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Separation of water-soluble *p*-sulfonated calixarenes 4, 6 and 8 and 4-hydroxybenzene sulfonate by use of capillary zone electrophoresis

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Abstract

A method for the separation of sulfonated calixarenes 4, 6 and 8 and the 4-hydroxybenzene sulfonate monomer by capillary zone electrophoresis with direct UV absorbance detection is described. The electroosmotic flow (EOF) decreases with addition of K^+ and reverses with addition of Mg^{2+}. The suppression of the EOF upon the addition of K^+ slightly improves the separation of the calixarenes and the monomer. However, the elution times of those compounds increase almost 35 min. The reversal of the EOF by addition of Mg^{2+} reduces the migration time and also improves the separation efficiency. The calixarenes are baseline separated in less than 14 min by use of a borate buffer with the addition of 4.0, 6.0 or 12.0 m*M* $MgCl_2$ at pH 8.3.

1. Introduction

Calixarenes are a class of cyclic phenols linked by methylene groups. These compounds can form a variety of host–guest type inclusion complexes [1–3]. Although the complexation properties of calixarenes have not been examined as extensively as those of cyclodextrins [4–6], calixarenes [1,2] have been used in the recovery of cesium and uranium, ion selective electrodes and field effect transistors. Other applications, such as phase transfer agents, hydrolysis catalysts and separation of organic molecules have also been reported. The most common forms of calixarenes are slightly soluble in several organic solvents, but are essentially insoluble in water. Therefore, experimental efforts have been directed toward enhancing the aqueous solubility of calixarenes. Several water-soluble derivatives of sulfonato- [7], amino- [8], nitro- [9], carboxyl- [10] and phosphonato- [11] calixarenes have been synthesized. The sulfonated calixarene derivatives are highly soluble in water and can be potentially useful as mimics of enzyme–substrate complexes [12].

Water-soluble sulfonated calixarenes are similar to cyclodextrins [4–6] and cyclophanes [13], in that they can form complexes with a variety of guests in aqueous solution [1,2] according to their size and hydrophobicity. Thus, the inclusion complexation abilities of calixarenes are often compared to those of cyclodextrins. The cavities of α-, β- and γ- cyclodextrins have inner

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diameters of 5.7, 7.8 and 9.5 Å, respectively [6]. In contrast, those of calixarene 4, calixarene 6 and calixarene 8 are 3.0, 7.6 and 11.7 Å, respectively [1]. The sizes of the calixarenes play important roles in their complexation capabilities.

In contrast to cyclodextrins, the calixarenes must be synthesized. The procedures for syntheses of calixarenes 4, 6 and 8 [14] are quite similar. Thus, it is sometimes difficult to obtain calixarenes with a specific number of phenol units. Since calixarenes have the same empirical formulas, it is also not possible to distinguish the differences by chemical analysis. Although NMR can provide some structural information about calixarenes, the low sensitivity of this technique is a problem for calixarene analysis. Therefore, separation methods can be very useful for detection and purification of calixarenes.

Capillary electrophoresis (CE) is a powerful separation technique for many compounds, especially charged compounds [15,16]. There are many advantages for the use of CE as a separation technique. These include high separation efficiency, high speed and low consumption of electrolytes and/or samples. The CE method has been used as a separation technique in many areas of research, including biological systems [17–20] to separate proteins, peptides, drugs and chiral compounds. It has also been employed to separate diverse species [21–23] such as inorganic acids, metal ions and surfactants.

In capillary zone electrophoresis (CZE), charged particles can be separated on the basis of differences in their electrophoretic mobilities (μ_{ep}) [16]. At pH 2–11, the inner walls of fused-silica capillaries are usually negatively charged. In the presence of an electric field, the electrolyte migrates toward the cathode as a flat flow profile with mobility μ_{eo}. The electroosmotic flow (EOF) forces all solutes (anionic, cationic, neutral) to migrate toward the cathode ends of the capillaries. In most instances, at high pH values μ_{eo} is larger than μ_{ep}. Therefore, anionic compounds are also carried to the cathode and detected [15,17]. The higher EOF gives shorter migration time, but poorer resolution. Cations, such as Ca^{2+}, Ba^{2+}, Mg^{2+}, Sr^{2+}, K^+ and Na^+, are often added to suppress the EOF and improve the separation efficiencies of anions [22]. The disadvantage of cation additives is that the migration times of the analytes in the capillaries are lengthened. To overcome this difficulty, cations are often used to reverse the electroosmotic flow at a certain pH.

In this manuscript, a procedure is described for separation of calixarenes and the phenol monomer by use of reversed electroosmotic flow CZE with UV absorbance detection. The electropherograms of calixarenes and the monomer as a function of different concentrations of Mg^{2+} also provide information about complex formation between the calixarenes and Mg^{2+}.

2. Experimental

2.1. Materials

Synthesis of water-soluble p-sulfonated calixarenes

The *p*-sulfonatocalixarenes 4, 6 and 8 (SCX4, SCX6 and SCX8) were synthesized by use of previously developed procedures of Gutsche et al. [14] and Shinkai et al. [7]. The synthesis is a three-step process to obtain the water-soluble sulfonated calixarenes. In the first step, *p-tert.*-butylcalixarenes are prepared according to the method of Gutsche et al. [14]. The second step involves debutylation of *p-tert.*-butylcalixarenes [24]. The last step involves sulfonation of the calixarenes in concentrated sulfuric acid (fuming sulfuric acid and 96% sulfuric acid) [7], producing the water-soluble sulfonated calixarenes. The structures of the calixarenes used in this study are provided in Fig. 1.

The sodium salt of the *p*-hydroxybenzene sulfonate monomer was purchased from Aldrich. The compounds $Na_2B_4O_7$, H_3BO_3, KCl and $MgCl_2$ were purchased from Fisher. All buffer solutions and samples were prepared by use of double ion-exchange deionized water and then passed through a 45-μm membrane filter.

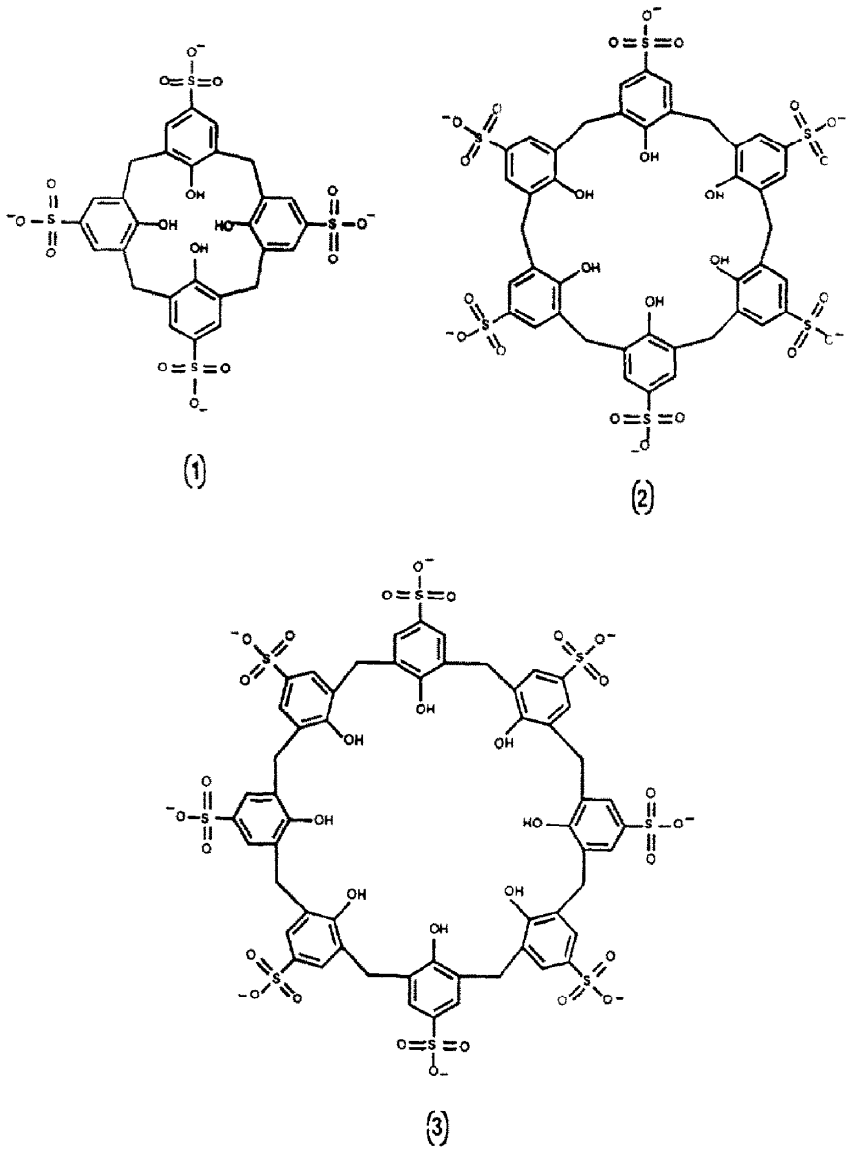

Fig. 1. Structures of *p*-sulfonated calixarenes: 1 = SCX4; 2 = SCX6; 3 = SCX8.

2.2. Apparatus

The CE instrument (CES1) was purchased from Dionex (Houston, TX, USA). Detection was by UV absorbance at 215 nm. Fused-silica capillaries (64 cm × 75 μm I.D.) were used. The distance between injection and detection was 60 cm. The applied electric field was 20 kV. All analyses were conducted at room temperature (21°C).

3. Results and discussion

For each separation buffer used in this study, the samples of calixarenes (about $1.0 \cdot 10^{-5}$ *M*):

SCX4, SCX6, SCX8, monomer (about $5 \cdot 10^{-5}$ *M*) and their mixtures were run at least three times, or until results were reproducible. The migration order of the mixture was assigned by testing the migration time of each component alone. The buffer was a combination of 50 m*M* H_3BO_3 and 10 m*M* $Na_2B_4O_7$. The pH of the buffer was 8.3. In this case, positive potential was employed. In other words, the anode was on the injection side. The electropherogram of the mixture of calixarenes and monomer under these conditions is shown in Fig. 2. The migration time of the monomer is about 8.3 min, which is far from the calixarenes (ca. 17 min). Although the calixarenes cannot be separated under these conditions, the elution order of the four analytes can still be determined, which is monomer, SCX4, SCX6 and then SCX8. This elution order can be explained by recalling that the inner surface of the capillaries contains $\equiv$Si–OH groups which are ionized to $\equiv$Si–O^- in alkaline and slightly acidic media (pH > 2). When an electric field is applied to the capillary, the positive counterions in the buffer migrate toward the cathode. Since the cations are solvated, the solvent flows with them, resulting in EOF. The direction of the EOF is identical for ionic and neutral molecules, despite their electric charges [16].

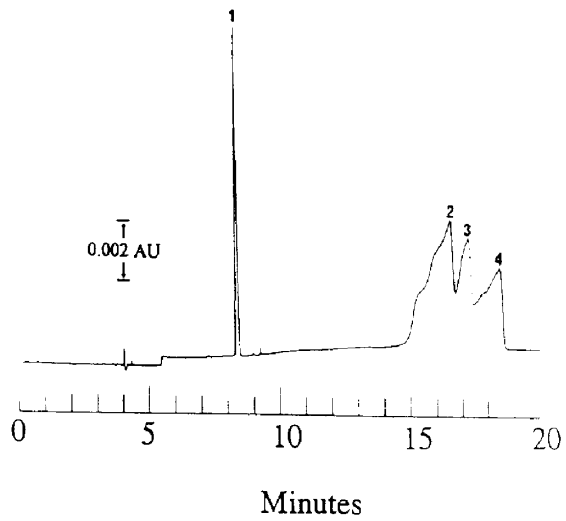

Fig. 2. Electropherogram of the calixarenes and phenol monomer; buffer: 50.0 m*M* boric acid and 10.0 m*M* sodium borate; pH 8.3. Peaks: 1 = monomer; 2 = SCX4; 3 = SCX6; 4 = SCX8.

It should also be noted that hydrolysis of the analytes and the pH of the buffer affects the separation efficiency and the elution order [25]. The pK_a values of the hydroxyl groups of the monomer unit, SCX4 and SCX6 have been reported. The pK_a values of SCX8 have not been found in the literature. Therefore, the discussions here regarding pK_a values are limited to monomer, SCX4 and SCX6. The pK_a values of SCX8 may be inferred from the trend observed relative to the three other analytes. As reported, for the monomer, the pK_a is 8.7 [26]; for SCX4: $pK_{a1} = 3$–4, $pK_{a2} = 11$ [27]; for SCX6: $pK_{a1} < 1$, $pK_{a2} = 3$, $pK_{a3} = 4$ and $pK_{a4} > 11$ [28]. The low pK_a values of the calixarenes are due to the formation of intramolecular hydrogen bonds which have been discussed previously [27,28]. Thus, at the buffer pH 8.3, the ionic forms of the analytes in the buffer solution is expected to be $[S]^-$; SCX4 as $[SCX4]^{5-}$; SCX6 as $[SCX6]^{9-}$. The charge-to-mass ratio for these three analytes are $5.78 \cdot 10^{-3}$, $6.76 \cdot 10^{-3}$ and $8.11 \cdot 10^{-3}$, respectively. If electrophoretic mobilities of ions are proportional to the values of charge to mass ratio [29], the mobilities of the analytes should be in the order of monomer < SCX4 < SCX6. Since all analytes are negatively charged, the electrophoretic mobilities of the analytes would be opposite to the direction of EOF. However, due to the dominant contribution of the EOF, the migration order of the analytes will be monomer, SCX4, SCX6 and then SCX8. The detection order in Fig. 2 agrees with this prediction. However, the separation of the calixarenes is not satisfactory. In the electropherogram, the peaks of calixarenes are very broad. To improve the separation of calixarenes, some manipulations of the buffer were undertaken.

3.1. K^+ and Mg^{2+} effects

The addition of cations in CE has been shown to decrease the EOF and improve the separation of many compounds such as sulfonate and sulfate surfactants [22]. Therefore, different amounts of

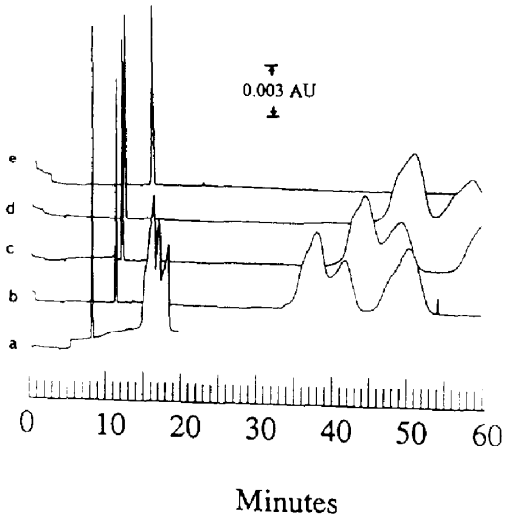

Fig. 3. Electropherograms of the calixarenes and monomer; buffer: borate buffer (as in Fig. 2) in presence of KCl: (a) 0.0; (b) 0.25 m*M*; (c) 0.5 m*M*; (d) 1.0 m*M*; (e) 2.0 m*M*.

K^+ and Mg^{2+} were used as additives to examine the effects of cations on the separation of the calixarenes. Fig. 3 provides the electropherograms of the mixtures of calixarenes and monomer under the condition of borate buffer with various amounts of KCl. The retention times of all four analytes increase by the addition of K^+. The retention time of the monomer increases from 8.3 to 16.1 min, while those of the calixarenes increase from 16 to 50 min. With 0.25 m*M* KCl, SCX8 can be separated from SCX6 and SCX4. By addition of 1.0 m*M* KCl, SCX6 and SCX4 can be barely separated. However, the migration times for the calixarenes are as long as 50 min. In addition, the peaks are broad and poorly resolved. Thus, this would not be an acceptable separation method for calixarenes.

Another cation, Mg^{2+}, was also investigated as an additive to the buffer. Initially, the injection was on the anode side and migration time was monitored for 100 min. No peaks appeared during this long period time, indicating that the EOF was reversed. The polarity of the electric field was then switched. Then, the cathode was placed on the injection side. The electropherograms of the analytes are shown in Fig. 4. Several details can be noted in these figures:

(1) As the amount of Mg^{2+} increases from 2.0 to 12.0 m*M*, the migration times of all the analytes were reduced. The separation by use of 4.0, 6.0 or 12.0 m*M* Mg^{2+} is satisfactory.

(2) The migration time of the monomer is very much a function of the concentration of Mg^{2+}.

(3) In all buffers with Mg^{2+}, the elution order for calixarenes is SCX4, SCX6 and SCX8.

At pH > 2.0 and in alkaline solution, the EOF is toward the negative electrode. By addition of Mg^{2+} at pH 8.3,

$$\equiv Si\text{–}O^- + Mg^{2+} \rightleftharpoons \equiv Si\text{–}OMg^+$$

is formed on the inner wall of the capillary [30]. Then the diffuse layer of solvent is negatively charged. Under an applied potential, the diffuse layer migrates toward the anode. The EOF is reversed under these conditions. Higher concentrations of Mg^{2+} in the buffer solution produces a greater amount of $\equiv Si\text{–}OMg^+$ on the wall. Thus, the reversed EOF is enhanced. This produces a shortening of the migration times for all analytes.

Reversed EOF usually results in a reversal of elution order of the analytes [31] unless there are other interactions between the analytes and the buffer components. As we discussed earlier, the order of electrophoretic mobilities of the calixarenes at pH 8.3 should be SCX8 > SCX6 > SCX4. The phenol monomer has the smallest mobility and is expected to be the last component to elute. In the electropherogram (Fig. 4a), the monomer is the last to elute. However, the elution order of calixarenes is not reversed because of the reversed EOF.

The characteristics of calixarenes should be considered to explain the above observations. Calixarenes are cyclic compounds, which can form complexes with metal ions. A number of studies on solid-state complexation of calixarenes have been reported [32]. The *p-tert.*-butylcalixarene amides have been used to extract metal ions from aqueous solution [33]. This suggests that the calixarenes may be capable of complexing Mg^{2+} ion. If water-soluble calixarenes can complex with Mg^{2+}, the positive Mg^{2+} would neutralize a corresponding number of negative

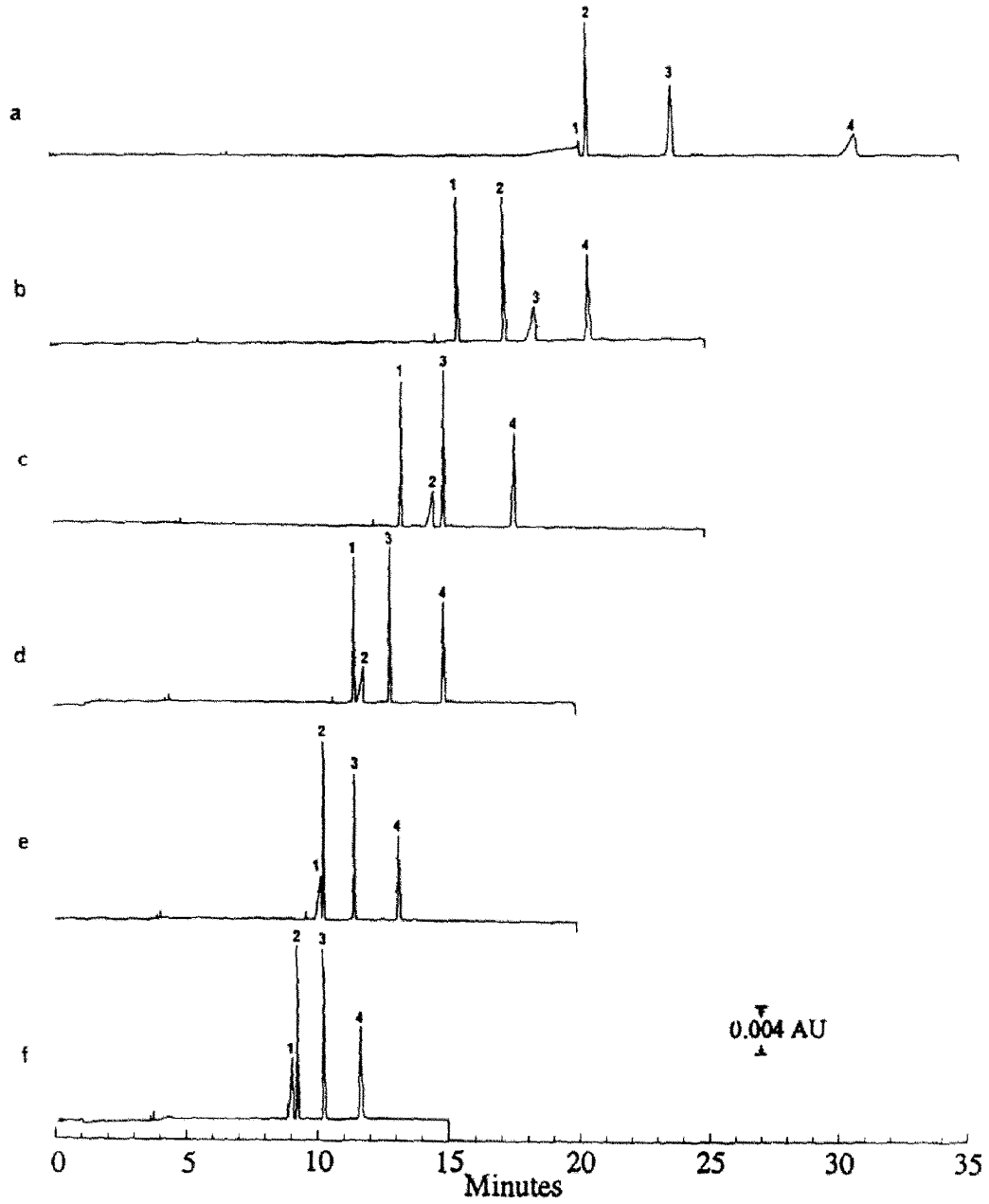

Fig. 4. Electropherograms of the calixarenes and monomer; buffer: borate buffer (as in Fig. 2) in the presence of (a) 2.0 m*M* $MgCl_2$ (peaks: 1 = SCX4; 2 = SCX6; 3 = SCX8; 4 = monomer), (b) 4.0 m*M* $MgCl_2$ (peaks: 1 = SCX4; 2 = SCX6; 3 = monomer; 4 = SCX8), (c) 6.0 m*M* $MgCl_2$ (peaks: 1 = SCX4; 2 = monomer; 3 = SCX6; 4 = SCX8), (d) 8.0 m*M* $MgCl_2$ (peaks: 1 = SCX4; 2 = monomer; 3 = SCX6; 4 = SCX8), (e) 10.0 m*M* $MgCl_2$ (peaks: 1 = monomer; 2 = SCX4; 3 = SCX6; 4 = SCX8) or (f) 12.0 m*M* $MgCl_2$ (peaks: 1 = monomer; 2 = SCX4; 3 = SCX6; 4 = SCX8).

charges on the calixarenes. Thus, the net negative charge density of calixarenes decreases and this suppresses the mobilities of the calixarenes. In other words, the more Mg^{2+} complexed by the calixarenes, the smaller the electrophoretic mobilities of the calixarenes. The phenol monomer does not complex with Mg^{2+} and does not change with the addition of Mg^{2+}. Thus, the net change in migration for the monomer, due to the increase of EOF, is larger than those for calixarenes. This results in the observed drastic changes in elution order of the monomer.

It is well known that calixarenes are size-selective host molecules [1,2]. It has been reported that extraction efficiencies (%) of *p-tert.*-butylcalixarene amides 4, 6 and 8 (non-water-soluble) for Mg^{2+} are less than 1.0, 11.8 and 14.2%, respectively. This suggests that the complexation capability with calixarene amide 8 is the strongest, followed by 6 and then 4. In Fig. 4, the elution order of the water-soluble calixarenes is SCX4, SCX6, SCX8. This is opposite to what was observed without Mg^{2+} in Fig. 2. This suggests that SCX8 may complex with the greatest amount of Mg^{2+}, followed by SCX6 and then by SCX4. This is the same trend observed for the calixarene amides.

3.2. Detection limit

For UV detection, the detection limit is typically on the order of 10^{-5} to 10^{-6} *M* depending on the absorptivities of the analytes [34]. The detection limit of the calixarenes and monomer can be as low as $10^{-7}M$. Fig. 5 shows an example of the electropherogram of SCX6 ($2.5 \cdot 10^{-7}$ *M*). With this low detection, CZE can be used to detect the impurities in the calixarene samples. Fig. 6 is an electropherogram of one batch of SCX8. The retention times of SCX4, SCX6 and SCX8 are 13.75, 15.25 and 18.43 min, respectively. In Fig. 6, there are two peaks whose retention times are about 15 and 18 min. The small peak is assigned to SCX6 and the larger one is SCX8. Thus, the appearance of a small amount of SCX6 can be noted in that SCX8 sample.

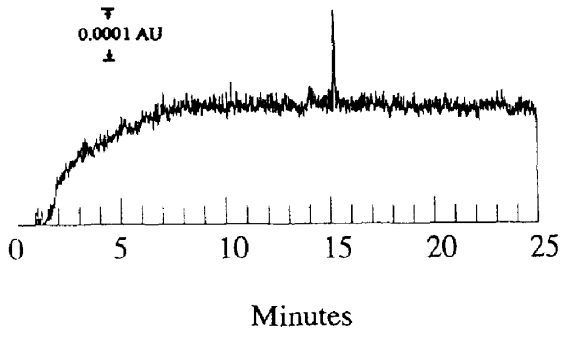

Fig. 5. Electropherogram of SCX6 at $2.5 \cdot 10^{-7}$ *M*.

4. Conclusions

CZE is a very effective method for separation of the calixarenes. The borate–boric acid buffer with 4.0, 6.0 or 12.0 m*M* Mg^{2+} is recommended for separation of these compounds. In addition, CZE is useful for examining the purity of the calixarene samples. CZE is also a potential

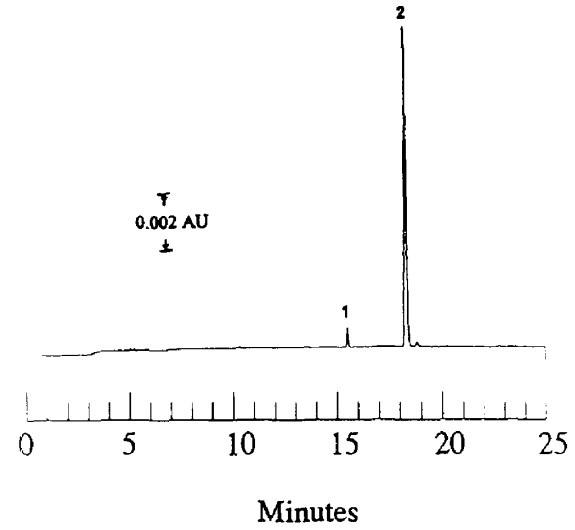

Fig. 6. Electropherogram of one batch of SCX8 sample which includes a small amount of SCX6. Peaks: 1 = SCX6; 2 = SCX8.

technique for studying the complexation abilities of calixarenes with cations and neutral compounds. However, the disadvantage of CZE is that it cannot be used as a preparative method. HPLC would be a better method in this regard.

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