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Use of calixarene compounds as selectivity modifiers in capillary electrophoresis separations

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Abstract

The use of calixarene compounds as selectivity modifiers and potassium bromide as electrolyte in capillary electrophoresis (CE) was studied. Calixarenes are macrocyclic oligomers having the shape of a conical vase whose inner cavity can accommodate various guest molecules. These compounds absorb in the ultraviolet region, with maxima at approximately 204 nm, and can be employed for the indirect determination of various types of compounds. The use of water soluble sulphonated calixarene as mobile phase in combination with different salts allowed us to develop electrophoretic methods for the determination of special compounds which are transparent in the UV region. The potential of this buffer was assessed by separating various groups of analytes including amino acids, biogenic amines and inorganic cations and anions. A system consisting of seven amino acids, used as model compounds, was chosen to optimize the buffer. The different instrumental variables affecting sensitivity and resolution of the mixture were carefully optimized.

Keywords: Buffer composition; Selectivity modifiers; Amino acids; Inorganic cations; Inorganic anions; Biogenic amines; Calixarenes

1. Introduction

The study of calixarene chemistry [1] is one of the growing areas in supramolecular chemistry. These compounds are very useful for analytical purposes. For example, calixarene derivatives have been used to efficiently purify C_{60} [2]; to make highly sensitive and selective electrodes [3] for Na^+ and K^+; and in sensors for Ca^{2+} [4]. However, the most common forms of calixarenes are not soluble in water. Therefore, much effort has been focused on the synthesis of water-soluble derivatives such as the highly soluble sulphonated calixarenes (SCXs) [5,6]. Water-soluble sulphonated calixarenes can selectively include various guest molecules according to size and hydrophobicity in a similar manner to cyclodextrins [7,8]. The properties of these two types of host molecules have been compared and summarized [9]. A detailed discussion about the separation mechanism can be found in Refs. [7–9]. One should note that the phenolic units of calixarenes are spectroscopically active in the UV region and that this is a significant difference from the oligosaccharide units of cyclodextrins.

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Although the use of calixarenes in capillary electrophoresis (CE) has been investigated, a literature search retrieved only six references to this type of compound. Thus, calixarenes were used as selectivity modifiers to separate various phenolic compounds in CE [10]. CE has proved a suitable technique for the separation of sulphonated calixarenes [11,12]; more recently, chiral acylcalix[4]arene was synthesised and used as a pseudo-stationary phase in CE [13]. Calixar[4]arene has been used to separate positional isomers in CE [14] and moderated-sized and polar-substituted polycyclic aromatic hydrocarbons (PAHs) were separated by capillary electrokinetic chromatography [15]

CE has become a popular separation technique over the past decade; almost 3500 papers on it have been published to date. There is a sizeable body of methods for separating a wide variety of analytes. However, some chemical compounds potentially useful, which could be used as constituents of the buffer have not yet been tested for this purpose. In CE, the background electrolyte is the most important variable in the ensuring efficient separation of most analytes. The buffer is responsible for pH, ionization of surface silanols and the magnitude of electroosmotic flow. An electrophoretic capillary method using sulphonated calixarenes in combination with different sodium or potassium salts as mobile phase is proposed here for the first time. The potential of the buffer studied was tested on, a group of amino acids. The most commonly used detection methods for amino acids involve is pre- or post-column derivatization of the analytes with fluorescent probes [16–18]. The derivatization process can be rather time consuming and requires considerable additional work. Underivatized amino acids can also be detected by indirect methods in CE [19]. The proposed buffer was found to provide several advantages over others, used for indirect photometric detection [20–22]. Only calixarenes improved the buffer solution, in the form of enhanced selectivity (as previously shown by Shohat and Grushka [10]), increased absorbance for indirect photometric detection and an acid pH for the buffer solution. This new system based on calixarene as a buffer constituent could be used in future studies for the separation and quantification of UV-transparent analytes in real samples.

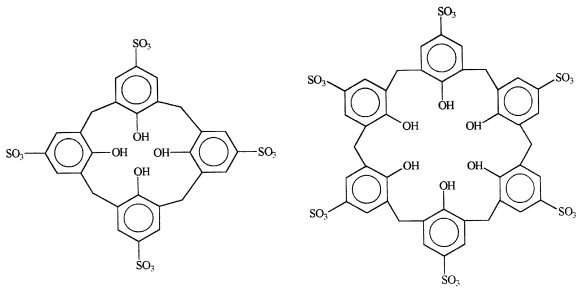

Fig. 1. *p*-Sulphonic calix[6]arene and *p*-sulphonic calix[4]arene.

2. Experimental

2.1. Reagents

SCX4 and SCX6 (Fig. 1) were purchased from Janssen Chemica and used as received. The analytes studied were as follows: histidine (His), lysine (Lys), arginine (Arg), glycine (Gly), alanine (Ala), serine (Ser) and threonine (Thr) as amino acids; methylamine, ethylamine, ethanolamine, morpholine, butylamine and hexylamine as biogenic amines; lithium, sodium, potassium, beryllium, magnesium, calcium, strontium and barium as cations; and chloride, nitrate, nitrite and sulphate as anions. All were obtained from Sigma. A 1000 $\mu g\ ml^{-1}$ stock standard solution of each analyte was prepared in water. Working standard solutions were prepared by diluting the stocks with purified water and adjusted to the desired pH with HCl.

The different salts used as ionic strength adjusters (Na_2SO_4, $NaNO_3$, NaF, NaCl, NaBr, NaI, KCl, KBr and KI) were delivered by Merck.

Doubly de-ionized water (18 mΩ) from a Milli-Q system (Millipore, Bedford, MA) was used in all standard and buffer solutions.

2.2. Apparatus

Experiments were carried out on a Beckman P/ACE 5500 CE instrument. The system comprised a 0–30 kV high-voltage built-in power supply, a diode array detector, and the GOLD software for system control and data processing. All capillaries used (bare fused-silica) were obtained from Beckman Instruments (Fullerton, CA, USA) and were 57 cm×

75 μm I.D. The temperature was controlled by using a fluorocarbon-based cooling fluid.

2.3. Electrophoretic procedures

Prior to first use, each new capillary was subjected to a standard wash cycle; subsequent runs were carried out according to the established procedure [23]. One hundred μg ml^{-1} stock solutions of the analytes were prepared in deionized water. Equal aliquots of each were mixed to obtain a mixture of seven at a final concentration of 10 μg ml^{-1} each.

The running buffer consisted of 0.2–0.8 m*M* SCX4 or SCX6 and 1–8 m*M* potassium bromide or another salt (pH 3.1). A voltage of 20 kV, a mean current of 14.8 μA and a temperature of 20°C were used. Samples were injected in the electrodynamic mode at 10 kV for 20 s or hydrodynamic mode for 20 s. Electropherograms were recorded at 204 nm. Separations were carried out from the positive to the negative electrode. The capillary was conditioned daily by flushing with water (5 min), followed by freshly prepared 0.1 *M* sodium hydroxide (5 min), ultrapure water (5 min) and fresh buffer (5 min). In order to optimize migration time and peak shape reproducibility, the capillary was flushed with ultrapure water (2 min) and fresh buffer (5 min) between analyses.

The peaks for each analyte were identified by spiking samples with known standards and inspecting the resulting changes.

3. Results and discussion

There are several advantages to using calix[4]arene and calix[6]arene in CE. First, the size of the cavity is more flexible than that of cyclodextrins. Second, calixarenes can be easily synthesised and derivatized. Finally, the strong absorbance of calixarenes, affords the separation and indirect detection of a wide range of analytes. This type of detection generally results in negative peaks (diminished absorbance from a high background). The different experimental and instrumental parameters were selected and optimized as described below.

3.1. Composition of the buffer

A new buffer system was optimized by using SCX4 or SCX6 compounds as selectivity modifiers in addition to different salts. The buffer was intended facilitate to the separation and quantification of groups of analytes which do not absorb in the UV region. The electrolyte system was based on sulphonated calixarene and a specific concentration of a salt. If the analytes are to migrate to the negative electrode, they must be positively charged at the acid pH of the buffer solution. Sulphonated groups in calixarene make the buffer solution acidic (pH 3.1); also, the ionic strength of the buffer is provided by the salt used. In order to determine the most suitable pH for this buffer, phosphate buffers containing calixarene and salts as additives were used to adjust the pH over the range 1.1–3.1. No advantage over the use of calixarene alone and a salt at a specific concentration as the buffer components was observed.

3.2. Effect of ionic strength

The effect of the type of salt used on the selectivity and sensitivity for a mixture of amino acids was investigated by using 0.2 m*M* SCX4 or SCX6 and different types of salts (Na_2SO_4, $NaNO_3$, NaF, NaCl, NaBr, NaI, KCl, KBr and KI) at 4 m*M* concentration. The influence of the type of salt on the resolution of the mixture of seven amino acids is shown in Fig. 2, together with the area of an amino acid (Gly) peak. No significant difference in the number of peaks in the electropherograms provided by the different salts were observed. All salts except NaF and KCl provided, seven peaks for the seven amino acids. If high sensitivity is required with real samples, then is KBr the salt of choice; in fact, it provided the largest area peak for Gly. NaCl gave a positive peak for histidine but resulted in poor sensitive for the other, negative peaks. Based on these results, KBr was finally chosen as the salt component of the buffer solution. The effect of the concentration of salt on the resolution of the mixture of amino acids was investigated by using 0.2 m*M* calixarene and KBr concentrations in the range 2–8 m*M*. The optimum concentration was 4 m*M* for both

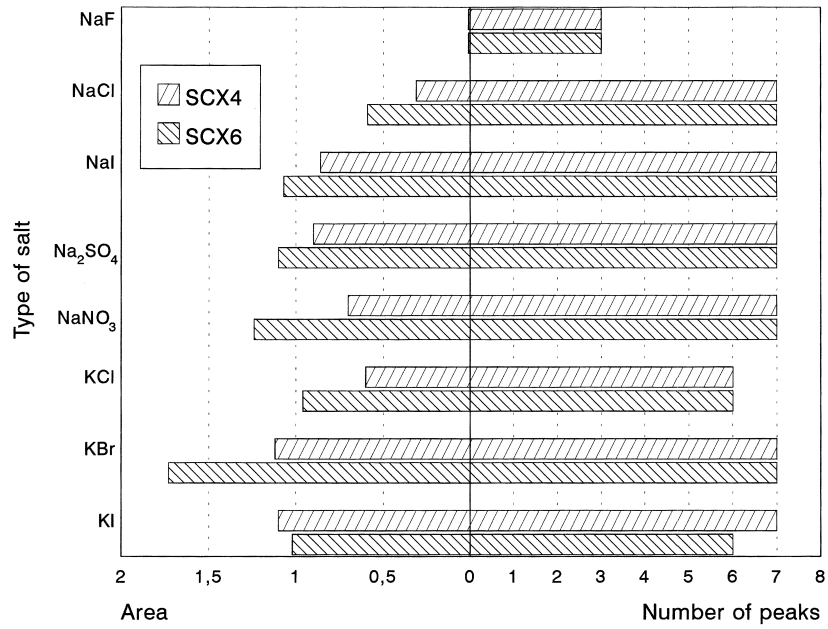

Fig. 2. Influence of the type of salt on the resolution of a mixture of seven amino acids. See text.

calixarenes. Lower concentrations precluded separation of some peaks. On the other hand, a concentration above 4 m*M* led to poor sensitivity.

3.3. Effect of calixarene concentration

The optimum composition of the run buffer was established by varying the contents in both calixarenes between 0.1 and 0.3 m*M*. The concentration chosen was 0.2 m*M*; above this level, the background of the electropherogram worsened as the SCX6 concentration was increased. On the other hand, lower SCX6 concentrations allowed no peak to be identified. Identical experiments with both calixarenes (SCX4 and SCX6) showed SCX6 to provide the better results (see Fig. 3).

3.4. Type of injection

The influence of the injection mode on the resolution and sensitivity in the determination of the test compounds was studied using two different buffers. When 0.2 m*M* SCX4 and 4 m*M* KBr buffer was used, electrokinetic injection for 65 s at 10 kV applied voltage or hydrodynamic mode for 20 s was possible and good sensitivity but bad resolution was achieved. Finally a 0.2 m*M* SCX6 and 4 m*M* KBr buffer resulted in better resolution between peaks, at

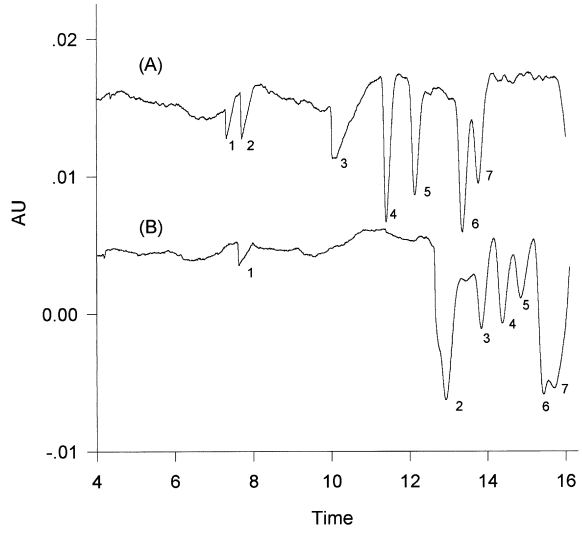

Fig. 3. Comparison of SCX6 and SCX4. Electropherogram for a standard mixture of amino acids; 1=histidine (His); 2=lysine (Lys); 3=arginine (Arg); 4=glycine (Gly); 5=alanine (Ala); 6=serine (Ser) and 7=threonine (Thr). Buffer: (A) 4 m*M* KBr+0.2 m*M* SCX6; and (B) 4 m*M* KBr+0.2 m*M* SCX4. (Time in min).

the expense of a restricted injection time (7 s) with hydrodynamic injection and a voltage of 10 kV for 20 s with electrokinetic injection.

3.5. Instrumental variables

Theory predicts that the use of the highest possible voltages will result in the shortest separation times. Experimentally, the optimum voltage was determined by performing runs at increasing voltages until resolution was found to suffer noted. The optimum value was 20 kV, which allowed optimal resolution of seven amino acids in a short time. The proposed method is based on the decrease of calixarene absorbance. The wavelength chosen was 204 nm.

Most separations were performed at 25°C (i.e., near room temperature). However, we also checked the effect of this variable over the range 10–30°C. At 10°C, migration times were longer and peak areas larger than at room temperature, as a consequence of that, broad and poorly integrated peaks were obtained. At 30°C, the background deteriorated and peak areas were smaller. A temperature of 20°C was chosen because migration times were fairly short and the background was good enough for the amino acid mixture to be resolved.

4. Analytical applications

The proposed method is recommended for the determination of analytes with no absorption in the UV region. Different groups of compounds were tested to validate the new buffer. Amino acids were selected as model compounds for this purpose. Minor adjustments were made when separating other analytes in order to ensure the best possible results. Different examples have been taken to demonstrate the analytical potential of the use of calixarene compounds in CE. There are briefly commented below.

A group of seven amino acids was separated in less than 16 min. Non-aromatic amino acids Gly, Ala, Ser, Thr, Lys and Arg exhibit low UV absorbance. Nevertheless, amino acids have been separated by CE and directly detected [24], after derivatization [25], or indirectly [19]. Only the three aromatic amino acids, namely phenylalanine (Phe), tyrosine (Tyr) and Tryptophan (Trp), absorb significantly in the UV region.

A rapid method for the separation and detection of amines [20] based on CE and indirect photometric detection was previously reported. The new system developed in this work also allows one to resolve a mixture of six biogenic amines (methylamine, ethylamine, ethanolamine, morpholine, butylamine, hexylamine) in 7 min (see Fig. 4).

The calixarene included in the buffer solution exhibited differential selectivity for with the different alkaline earths tested. The experimental conditions had to be reoptimized to ensure acceptable separation of this group of analytes. The concentration of SCX4 (or SCX6) was 0.05 m*M* and that of the salt 4 m*M*. As can be seen from Fig. 5, the peaks for Be^{2+} and Mg^{2+} were higher than for Ca^{2+} and Sr^{2+} on account of the lower atomic weight and size of the former two. The effect was similar with both calixarenes.

Finally, a group of anions (sulphate, nitrite, nitrate and chloride) was examined in order to demonstrate the applicability of this new buffer for to the determination of anions (under the same conditions as for cations). Note that the positive electrode was placed at the end for this purpose. The best results were achieved with SCX6 (see Fig. 6).

5. Conclusions

We investigated calixarenes as electrophoretic buffer components. These compounds can be used as additives in CE with a view to the indirect determination of analytes which do not absorb in the UV region. Separation of different analyte groups was accomplished by using 4 m*M* SCX4 or SCX6 in conjunction with various sodium and potassium salts. The ensuing method is rapid, simple and highly suitable for the separation and determination of amino acids, biogenic amines, cations and anions. Future further research into the topic may go in two different directions, viz. expanding the use of SCX in buffer systems for real samples and achieving chiral separations by CZE using cyclodextrins, crown ethers and calixarenes.

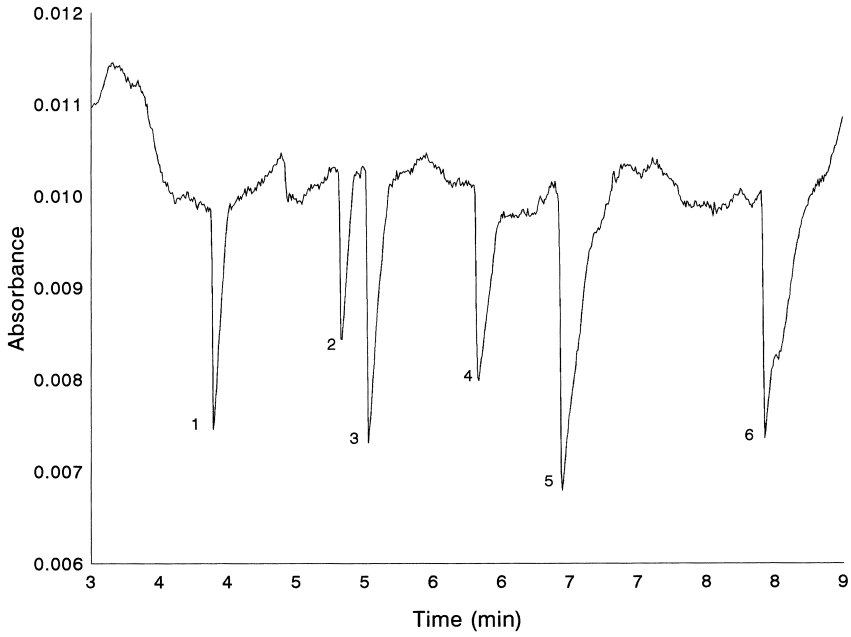

Fig. 4. Electropherogram for a standard mixture of biogenic amines; 1=methylamine, 2=ethylamine, 3=ethanolamine, 4=morpholine, 5=butylamine, 6=hexylamine.

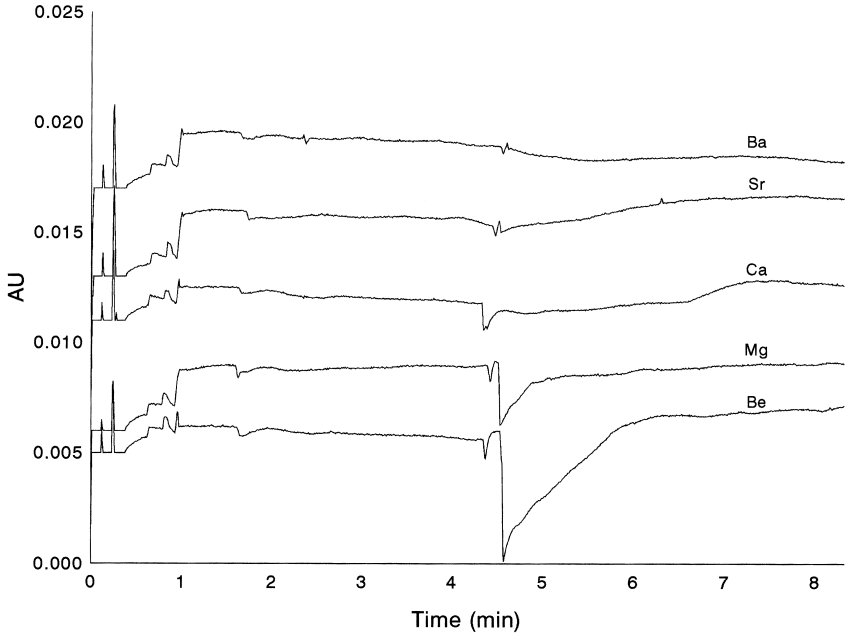

Fig. 5. Electropherograms for different behaviour of the alkaline earth metals. Buffer: 0.05 m*M* SCX6+4 m*M* KBr (for details see Section 3).

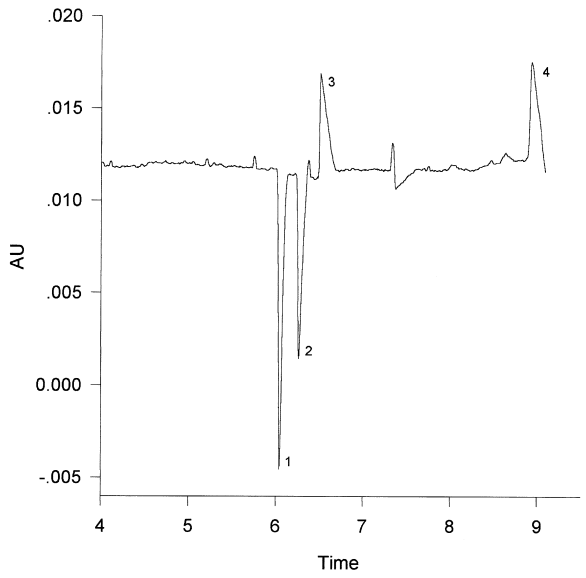

Fig. 6. Electropherogram for a standard mixture of anions: 1= chloride, 2=sulphate, 3=nitrate, 4=nitrite. Time in min.

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