



ELSEVIER

Journal of Chromatography A, 934 (2001) 113–122

JOURNAL OF
CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Complex formation analysis of water-soluble calixarenes by capillary zone electrophoresis

Koji Oshita, Toshio Takayanagi, Mitsuko Oshima, Shoji Motomizu*

Department of Chemistry, Faculty of Science, Okayama University, 3-1-1 Tsushimanaka, Okayama 700-8530, Japan

Received 11 May 2001; received in revised form 17 September 2001; accepted 17 September 2001

Abstract

The equilibria of complex formation reactions between various kinds of cations and anionic calixarenes in an aqueous solution were analyzed by capillary zone electrophoresis on the basis of the change in electrophoretic mobility of the calixarenes. The apparent electrophoretic mobility of the calixarenes decreased with increasing concentrations of alkali metal ions and quaternary ammonium ions. Equilibrium constants were determined by a non-linear least-squares analysis using the changes in apparent electrophoretic mobility. The complex formation constants obtained with alkali metal ions were in the order of $10^{2.3}$ – $10^{3.0}$, and those with quaternary ammonium ions were $10^{2.9}$ – $10^{4.2}$, providing less selectivity among them. The selectivity of the calixarene toward alkali metal and quaternary ammonium ions in aqueous solution involves flexible structure, and compensative interactions of electrostatic and hydrophobic interactions. From the results obtained in this work, the electrophoretic method has proved to be useful for analyzing the reactivity of anionic calixarene in aqueous solution. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Complexation; Calixarenes; Metal cations; Quaternary ammonium compounds; Organosulphur compounds

1. Introduction

Calixarenes are cyclic oligomers which can be synthesized by the condensation of *p*-alkylphenol and formaldehyde. Calixarenes possess an hydrophobic cavities, and can form a wide variety of host–guest type of inclusion complexes; therefore, calixarenes have attracted much attention as a third inclusion compound next to crown ethers and cyclodextrins [1]. In addition to the size selectivity of such compounds, crown ethers can incorporate cations, especially alkali and alkaline earth metal ions, based

on the electrostatic interaction, whereas cyclodextrins can incorporate various guest molecules based on the hydrophobic interaction. In the case of calixarenes, various kinds of interaction would participate in the molecular recognition; they are hydrogen bonding, coordination bonding, electrostatic interaction, aromatic–aromatic interaction and cation–aromatic interaction [2]. Furthermore, phenolic hydroxyl groups of the calixarenes can be modified to other functional groups, and therefore several kinds of interaction can be used for selectivity enhancement.

Most of the calixarenes synthesized and investigated so far are hydrophobic ones and slightly soluble in water, and therefore, the reactivity of calixarenes has been studied mainly in hydrophobic

*Corresponding author. Tel.: +81-86-251-7846; fax: +81-86-251-7846.

E-mail address: motomizu@cc.okayama-u.ac.jp (S. Motomizu).

media, such as in solvent extraction and in ion elective electrodes [3]. Several water-soluble derivatives of sulfonate-, amino-, nitro-, carboxyl- and phosphonate-calixarenes have been synthesized [4]. In the research field of molecular recognition in analytical chemistry, interest must be directed towards reactions in aqueous media, because most of the objectives in analytical chemistry involve such aqueous media. In this study, the reactivity of some water-soluble calixarenes has been investigated through the mobility changes in capillary zone electrophoresis (CZE) using *p*-sulfocalix[6]arene (calix[6]–SO₃) and *p*-sulfocalix[8]arene (Calix[8]–SO₃), where alkali metal ions and quaternary ammonium ions were examined as guest molecules. Complex formation constants were determined by using the change in electrophoretic mobility of the calixarenes. The contribution of the cavity size of the calixarenes as well as and other interactions affecting the reactivity are discussed. The contribution of functional groups was also investigated using carboxymethoxy-*p*-sulfocalix[6]arene (Calix[6]–SO₃–CO₂).

2. Experimental

2.1. Apparatus

An Applied Biosystems (Foster City, CA, USA) 270A capillary electrophoresis system equipped with a UV detector was used. A fused-silica capillary (50 μm I.D.) was purchased from GL Sciences, and was used after being cut. The detection window was made by burning the polyimide coating of the capillary; the size of a capillary was 72 cm (50 cm effective length) from the sample injection point to the detection point. A Hitachi (Tokyo, Japan) D-2000 Chromato-Integrator was used for recording the electropherograms.

2.2. Reagents

Boric acid and alkali metal hydroxides, such as LiOH, NaOH, KOH, RbOH or CsOH, were used for preparing the migrating buffer solutions. Quaternary ammonium chloride, such as tetramethylammonium chloride (TMA⁺Cl[–]), tetraethylammonium chloride

(TEA⁺Cl[–]), tetrapropylammonium chloride (TPA⁺Cl[–]) and tetrabutylammonium chloride (TBA⁺Cl[–]), were used for the reaction analysis with the water-soluble calixarenes.

A series of migrating solutions containing certain amounts of H₃BO₃–alkali metal hydroxide buffer (M⁺–borate buffer) (pH 9.00) were used for the equilibrium analysis of calixarenes with alkali metal ions. The migrating buffer solutions were prepared by adding alkali metal hydroxide solution to boric acid solution. The concentrations of alkali metal ion in these buffer solutions were calculated from the amount of alkali metal hydroxide added. A series of certain concentrations of quaternary ammonium chloride were added to the K⁺–borate buffer for the equilibrium analysis of the organic cations. Components of these buffer solutions are summarized in Table 1.

The calixarenes examined are shown in Fig. 1. *p*-Sulfocalix[6]arene (Calix[6]–SO₃) and *p*-sulfocalix[8]arene (Calix[8]–SO₃) were purchased from Dojindo Laboratory (Kumamoto, Japan) and used as received. Carboxymethoxy-*p*-sulfocalix[6]arene (Calix[6]–SO₃CO₂) was synthesized according to the literature in four steps; hexamization of *tert*-butylphenol, dealkylation, sulfonation and esterification with chloroacetic acid [5–8]. *p*-Phenolsulfonic acid (PS) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan) and used without further purification.

2.3. Procedure for CE measurements

A solution containing 1·10^{–5} M calixarene and 6·10^{–5} M PS was used as a sample solution, and 3% (v/v) ethanol was added to monitor the electroosmotic flow (EOF). After the migrating solution was filled into a cathodic and an anodic reservoir, as well as into the capillary, the sample solution was injected into the capillary from the anodic end hydrodynamically for 3 s (injection volume: about 9 nl). Then a voltage of 20 kV was applied, and the analytes, calixarenes, were detected photometrically at 210 nm. Throughout the experiments, the capillary was held in a thermostated compartment controlled at 35.0°C. The apparent electrophoretic mobility of calixarenes ($-\mu'_{ep}$) is defined by Eq. (1):

Table 1
Component of the buffers used to make the CE measurements

Species	Concentration of species including buffer (mM)						
Component of the buffer used for the equilibrium analysis of calixarenes with alkali metal ions							
M ⁺ -borate buffer (pH 9.00) ^a	1.00	2.00	4.00	6.00	8.00	10.0	12.0
Total borate ion (H ₃ BO ₃ + H ₂ BO ₃ ⁻) ^a	1.00	2.00	4.00	6.00	8.00	10.0	12.0
Alkali metal ion ^b	0.365	0.730	1.46	2.19	2.92	3.65	4.38
Component of the buffer used for the equilibrium analysis of calixarenes with quaternary ammonium ions							
K ⁺ -borate buffer (pH 9.00) ^a	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total borate ion (H ₃ BO ₃ + H ₂ BO ₃ ⁻) ^a	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Potassium ion ^b	0.365	0.365	0.365	0.365	0.365	0.365	0.365
Quaternary ammonium ion ^c	0.00	0.100	0.200	0.300	0.400	0.500	

^a Calculated from the amount of boric acid added on preparing the buffer.

^b Calculated from the amount of alkali metal hydroxide added on preparing the buffer.

^c Calculated from the amount of quaternary ammonium chloride added on preparing the buffer.

$$-\mu'_{\text{ep}} = -\mu'_{\text{ep, calix}} - \mu'_{\text{ep, EOF}} \quad (1)$$

By measuring the migration time of calixarenes and ethanol as an EOF marker, $-\mu'_{\text{ep, calix}}$ (the apparent mobility of calixarene) and $\mu'_{\text{ep, EOF}}$ (the velocity of EOF, reduced to the mobility) were calculated, and $-\mu'_{\text{ep}}$ was obtained using Eq. (1).

3. Results and discussion

3.1. Equilibrium analysis of reactions of water-soluble calixarenes with alkali metal ions

Calixarenes are reactive with various types of

guest molecules. The water-soluble calixarenes used in this work possess sulfonate groups and are highly charged; therefore, suspected interacting substances must be removed from the migrating solutions. First, the reaction with alkali metal ions was investigated; migrating solutions containing an alkali metal ion with the buffer components were used. As a counter anion, borate was selected due to its lesser reactivity with anionic and cationic species. The electrophoretic mobility of calixarenes was measured using a series of the migrating buffers, where the pH of the migrating solution was well controlled and the concentration of alkali metal ion was varied. As much as 1 mM concentration of the buffer was allowed from the viewpoint of the stability of the EOF. Based on the experimental conditions of the

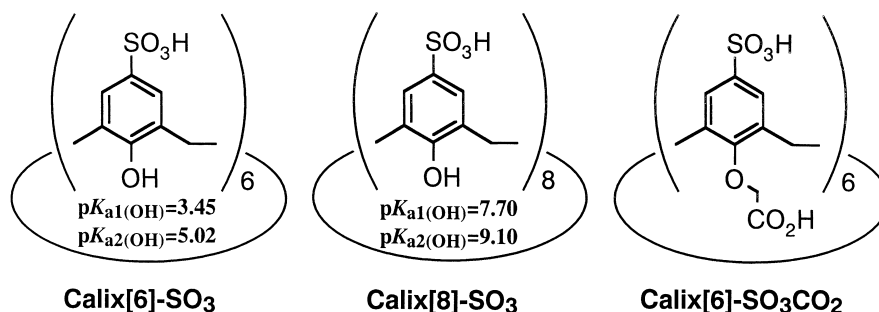


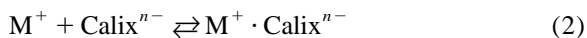
Fig. 1. Structures of *p*-sulfocalix[6]arene (Calix[6]-SO₃), *p*-sulfocalix[8]arene (Calix[8]-SO₃) and carboxymethoxy-*p*-sulfocalixarene (Calix[6]-SO₃CO₂).

equilibrium analysis, the concentrations of interacting ions should be in large excess to that of an analyte, a calixarene. Therefore, the migrating solution adopted in this study was prepared to satisfy these conditions.

Changes in apparent electrophoretic mobility of the calixarenes with increasing concentrations of alkali metal ions are shown in Fig. 2. The electrophoretic mobility of PS was almost constant at $-4.4 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ in the buffer concentration ranges examined, which indicates that PS did not interact with the cations existing in the migrating solution, and that the measurement of the electrophoretic mobility was satisfactorily carried out.

The complex formation reaction and its equilibrium constant between alkali metal ion, M^+ , and

anionic calixarene, Calix^{n-} , are shown in Eqs. (2) and (3), respectively:



$$K_C = \frac{[M^+ \cdot \text{Calix}^{n-}]}{[M^+][\text{Calix}^{n-}]} \quad (3)$$

where K_C is a complex formation constant. The apparent electrophoretic mobility of the calixarene, $-\mu_{ep}'$, is given in Eq. (4):

$$\begin{aligned} -\mu_{ep}' &= \frac{[\text{Calix}^{n-}](-\mu_{ep,\text{calix}}) + [M^+ \cdot \text{Calix}^{n-}](-\mu_{ep,\text{calix}-M})}{[\text{Calix}^{n-}] + [M^+ \cdot \text{Calix}^{n-}]} \\ &= \frac{(-\mu_{ep,\text{calix}}) + K_C[M^+](-\mu_{ep,\text{calix}-M})}{1 + K_C[M^+]} \quad (4) \end{aligned}$$

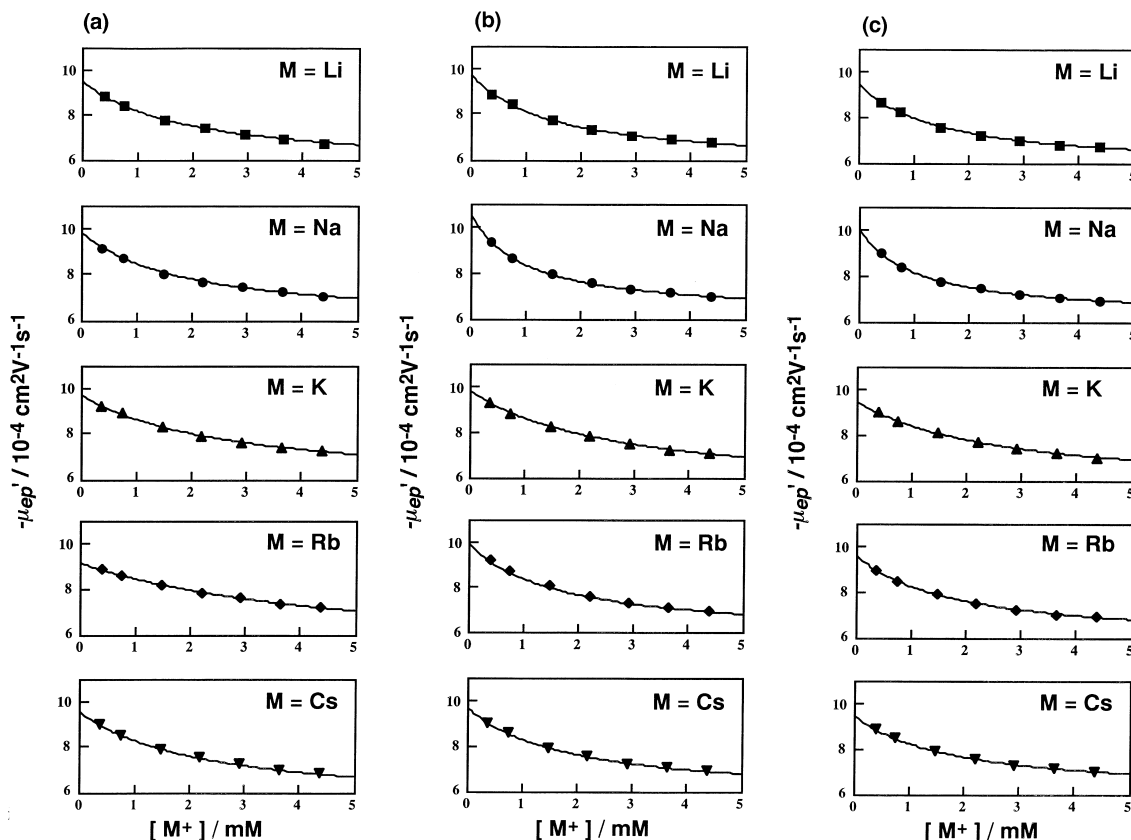


Fig. 2. Changes in electrophoretic mobility of water-soluble calixarenes with increasing concentrations of alkali metal ions. Migrating solution: 1–12 mM $\text{H}_3\text{BO}_3\text{-MOH}$ (pH 9.00). Sample solution: $1 \cdot 10^{-5}$ water-soluble calixarenes + $6 \cdot 10^{-5}$ *M* *p*-phenolsulfonate + 3% (v/v) ethanol. CE conditions: applied voltage 20 kV; detection wavelength, 210 nm; capillary temperature, 35.0°C; injection period, 3 s. Calixarenes: (a) Calix[6]- SO_3^- ; (b) Calix[8]- SO_3^- ; (c) Calix[6]- SO_3CO_2^- .

Table 2
Complex formation constants (K_C) of calixarenes and crown ether and thermodynamic equilibrium constant (K_C^0) of calixarenes

Alkali metal ions or quaternary ammonium ions	$\log K_C$ ($\log K_C^0$) ^a			
	Calix[6]–SO ₃	Calix[8]–SO ₃	Calix[6]–SO ₃ CO ₂	18-Crown-6 ^b
Li ⁺	2.71±0.03 (2.71±0.03)	2.83±0.03 (2.83±0.03)	2.83±0.02 (2.84±0.02)	–
Na ⁺	2.72±0.07 (2.73±0.06)	3.00±0.04 (3.01±0.04)	2.98±0.05 (2.99±0.05)	0.80
K ⁺	2.55±0.05 (2.55±0.05)	2.56±0.03 (2.56±0.03)	2.57±0.05 (2.57±0.05)	2.03
Rb ⁺	2.29±0.04 (2.28±0.04)	2.78±0.05 (2.76±0.06)	2.73±0.05 (2.73±0.05)	1.56
Cs ⁺	2.64±0.05 (2.64±0.05)	2.72±0.03 (2.72±0.03)	2.73±0.04 (2.73±0.04)	0.99
TMA ⁺	4.01±0.25 (4.02±0.22)	4.23±0.30 (4.30±0.21)	2.94±0.12 (2.94±0.13)	–
TEA ⁺	3.79±0.13 (3.79±0.12)	3.95±0.12 (4.04±0.16)	3.10±0.11 (3.10±0.11)	–
TPA ⁺	3.64±0.11 (3.64±0.09)	3.96±0.15 (4.00±0.22)	3.30±0.10 (3.30±0.11)	–
TBA ⁺	3.59±0.11 (3.59±0.10)	3.72±0.08 (3.72±0.13)	2.92±0.08 (2.93±0.08)	–

^a Values in parentheses are thermodynamic complex formation constants; Error: 3σ .

^b Cited from Ref. [15].

where $-\mu_{\text{ep,calix}}$ and $-\mu_{\text{ep,calix-M}}$ are the electrophoretic mobilities of free calixarenes and of the complex, respectively. The formation constants were determined by a non-linear least-squares analysis using the mobility change in a similar manner to our previous study [9]. The formation constants and electrophoretic mobility obtained are summarized in Tables 2 and 3, respectively. The curves shown in Fig. 2 are the simulated results obtained by using the values obtained, K_C and $-\mu_{\text{ep,calix-M}}$. The experimental and simulated results agreed well with each other, however, the trends in variations of

$-\mu_{\text{ep,calix-M}}$ in Table 3 are only slightly consistent with alkali metal ions, because, $-\mu_{\text{ep,calix-M}}$ values do not decrease significantly with the large increase in volumes and the masses of the alkali metal ion.

Under the present experimental conditions, ionic strength is not always constant, and the activity of the analytes and the measurement of the mobility must be reconfirmed. The electrophoretic mobility of the monomer unit, PS, is constant, and therefore, the measurements in these conditions are regarded as correct ones. Further, the formation constants were also determined by using the activities of alkali metal

Table 3
Electrophoretic mobility of calixarenes and their complexes

Interacting reagent	$-\mu_{\text{ep,calix}}$ (10^{-4} cm ² V ⁻¹ s ⁻¹) ^a		
	Calix[6]–SO ₃	Calix[8]–SO ₃	Calix[6]–SO ₃ CO ₂
None	9.53 (9.54)	9.83 (9.94)	9.58 (9.59)
Alkali metal ions			
Li ⁺	5.61±0.12 (5.53±0.11)	5.70±0.09 (5.62±0.10)	5.73±0.07 (5.66±0.07)
Na ⁺	5.84±0.25 (5.77±0.25)	6.19±0.12 (6.14±0.12)	6.19±0.14 (6.14±0.14)
K ⁺	5.64±0.22 (5.52±0.23)	5.32±0.20 (5.17±0.15)	5.52±0.20 (5.40±0.21)
Rb ⁺	4.90±0.25 (4.67±0.27)	5.79±0.20 (5.71±0.21)	5.76±0.18 (5.67±0.19)
Cs ⁺	5.37±0.25 (5.27±0.25)	5.68±0.11 (5.59±0.11)	5.94±0.13 (5.85±0.14)
Quaternary ammonium ions			
TMA ⁺	7.74±0.23 (7.73±0.23)	8.23±0.18 (8.23±0.29)	6.89±0.22 (6.78±0.23)
TEA ⁺	6.62±0.28 (6.61±0.27)	7.49±0.15 (7.53±0.29)	6.13±0.26 (6.12±0.28)
TPA ⁺	5.89±0.32 (5.86±0.31)	7.08±0.25 (7.09±0.36)	5.48±0.25 (5.33±0.33)
TBA ⁺	5.68±0.38 (5.66±0.38)	6.12±0.24 (6.05±0.40)	4.80±2.62 (4.80±0.16)

^a Values refer to $-\mu_{\text{ep,calix}}$, $-\mu_{\text{ep,calix-M}}$ or $-\mu_{\text{ep,calix-Q}}$; error: 3σ . Values in parentheses are the electrophoretic mobility of the calixarenes and their complexes determined with activity of alkali metal ions and/or quaternary ammonium ions.

ions in a similar manner, where the activities were calculated by the Debye–Huckel equation. Eqs. (3) and (4) are rewritten as Eqs. (5) and (6), respectively, by using the activities:

$$K_C^O = \frac{a_{M^+} \cdot a_{\text{Calix}^{n-}}}{a_{M^+} \cdot a_{\text{Calix}^{n-}}} \quad (5)$$

$$-\mu'_{ep} = \frac{(-\mu_{ep,calix}) + K_C^O a_{M^+} (-\mu_{ep,calix-M})}{1 + K_C^O a_{M^+}} \quad (6)$$

where K_C^O is a thermodynamic equilibrium constant; the values obtained are also summarized in Table 2. The equilibrium constants obtained on the basis of activity of alkali metal ions agreed well with those obtained on the basis of the concentration of alkali metal ions. The results indicate that the analysis of the complex formation reaction on the basis of the concentration of alkali metal ions is reasonable.

Fig. 3 shows the plot of $-\mu_{ep,calix-M}$ against the crystal ionic diameter of alkali metal ions. In spite of the large difference in the volume and the mass of alkali metal ions, the variation of $-\mu_{ep,calix-M}$ values with the type of alkali metal ion is slight or almost non-existent. Therefore, it is difficult to consider that the reaction of calixarenes with alkali metal ions is a simple complexing reaction. In general, the mobility is effected by the charge, mass, and volume of the species. The values should decrease with increasing apparent mass of calixarene complex with alkali metal ions. However, the observed results do not agree with the expectations since $-\mu_{ep,calix-M}$ should become smaller with heavier alkali metal ions. In this case, it is expected that $-\mu_{ep,calix-M}$ of calixarene is mainly concerned with the mass and the volume of complex, because alkali metal ions would be included in the cavity of calixarene. In aqueous solutions, lithium and sodium ions are highly hydrated among alkali metal ions, and therefore, the differences in the mass and the ionic diameter among alkali metal ions becomes smaller by the hydration. In addition to these effects of the mass and the volume of alkali metal ions, it is also expected that the structure of the complex of calixarene with alkali metal ions affects $-\mu_{ep,calix-M}$. Shinkai et al. synthesized many kinds of calixarene derivatives and analyzed their con-

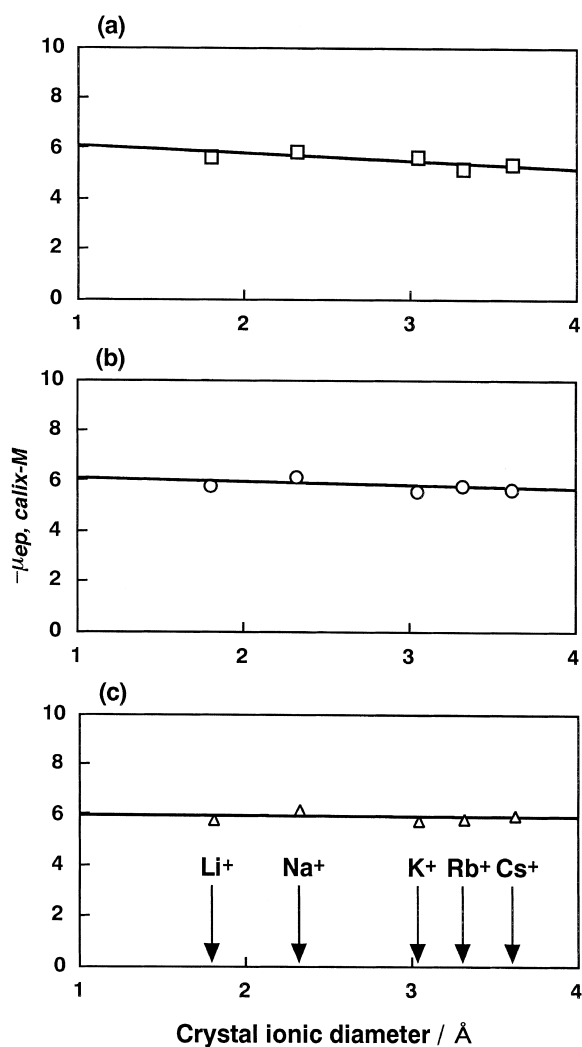


Fig. 3. Plots of $-\mu_{ep,calix-M}$ against crystal ionic diameter of alkali metal ions. □, Calix[6]-SO₃; ○, Calix[8]-SO₃; Δ, Calix[6]-SO₃CO₂.

formations [10]. Calixarene made up of phenol and methylene units, having a flexible structure, which confirmed that calixarene forms various complex conformations such as Cone, Pinched cone, Partial Cone, and Alternate with each alkali metal ions. By forming such flexible and convertible structures, the volume of the calixarene complex formed with alkali metal ions is not rigid. Therefore values of

$-\mu_{ep,calix-M}$ do not seem to be related to the ionic diameter.

3.2. Equilibrium analysis of the reaction of water-soluble calixarenes with quaternary ammonium ions

When the solutions of H_3BO_3 partly neutralized with quaternary ammonium (Q^+) hydroxide were used as the migrating buffers with the total borate concentration of 1 mM, the mobility of the calixarenes changed with increasing concentrations of Q^+ . The result indicates that some parts of the calixarene can form a complex with quaternary ammonium ions even at such low concentrations. Therefore, in the equilibrium analysis of the reaction

of calixarenes with Q^+ , the solutions containing 1 mM H_3BO_3 –KOH buffer (pH 9.00) with various amounts of $Q^+ \cdot Cl^-$ were adopted, because of a similar reactivity of potassium ion with the respective calixarene derivatives and simple comparison of contribution of quaternary ammonium ion being easily achieved.

Changes in apparent electrophoretic mobility of calixarenes with increasing concentrations of quaternary ammonium ions are shown in Fig. 4. The electrophoretic mobility decreased significantly in the concentration range of quaternary ammonium ion at sub-mM level, while the electrophoretic mobility of PS was almost identical in the concentration ranges examined.

The complex formation reaction and its equilibrium constant between quaternary ammonium ion,

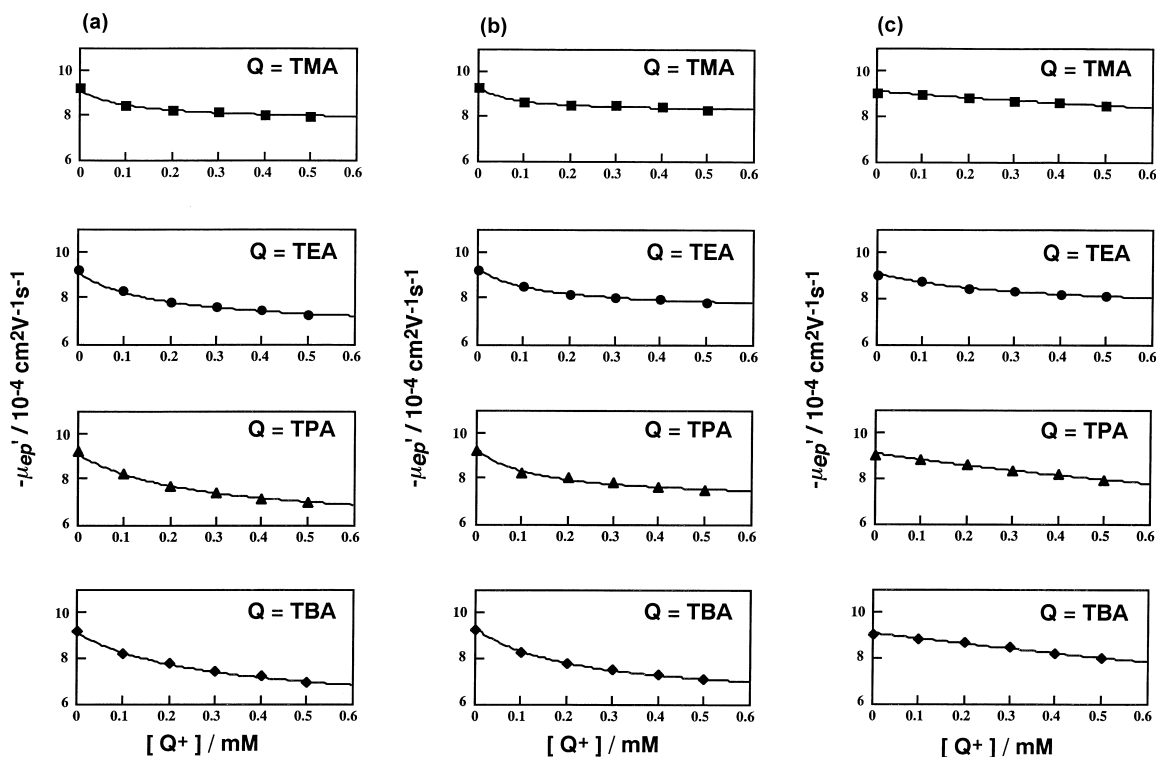
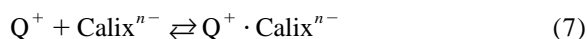


Fig. 4. Changes in electrophoretic mobility of water-soluble calixarenes with increasing concentrations of quaternary ammonium ions. Migrating solution: 1 mM H_3BO_3 –KOH buffer (pH 9.00)+0–0.5 mM quaternary ammonium salt. Sample solution and CE conditions as in Fig. 2. Calixarene: (a) Calix[6]– SO_3 ; (b) Calix[8]– SO_3 ; (c) Calix[6]– SO_3CO_2 .

Q^+ , and anionic calixarene, $Calix^{n-}$, are written as in Eqs. (7) and (8), respectively:



$$K_C = \frac{[Q^+ \cdot Calix^{n-}]}{[Q^+][Calix^{n-}]} \quad (8)$$

The complex formation constants of quaternary ammonium ions were also determined using the apparent electrophoretic mobility by applying a non-linear least-squares analysis represented in Eq. (9):

$$\begin{aligned} & -\mu'_{ep} \\ &= \frac{[Calix^{n-}](-\mu_{ep,calix}) + [K^+ \cdot Calix^{n-}](-\mu_{ep,calix-K}) + [Q^+ \cdot Calix^{n-}](-\mu_{ep,calix-Q})}{[Calix^{n-}] + [K^+ \cdot Calix^{n-}] + [Q^+ \cdot Calix^{n-}]} \\ &= \frac{(-\mu_{ep,calix}) + K_{C,K}[K^+](-\mu_{ep,calix-K}) + K_C[Q^+](-\mu_{ep,calix-Q})}{1 + K_{C,K}[K^+] + K_C[Q^+]} \quad (9) \end{aligned}$$

where the equilibrium constant, obtained between K^+ and the corresponding calixarene, $K_{C,K}$, the concentration of K^+ , and $(-\mu_{ep,calix-K})$ value were handled as known values, $[Q^+]$ and $-\mu'_{ep}$ were as input variables, and the values of K_C and $(-\mu_{ep,calix-Q})$ were optimized. The formation constants thus obtained are also summarized in Tables 2 and 3. The curves in Fig. 4 are the results simulated using the experimentally obtained values. The experimental and the simulated results agreed well with each other, which indicates that the analysis of complex formation reaction is reasonable.

The equilibrium constants were also evaluated on the basis of the activities, in a similar manner as the alkali metal ions. By using the activities, Eqs. (8) and (9) are rewritten as in Eqs. (10) and (11), respectively:

$$K_C^O = \frac{a_{Q^+ \cdot Calix^{n-}}}{a_{Q^+} \cdot a_{Calix^{n-}}} \quad (10)$$

$$\begin{aligned} & -\mu'_{ep} \\ &= \frac{(-\mu_{ep,calix}) + K_{C,K}^O a_{K^+}(-\mu_{ep,calix-K}) + K_C^O a_{Q^+}(-\mu_{ep,calix-Q})}{1 + K_{C,K}^O a_{K^+} + K_C^O a_{Q^+}} \quad (11) \end{aligned}$$

The formation constants, K_C^O , and the electrophoretic mobility obtained are also summarized in Tables 2 and 3, respectively. The values obtained with the concentration or the activities of quaternary ammonium ions and potassium ion agreed well with the

values obtained with their concentrations. The results indicate that the analysis of the complex formation reaction with concentration of quaternary ammonium ions and potassium ion is reasonable.

3.3. Comparison of the reactivity of alkali metal ions with quaternary ammonium ions

The reactivity of the water-soluble calixarenes can be considered from the formation constants determined in this work. Acid dissociation constants of the calixarenes were reported as $pK_{a1}=3.45$ and $pK_{a2}=5.02$ for the hydroxy groups of Calix[6]-SO₃, and $pK_{a1}=7.70$ and $pK_{a2}=9.10$ for the hydroxyl groups of Calix[8]-SO₃ [11–13]. These pK_a values are smaller than the pK_a value for hydroxyl group of the monomer (*p*-phenolsulfonic acid). Such smaller pK_a values for calixarenes are due to stabilization through intramolecular hydrogen bond between hydroxyl groups of calixarene, which promotes the acid dissociation reaction. From these constants, the average charges of the calixarenes are calculated to be -8 and -9.5 at pH 9.00 for Calix[6]-SO₃ and Calix[8]-SO₃, respectively. Similarly, the average charge of Calix[6]-SO₃CO₂ is considered to be -12 at pH 9.00, whereas the average charge of PS is calculated to be about -1.5 under the experimental conditions [14]. The experimental results show that the electrophoretic mobility of the calixarenes was larger than that of their monomer unit, PS. These results can be attributed to the condensed charge density and the condensed molecular volume of the calixarene as a cyclical structure.

Fig. 5 shows the plots of the logarithmic values of K_C against the crystal ionic diameters of alkali metal ions. The complex formation constants of calixarenes with alkali metal ions are in the order of $10^{2.5}$ – $10^{3.0}$ (Table 2). Crown ethers are well known to incorporate alkali metal ions in their cavity in an aqueous solution [15], and the equilibrium constants of 18-crown-6 are 10^1 – 10^2 , as is listed in Table 2. Therefore, the calixarenes are proved to be more reactive than the crown ether in the magnitude of $10^{0.5}$ – 10^2 . Such high reactivity in the calixarenes can be attributed to the large number of the charge which enhances the electrostatic interaction, in addition to the incorporation ability of alkali metal ions

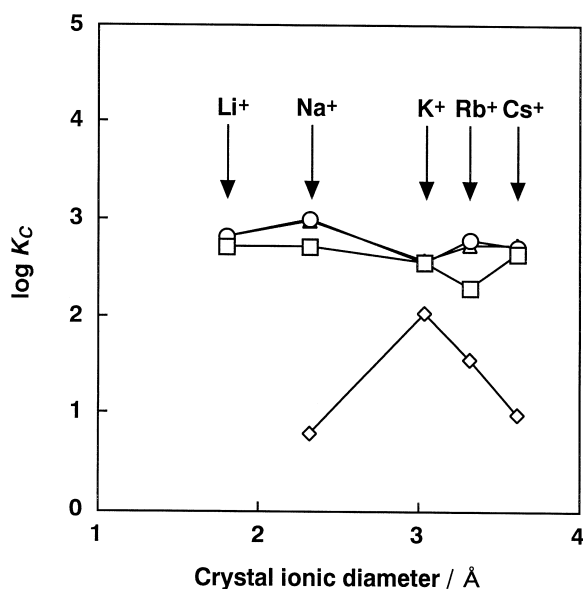


Fig. 5. Plots of K_c against crystal ionic diameter of alkali metal ions. □, Calix[6]-SO₃; ○, Calix[8]-SO₃; △, Calix[6]-SO₃CO₂; ◇, 18-Crown-6.

into the cavity of calixarenes. From the comparison of the formation constants between alkali metal ions and calixarenes, it is noticed that the differences in the formation constants are small, and show less selectivity towards alkali metal ions. Shinkai et al. synthesized flexible calixarene, methoxy-*p*-*tert*-butylcalix[4]arene, and determined the complex formation constants with alkali metal ions [16]. The flexible calixarene was less selective toward alkali metal ions, while the rigid ones reacted with alkali metal ions selectively, as synthesized by Shinkai et al. [17]. For instance, calix[4]arene crown ether possessing a short crown loop such as $-(\text{CH}_2\text{CH}_2\text{O})_2\text{CH}_2\text{CH}_2-$ on the narrower rim (hydroxyl side of calixarene), a rigid one, showed Na⁺ selectivity with respect to K⁺. Therefore, the trend of less selectivity obtained in this work is affected by a smaller difference in both ionic diameter of alkali metal ions and the very flexible structure of the calixarenes.

The reactivity of the calixarenes with quaternary ammonium ions is much higher than with alkali metal ions (Table 2). The results suggest that the quaternary ammonium ions can interact with the hydrophobic moiety of the calixarenes through hy-

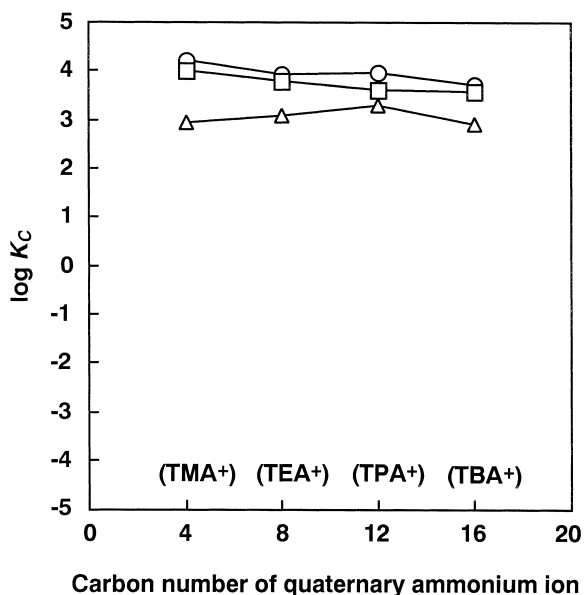


Fig. 6. Plots of $\log K_c$ against carbon number of quaternary ammonium ions. □, Calix[6]-SO₃; ○, Calix[8]-SO₃; △, Calix[6]-SO₃CO₂.

drophobic interactions, in addition to the electrostatic interactions. Fig. 6 shows the plots of $\log K_c$ values against the carbon number of quaternary ammonium ions. The formation constants of Calix[6]-SO₃CO₂ are smaller than those of Calix[6]-SO₃ and Calix[8]-SO₃. In the case of Calix[6]-SO₃CO₂, carboxyl groups are attached to the narrower rim (hydroxyl side of the calixarene), and the ring size of narrower rim should be larger, because the carboxyl groups show steric hindrance and electrostatic repulsion. Therefore, the ring size of wider rim (sulfonate side of the calixarene), the opposite side of the narrower rim, should become smaller, and therefore, the ring size of Calix[6]-SO₃CO₂ should be smaller than those of Calix[6]-SO₃ and Calix[8]-SO₃. The smaller the ring size, the smaller the complex formation constant, therefore it is difficult to incorporate quaternary ammonium ion into Calix[6]-SO₃CO₂. Furthermore, from a comparison of the formation constants, it is found that the difference in the formation constants is small among the quaternary ammonium ions, and the selectivity toward them is small.

Small quaternary ammonium ions would be easy to incorporate into calixarene. Therefore, the effect

of the electrostatics can be accepted, because the distance between the negative charge and the positive charge is shortened by the incorporation. In contrast, the bulky quaternary ammonium ion is difficult to incorporate, and a non-incorporated complex of simple ion associate is expected in the aqueous solution; the reaction in this case is effected by hydrophobicity. Less selectivity among quaternary ammonium ions should be attributed to such compensating effects of electrostatics and hydrophobicity.

In conclusion, we have succeeded in analyzing the equilibrium reaction between anionic calixarene and pairing cations. However, the calixarenes did not show any significant selectivity among the alkali metal ions or the quaternary ammonium ions in aqueous solution. Less selectivity toward alkali metal ions can be explained by the flexible conformation of the calixarene, and that toward quaternary ammonium ions is attributed to the complicated interactions such as the compensation of electrostatic and hydrophobic interactions.

Acknowledgements

This study was supported in part by Grant-in-Aid No.13440220 from the Ministry of Education, Science, Sports and Culture, Japan.

References

- [1] C.D. Gutsche, *Acc. Chem. Res.* 16 (1983) 161.
- [2] D.J. Cram, M. Cram (Eds.), *Container Molecules and Their Guests*, Royal Society of Chemistry, London, 1994, p. 20, Chapter 2.
- [3] Y.A. Zolotov, *Macrocyclic compounds*, in: *Analytical Chemistry*, Wiley, Chichester, 1997, p. 63, Chapter 3.
- [4] C.D. Gutsche, in: *Calixarenes*, Royal Society of Chemistry, London, 1989, p. 77, Chapter 3.
- [5] C.D. Gutsche, B. Dhawan, K.-H. No, R. Muthukrishnan, *J. Am. Chem. Soc.* 103 (1981) 3782.
- [6] C.D. Gutsche, L.-G. Lin, *Tetrahedron* 42 (1986) 1633.
- [7] S. Shinkai, K. Araki, T. Tsubaki, T. Arimudra, O. Manabe, *J. Chem. Soc., Perkin Trans. I* (1987) 2297.
- [8] S. Shinkai, S. Mori, H. Koreishi, T. Tsubaki, O. Manabe, *J. Am. Chem. Soc.* 108 (1986) 2409.
- [9] T. Takayanagi, E. Wada, S. Motomizu, *Analyst* 122 (1997) 57.
- [10] A. Ikeda, S. Shinkai, *Chem. Rev.* 97 (1997) 1713.
- [11] J.L. Atwood, D.L. Clark, R.K. Juneja, G.W. Orr, K.D. Robinson, R.L. Vincent, *J. Am. Chem. Soc.* 114 (1992) 7558.
- [12] Y. Zhang, I.M. Warner, *J. Chromatogr. A* 688 (1994) 293.
- [13] I. Rodriguez, H.K. Lee, S.F.Y. Li, *Talanta* 45 (1998) 683.
- [14] C.D. Gutsche, in: *Calixarenes Revisited*, Royal Society of Chemistry, London, 1998, p. 32, Chapter 3.
- [15] T. Okada, *J. Chromatogr. A* 834 (1999) 73.
- [16] A. Ikeda, S. Shinkai, *J. Am. Chem. Soc.* 116 (1994) 3102.
- [17] H. Yamamoto, S. Shinkai, *Chem. Lett.*, (1994) 1115