USE OF AN AMPHIPHILIC CALIX[4]ARENE WITH SULFONATE FUNCTIONALITY AT THE LOWER RIM AS A BUFFER ADDITIVE IN CAPILLARY ELECTROPHORESIS

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SUMMARY

An amphiphilic calix[4]arene with sulfonate groups on the lower rim has been investigated as a buffer additive for separation of positional isomers by capillary electrophoresis. Addition of the compound to the running buffer led to successful separation of positional isomers of nitrophenols, dinitrobenzenes, and benzenediols. Association constants between the calixarene and the analytes indicated that size-selective host–guest interactions between them played a prominent role in determining the separation behavior.

INTRODUCTION

Capillary electrophoresis (CE) is a powerful and versatile separation technique by which a variety of compounds can be analyzed with much lower consumption of samples and running buffer than in liquid chromatography [1]. CE is further promoted by use of a selector. For example, macrocyclic molecules such as crown ethers [2,3], cyclodextrins [4,5], and calixarenes [6–9] have been investigated for selective separation of a variety of enantiomers or positional isomers.

We have previously reported that sulfonated β-cyclodextrin acted as a good buffer additive in the CE separation of endocrine-disrupting chemicals commonly detected in natural water [10]. The ability of cyclodextrins to act as hosts arises from their well-defined hydrophobic cavities;

these enable size-selective separation of neutral analytes as a result of host–guest complexation.

Calixarenes also occupy a prominent position in host–guest chemistry [11–13]. Their use as adsorbents [14] and as selectors in CE [9] has recently been studied using the water-soluble calix[4]arene **2** with sulfonate groups on the upper rim, as shown in Fig. 1. Calixarene also has another functionalization site on the lower rim, however; modification of this can further expand its versatile properties as a host.
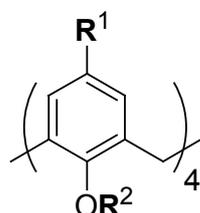
1: R^1 = *tert*-Bu, R^2 = $CH_2CH_2CH_2SO_3Na$
2: R^1 = SO_3Na, R^2 = H

Fig. 1

The structures of calix[4]arenes **1** and **2**

In this study, with these points in mind, we have examined calixarene **1** (Fig. 1), with sulfonate groups at the lower rim, as a buffer additive in CE. Calixarene **1** is more amphiphilic than **2**, because the former has hydrophobic *tert*-butyl groups and hydrophilic sulfonate groups on the upper and lower rims, respectively, whereas in the latter calixarene sulfonate groups are present on the upper rim in addition to hydrophilic hydroxyl groups on the lower rim. Indeed, because of the different arrangement the separation behavior of calixarene **1** is different from that of **2**.

The purpose of this study was to examine the performance of **1** as a novel buffer additive in the CE of positional isomers such as nitrophenols, dinitrobenzenes, and benzenediols. Here, we report three findings:

1. successful separation of the positional isomers;
2. selectivity of **1** for positional isomers; and
3. a separation mechanism.

EXPERIMENTAL

Apparatus

CE experiments were performed with a Matsusada-Precision (Tokyo, Japan) HCZE-30PNO.25 high-voltage power supply, a Jasco (Tokyo, Japan) CE-970 UV–visible detector, and a Hitachi (Tokyo, Japan) D-2500 integrator. Fused-silica capillary tubing (25 μm i.d.) was purchased from GL-Science (Tokyo, Japan). The total length of the capillary was 50 cm (41.5 cm from inlet to detector). Sample injection was performed hydrodynamically, from the positive end, for 10 s. Before use, capillaries were conditioned by washing with 0.1 M NaOH for 5 min, with water for 5 min, and with the running buffer for 5 min. A constant potential of 25 kV was applied. Nitrophenols were monitored at 335 nm, dinitrobenzenes at 300 nm, and benzenediols at 280 nm. Experiments were performed at 25°C, and the effective mobilities of the solutes were evaluated by use of the standard procedure (section 'Electroosmotic Flow Mobility', below).

Reagents

Calixarene **1** was prepared by a procedure described elsewhere [15]. All analytes were purchased from Wako (Osaka, Japan) and were of HPLC grade. The aromatic compounds used were *o*, *m*, and *p*-nitrophenol, *o*, *m*, and *p*-dinitrobenzene, and *o*, *m*, and *p*-benzenediol. Stock solutions (20 mM) of each were prepared in 1:1 (*v/v*) acetonitrile–water. Acetonitrile was used as electroosmotic flow (EOF) marker. An aqueous solution of NaH_2PO_4 and Na_2HPO_4 was prepared as running buffer. After addition of appropriate amounts of 0.1 M stock solution of the calixarene to the buffer the resulting solutions were passed through a 0.2-μm cellulose acetate filter before use.

RESULTS AND DISCUSSION

Electroosmotic Flow Mobility

To evaluate the effective electrophoretic mobility of the solutes, electroosmotic flow (EOF) mobility (μ_{eo}) was determined in the absence and presence of calixarene **1** in the running buffer. The initial μ_{eo} of 2.16×10^{-4} cm^2 V^{-1} s^{-1} at pH 5 in the absence of **1** gradually changed to 2.27–2.31×10^{-4} cm^2 V^{-1} s^{-1} in the presence of 2.5–15 mM **1**. This increase in μ_{eo} could be attributed to reinforcement of the electric double layer

on the capillary wall, because the apparent number of negative charges on the capillary wall was increased by addition of calixarene **1** containing negatively charged sulfonate groups on the upper rim.

Separation of Positional Isomers

Nitrophenol isomers (NP) are almost uncharged at pH ≈ 5 (pK_a of *o*, *m*, and *p*-NP are 7.05, 8.09, and 6.90, respectively [16]), so their separation by conventional CZE is not feasible at this pH. Indeed, all the isomers were co-eluted under electrophoretic conditions with no additive, as shown in Fig. 2A. In contrast, on the basis of host–guest interactions between **1** and the analyte samples, the NP were resolved as the concentration of **1** in the running buffer was increased (Figs 2B and 2C). It is also
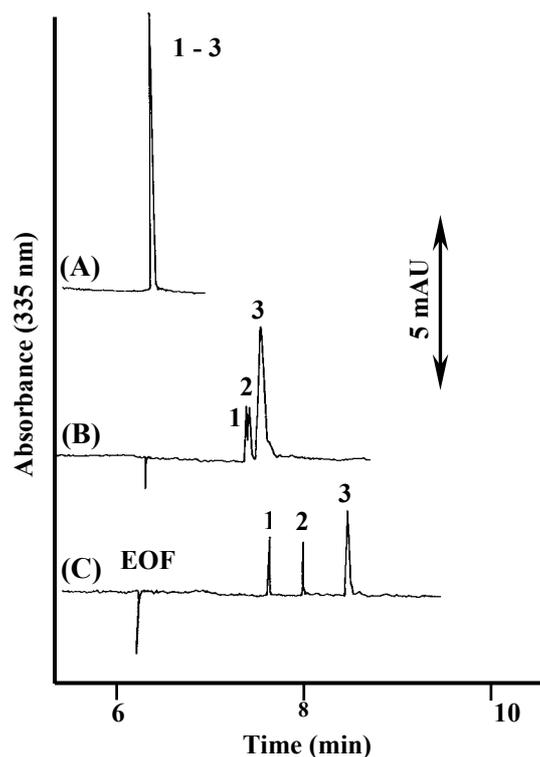

Fig. 2

Electropherograms obtained from the nitrophenols (1 mM) in the presence of (A) 0 mM, (B) 1.5 mM, and (C) 10 mM **1** in 10 mM phosphate buffer at pH 5. Analyte peaks: 1, *o*-nitrophenol; 2, *m*-nitrophenol; 3, *p*-nitrophenol. Separation conditions: capillary length, 25 μm i.d. × 50 cm (41.5 cm to detector); applied potential 25 kV; UV detection at 335 nm

of interest that the order of migration o-NP < m-NP < p-NP in the presence of **1** is in striking contrast with that reported for the analogous calixarene **2** – with this additive the positional isomers of the NP migrated in the opposite order, i.e. p-NP < m-NP < o-NP [9].

The order of migration of the NP was the opposite of that of the dinitrobenzenes (DNB) and benzenediols (BD), which are also neutral at pH 5. In the presence of **1** (7.5 mM) the positional isomers of the DNB were perfectly separated at pH 5 and migrated in the order m-DNB < p-DNB < o-DNB, as shown in Fig. 3A. In addition, in the presence of **1** (15 mM) the order of migration of the BD was p-BD < m-BD < o-BD, as shown in Fig. 3B.
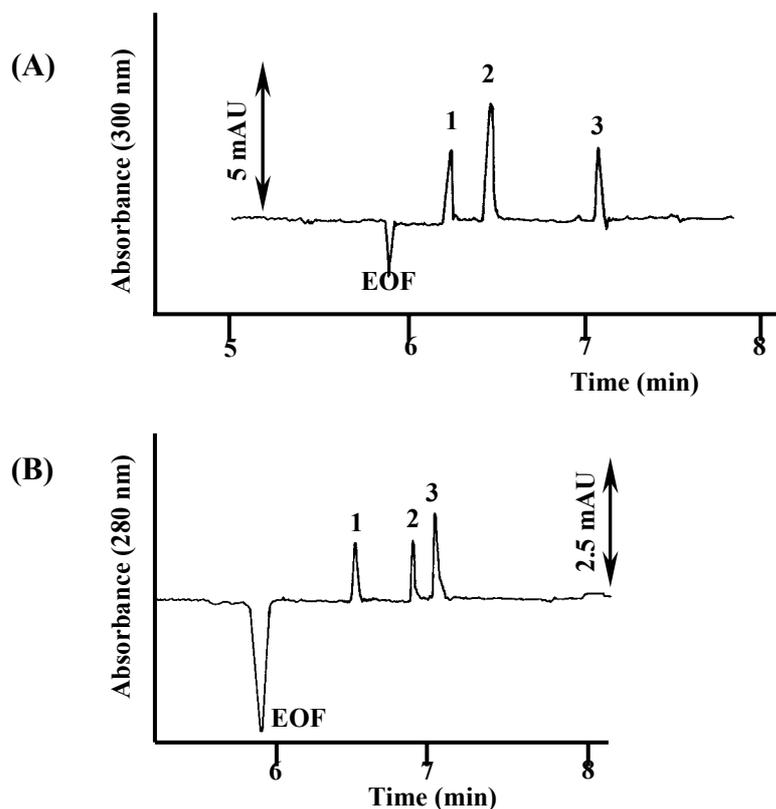

Fig. 3

Electropherograms obtained from (A) dinitrobenzenes and (B) benzenediols in the presence of **1** in 10 mM phosphate buffer at pH 5. Conditions for (A): concentration of **1** 7.5 mM; UV detection at 300 nm; peaks: 1, m-dinitrobenzene; 2, p-dinitrobenzene; and 3, o-dinitrobenzene. Conditions for (B): concentration of **1** 10 mM; UV detection at 280 nm; peaks: 1, p-benzenediol; 2, m-benzenediol; and 3, o-benzenediol. Other conditions were as for Fig. 2

Separation Mechanism

As shown in Figs 2 and 3, the selectivity of **1** for the positional isomers was strongly affected by the types of functional group present in the compounds. To clarify the observed separation behavior, association constants (K_{ass}) between **1** and the analytes were determined by the method reported by Kuhn et al. [17], who successfully applied the well-established Benesi–Hildebrand method [18] to CE. As summarized in Table I, the K_{ass} values increased in the order $o < m < p$ for the NP, $m < p < o$ for the DNB, and $p < m < o$ for the BD.

Table I

Association constants (K_{ass}, M^{-1}) for the positional isomers and calixarene **1**

Solutes	Concentration range estimated (mM)	K_{ass} (M^{-1})
o-Nitrophenol	1.5–10	18.1
m-Nitrophenol	1.5–10	22.9
p-Nitrophenol	1.5–10	36.4
o-Dinitrobenzene	2.5–10	13.1
m-Dinitrobenzene	2.5–10	8.9
p-Dinitrobenzene	2.5–10	9.5
o-Benzenediol	5.0–15	18.9
m-Benzenediol	5.0–15	14.1
p-Benzenediol	5.0–15	12.5

Among the NP, hydrophobic adsorption was observed for *p*-NP. The solubility of *o*, *m*, and *p*-NP was 0.2, 1.4, and 1.7 g, respectively, per 100 g water [19]. Contrary to our initial expectations, the order of hydrophobicity (*p*-NP < *m*-NP < *o*-NP) was inversely proportional to that of their associability with **1** (*o*-NP < *m*-NP < *p*-NP, Table I). This clearly indicated that for the NP the molecular size of each analyte rather than the degree of hydrophobicity has the decisive effect on selectivity for the positional isomers in size-selective supramolecular encapsulation into the hydrophobic cavity of **1**. In contrast with the NP, with the BD the order of their solubility [20] was more important than the molecular size of each analyte. The concentration of **1** required to separate the BD was higher than that required to separate the NP, and the association constants of the BD with **1** were, consequently, lower than those of the NP, as listed in Table I. It seems that the selectivity of the molecular size of **1** is low and so the hydrophobic interaction is enhanced. For the DNB there was no relationship

between the association constants ($m < p < o$) and either solubility or steric size. The solubilities of o, m, and p-DNB were 0.015, 0.5, and 0.008 g, respectively, in 100 g water ($p < o <$ m) [20]. The steric hindrance arising from the two nitro groups is too large to accommodate the DNB in the hydrophobic cavity of **1**, thus hampering association of **1** with the DNB, compared with the NP and BD, which bear less bulky substituents.

CONCLUSIONS

We have demonstrated that the water-soluble, amphiphilic calixarene **1** could be used as a selector in CE. Addition of **1** to the running buffer led to successful separation of positional isomers. Size-selective host–guest interactions were found to have an important effect on separation behavior. A principal weakness in the use of **1** as a CE additive is the unavoidable interference with UV-detection of the analytes owing to the UV-absorption of **1**; this eventually leads to much background noise and, hence, an unfavorable decrease in sensitivity. Nonetheless, this inherent defect of **1** may be minimized or eliminated by use of a different detection system.

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