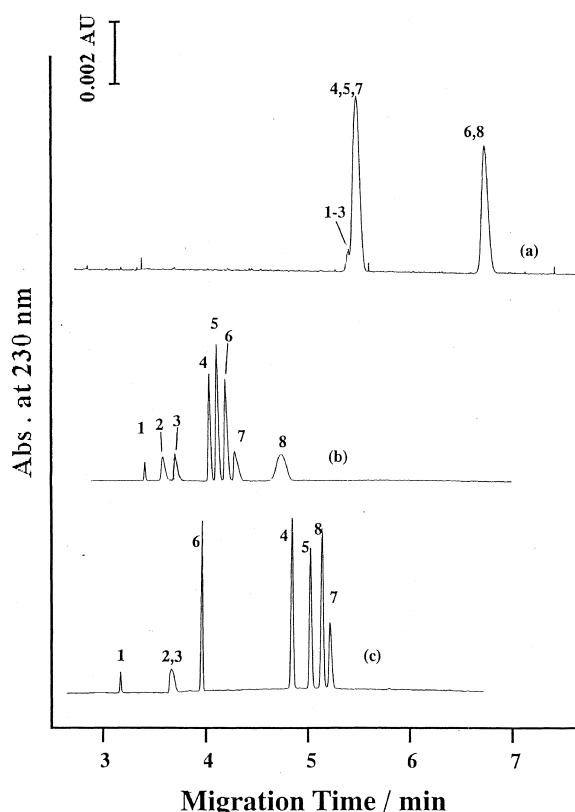


Fig. 2. Electropherograms of eight aromatic anions in the presence of diazacrown **1** in 10 mM phosphate buffer (pH 7): [**1**] = 0 (a), 2 (b) and 15 (c) mM. Plot numbers: PH (1), *t*-PH (2), *i*-PH (3), 2,6-NDS (4), 2,7-NDS (5), 1,5-NDS (6), 2,3-NDC (7) and 2,6-NDC (8). Capillary length: 60 cm (51.5 cm to the detector). Voltage:  $-25$  kV. Detection: UV at 230 nm.

analyte anions were determined according to the method reported by Kuhn et al. [5] or Rundlett et al. [10], who successfully applied the well-established Benesi–Hildebrand method [11] to a CE system. Table 1 shows that the constants increased in the order of  $\text{PH} < 2,3\text{-NDC} < t\text{-PH} < i\text{-PH} < 2,6\text{-NDC} < 2,6\text{-NDS} < 2,7\text{-NDS} < 1,5\text{-NDS}$ . This result demonstrates that sulfonate groups in the anions tend to show greater ion association than carboxyl groups, mainly due to the ion-exchange selectivity with the counter-cation e.g. a quaternary ammonium ion [12]. In addition, the constants in Table 1 also indicate that the distance between the charged sites would be an additional factor in determining the association constants, as pointed out by Takayanagi and co-

Table 1

Ion association constants for aromatic anions and their calculated interatomic distances of charged sites

Anion	Ion association constant ( $M^{-1}$ )	O–O distance <sup>a</sup> (difference) <sup>b</sup> (Å)
PH	20.3±0.08	3.79±0.81 (–2.35±0.88)
i-PH	68.8±0.10	6.28±0.73 (–0.04±0.81)
t-PH	61.6±0.10	7.25±0.18 (+0.63±0.38)
2,3-NDC	30.6±0.09	3.80±0.79 (–2.52±0.86)
2,6-NDC	80.3±0.08	9.39±0.25 (+3.07±0.42)
1,5-NDS	126.5±0.07	8.33±0.47 (+2.01±0.58)
2,6-NDS	89.7±0.04	9.53±0.29 (+3.21±0.45)
2,7-NDS	95.1±0.04	8.82±0.67 (+2.50±0.75)

<sup>a</sup> The average distances of 1200 conformers generated by the mixed-mode procedure of stochastic molecular dynamics and Monte Carlo simulation implemented in Macro Model version 5.5 MM2\* force field and GB/SA solvation model for water were used for the calculations, and sampling was carried out every 1 ps for a period of 1.2 ns after the initial 300-ps equilibration.

<sup>b</sup> Values in parentheses are the differences in the interatomic distances of charged sites in **1** and the relevant anions.

workers [13,14]. The latter group studied the separation behaviour of divalent aromatic anions by CE using divalent quaternary ammonium ions [13], and concluded that ion association between them would be facilitated when the spatial interatomic distance of charged sites in anion is  $\sim 2$  Å longer than that in the cation [13]. Our experimental results are in agreement with their conclusion [13], and the favourable  $O^- - O^-$  distance in the anion was  $\sim 2$  Å longer than the  $N^+ - N^+$  distance of diazacrown **1** ( $6.32 \pm 0.34$  Å), judging from the largest association constant in 1,5-NDS. Qualitatively, the greater the differences from the optimum distance of  $\sim 8.3$  Å in anion, the smaller were the association constants with diazacrown **1** (Table 1). Although this was not true in the case of some 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 result, it is very likely that a combination of ion-exchange selectivity and interatomic distance plays an important role in determining the degree of ion associability and, thus, in improving the separation of aromatic anions. Further investigations aimed at a more detailed knowledge of the mechanism are currently in progress.

## 4. Conclusion

The use of cationic diazacrown **1** in CE was investigated, for the first time, through the successful separation of positional isomers of aromatic anions, and it was clearly demonstrated that diazacrown **1** has the ability to greatly improve the separation of the positional isomers of aromatic anions. From the determinations of the ion association constants, ion-exchange selectivity with counter-cation and interatomic distances were found to be important factors for separating the analytes, although further study will be required to fully understand the observed separation behaviour. As a final point, great emphasis should be placed on the fact that complete separation of positional isomers could be accomplished in a short analytical time by using small amounts of diazacrown **1** as an additive ( $< 2$  mM).

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