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Journal of Chromatography A, 910 (2001) 311–318

JOURNAL OF CHROMATOGRAPHY A

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Study of a novel cationic calix[4]arene used as selectivity modifier in capillary electrophoresis with electrochemical detection

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Received 12 September 2000; received in revised form 9 November 2000; accepted 29 November 2000

Abstract

The use of a novel cationic calixarene, *p*-(quaternary ammonium) calix[4]arene, as selectivity modifier in capillary electrophoresis coupled with electrochemical detection was reported. The calixarene displayed good selectivity for the positional isomers of benzenediol and aminophenol and their successful separation was obtained under optimum conditions. The interaction mechanism between *p*-(quaternary ammonium) calix[4]arene and the solutes is discussed using the molecular modeling method. The detection limits by electrochemical detection for the most solutes studied here were below picogram level, which was ~2 orders of magnitude lower than those reported in the literature using UV detection. The results showed that electrochemical detection is especially suitable for an electrophoresis system where calixarenes are used as modifier.

Keywords: Selectivity; Buffer composition; Calixarenes; Benzenediols; Aminophenols; Phenols

1. Introduction

In order to enhance the selectivity of capillary electrophoresis (CE) in the separation of structurally similar solutes, many modifiers have been exploited. In this respect, macrocyclic compounds have been shown to be quite unique. The enhancement of the selectivity involves the formation of host–guest complexes between macrocyclic reagents and solutes. Some examples of the kinds of modifier used successfully in CE include crown ether [1,2], macrocyclic antibiotics [3,4], macrocyclic dioxopolyamine [5,6] and various cyclodextrins (CDs) [7–12].

Calixarenes are another class of macrocyclic molecules, generally made up of phenolic units *meta* linked by methylene bridges. They possess basket-shaped cavities similar to CDs, and therefore are expected to form host–guest-type complexes with organic guests in water by their hydrophobic forces. However, the studies of the host–guest chemistry of calixarenes has been very limited in contrast with that of CDs. This is mainly due to the fact that the early-synthesized calixarenes were water-insoluble, and their interaction with organic solutes was retarded by the competing complexation from the organic solvent [13]. Nevertheless, after Gutsche and Alam [14] and Shinkai and co-workers [13,15–17] synthesized water-soluble calixarenes and verified that they could form a variety of host–guest-type

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0021-9673/01/$ – see front matter
PII: S0021-9673(00)01216-4

complexes with organic guests in water, the status of calixarenes as the third generation of supramolecules, after CDs and crown ethers, has been established. The first report involving the use of calixarenes as a selectivity modifier in CE separation appeared in 1994, in which Shohat and Grushka first studied the effect of *p*-sulfonic calix[6]arene on the migration of phenol compounds [18]. After that, Baechmann's group synthesized a series of resorcarenes and studied systematically their applications as pseudostationary phases in electrokinetic chromatography [19–22]. Sun et al. also studied the separation of polycyclic aromatic hydrocarbons (PHAs) employing *p*-(carboxyethyl) calix[*n*]arenes as running buffer additives in capillary electrokinetic chromatography [23]. Waner et al. reported the application of calix[4]arenes with a chiral group in the chiral separations for binaphthyl derivatives [24]. Zhao et al. separated phenolic positional isomers using *p*-sulfonic calix[4]arene [25]. Arce et al. used the same calixarene in CE for the separation of amino acids, biogenic amines and inorganic ions [26]. It can be seen from the literature cited above that calixarenes used in CE have been generally restricted to the anionic type and their applications in CE have been very limited in contrast with the extensive employment of CDs. The reasons for this may relate to the following factors: (1) few water-soluble calixarenes are commercially available and they are expensive; (2) their syntheses are comparatively difficult with the low yields; (3) calixarenes have high UV absorption, which leads to high baseline noise and limits the sensitivity of conventional UV detection. Therefore, in order to expand the applications of calixarenes in CE, novel calixarenes should be exploited, and other detection methods should be developed.

In the present research, we synthesized a novel cationic calix[4]arene (see Fig. 1) and investigated its effect on the selectivity of CE. Positional isomers of benzenediol and aminophenol were used as test solutes. Molecular modeling depictions and interaction energy data for the calixarene–solute complexes were used to explain the observed separation behaviors. Electrochemical detection (ED) was employed to remedy the inadequacy of direct UV detection resulted from the strong UV absorption of calixarenes.

2. Experimental

2.1. Syntheses

The structure of newly synthesized *p*-(quaternary ammonium) calix[4]arene, 5,11,17,23-tetrakis [(*N*,*N*-dimethyl,*N*-ethyl) - ammoniomethylene]-25,26,27,28-tetrahydroxycalix[4]arene bromide, is shown in Fig. 1. It was synthesized following a procedure similar to that described by Gutsche et al. [27]. The synthesis route is depicted in Fig. 2. $\delta_H(^2H_2O$, 60 MHz) for the product: 1.14(t, 12H, $N^+(CH_3)_2CH_2C\underline{H}_3$), 2.56(m, 32H, $N^+(C\underline{H}_3)_2C\underline{H}_2CH_3$), 3.56(m, 12H, $ArC\underline{H}_2Ar$, $ArC\underline{H}_2N^+(CH_3)_2CH_2CH_3$), 4.28(d, 4H, $ArC\underline{H}_2Ar$), 7.02(s, 8H, $Ar\underline{H}$).

2.2. Apparatus

Separations were performed with a laboratory-built CE system, which consists of a 30-kV high-voltage power supply (Shanghai Institute of Atomic Nucleus, Academy of Sciences of China, Shanghai, China) and a 50 cm×50 μm I.D. uncoated fused-silica capillary (Yongnian Optical Fiber Factory, Hebei, China). Detection was carried out using an ED system assembled in the laboratory. It is composed of an electrochemical cell, whose construction

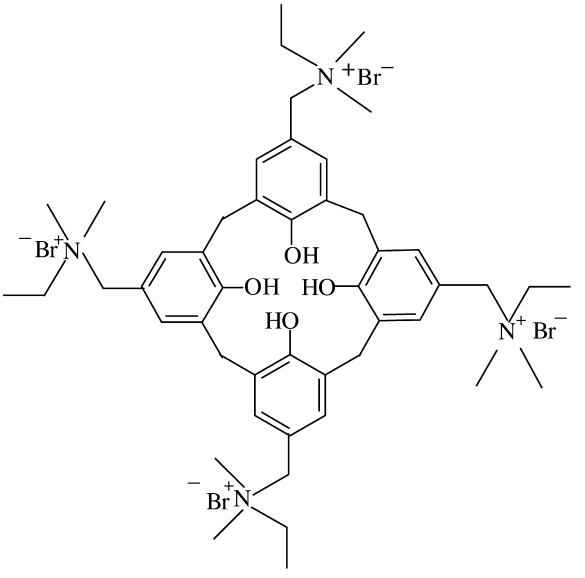

Fig. 1. Structure of *p*-(quaternary ammonium) calix[4]arene.

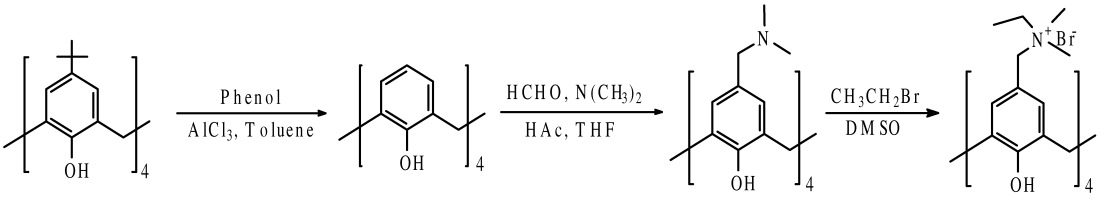

Fig. 2. Synthesis route of *p*-(quaternary ammonium) calix[4]arene.

has been described elsewhere [28,29], and an amperometric detector (Shanghai Institute of Organic Chemistry). A conventional three-electrode mode was used. A bound carbon fiber disk electrode (diameter 150 μm), a saturated calomel electrode (SCE) and a platinum wire were used as a working electrode, a reference electrode and a counter electrode, respectively. The electrodes were connected to the amperometric detector, which provided the applied constant potential and measured the resulting current. A chart recorder (Shanghai Dahua factory, Shanghai, China) was used for recording all data. The entire CE–ED system used here is similar to that described previously [30].

Cyclic voltammetry (CV) experiments were performed with CHI660 electrochemistry working station (CH Instruments, USA) with a three-electrode system as described above.

2.3. Reagents

All reagents were of analytical grade and water was doubly distilled. *o, m, p*-benzenediols and *o, m, p*-aminophenols (Shanghai Reagent factory, Shanghai, China) were used as received and dissolved in water to prepare 1.0 m*M* standard solutions. To retard oxidation of the aminophenol solutions in air, 1.0 m*M* vitamin C was added. The running buffer consisted of 30.0 m*M* Na_2HPO_4 adjusted to the desired pH with concentrated H_3PO_4. Weighed amounts of *p*-(quaternary ammonium) calix[4]arene were dissolved in a definite volume of the buffer solution and ultrasonicated for 5 min. All solutions were filtered through a 0.25-μm membrane filter.

2.4. Electrophoretic procedures

Prior to first use, a new capillary was washed successively with 0.1 *M* NaOH, deionized water and the running buffer, each for 5 min, and then equilibrated with the running buffer under the separation voltage about 2 h until the migration times of solutes did not change significantly. Whenever the running buffer was altered, the above steps were repeated. Before every run, the capillary was washed with the running buffer for 2 min. The buffer in the anodic and cathodic reservoirs was renewed every two runs in order to keep the same pH between the two reservoirs.

The negative voltage was applied, i.e. the detection was at the anode because the direction of electroosmotic flow (EOF) was observed towards the anode in our experiments when the concentration of *p*-(quaternary ammonium) calix[4]arene in the running buffer was enough. The reason may be that the cationic *p*-(quaternary ammonium) calix[4]arene was absorbed onto the inner wall of capillary, and so, made it charge positively. Sample introduction was performed electrokinetically at the cathode. Peak identification for each solute was based on peak height increase by spiking samples with known standards.

2.5. Molecular modeling procedures

Molecular modeling studies were performed with CS CHEM3D Pro Version 5.0 for Windows (CambridgeSoft). Molecular models of *p*-(quaternary ammonium) calix[4]arene and the solutes were first constructed. Then the conformation of *p*-(quaternary ammonium) calix[4]arene, which was shown to exist predominantly in coned form by the ^1H NMR spectroscopic data given above [31], was optimized. Last, the configuration of calixarene–solute complex was optimized to the lowest energy state to create the most stable depiction of the complex and to determine the interaction energy.

3. Results and discussions

3.1. Benzenediols

Benzenediols are nearly uncharged at pH 5.0 (pK_a values for *o*-, *m*-, *p*-benzenediols are 9.35, 9.44 and 9.91, respectively [32]). Therefore, it is impossible to separate them by conventional CE at this pH. The results showed all isomers coeluted under the experimental conditions in absence of the modifier ($R_s = 0$, in Fig. 3). Because of the guest–host-type interaction between the calixarene and the solutes, the separation occurred when *p*-(quaternary ammonium) calix[4]arene was added. Fig. 3 shows the effect of *p*-(quaternary ammonium) calix[4]arene concentration on the R_s values of benzenediols. R_s increased as *p*-(quaternary ammonium) calix[4]arene concentration increased. As *p*-(quaternary ammonium) calix[4]arene concentration reached 8 m*M*, three isomers were baseline resolved. Typical electropherograms are shown in Fig. 4.

In all experimental cases, the migration order of benzenediols was found to be $p<m<o$ isomers. This migration order indicates that the interaction of *p*-(quaternary ammonium) calix[4]arene with benzenediols strengthens in the order of *para*, *meta* and *ortho* isomers, and that the migration direction of *p*-(quaternary ammonium) calix[4]arene was opposite to that of EOF (the direction of the EOF was towards the anode as described in Experimental). This order was identical with that reported in the literature where *p*-sulfonic calix[4]arene was used as modifier [18,25]. The same order showed that the effect of *p*-(quaternary ammonium) calix[4]arene on the separation selectivity for benzenediols was similar to that of *p*-sulfonic calix[4]arene in quality. Because the structural difference between *p*-(quaternary ammonium) calix[4]arene and *p*-sulfonic calix[4]arene is only in the outer substituents of the calixarenes, it seemed to suggest that the outer substituents of the calixarene did not significantly affect the separation selectivity for benzenediols, and further, that the sites of the interaction of the calixarene with the solutes might be in the calixarene cavity rather than on its outer functional groups.

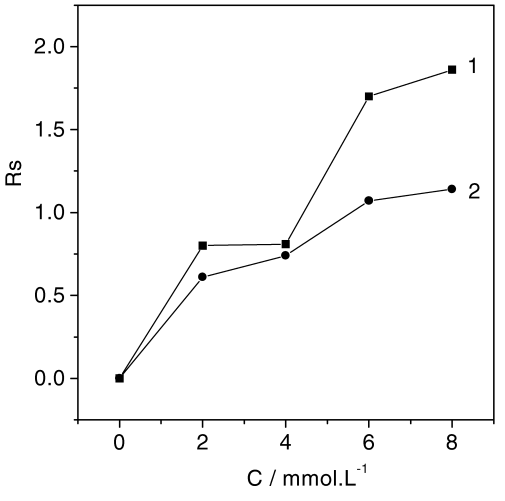

Fig. 3. Effect of *p*-(quaternary ammonium) calix[4]arene concentration on resolutions (1) *p*-benzenediol and *m*-benzenediol, (2) *m*-benzenediol and *o*-benzenediol. Conditions: phosphate buffer (30.0 m*M*, pH 5.0); separation voltage, −12 kV; injection, −12 kV, 5 s; detection potential, +0.7 V (vs. SCE).

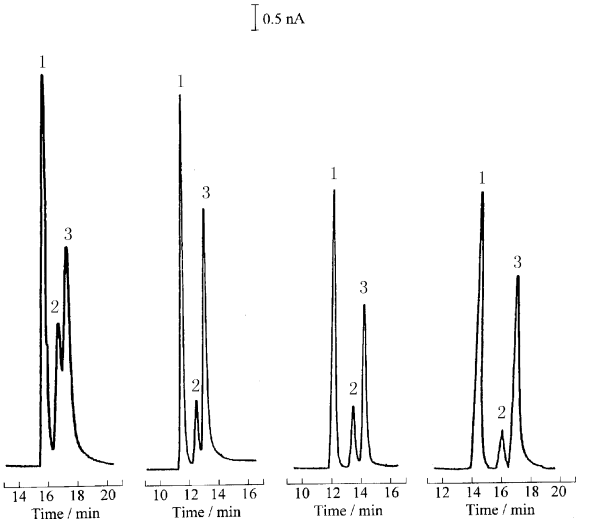

Fig. 4. Electropherograms of benzenediols. Peaks: 1=*p*-benzenediol, 2=*m*-benzenediol, 3=*o*-benzenediol. Conditions: solutes, 0.2 m*M*; *p*-(quaternary ammonium) calix[4]arene, (A) 2.0 m*M*, (B) 4.0 m*M*, (C) 6.0 m*M* and (D) 8.0 m*M*. Other conditions as in Fig. 2.

3.2. Aminophenols

The effect of *p*-(quaternary ammonium) calix[4]arene on the separation selectivity for aminophenols was different from that for benzenediols. The experiments showed that *o*-, *m*-, *p*-aminophenols could be separated partially at pH 5.0

without *p*-(quaternary ammonium) calix[4]arene for they were partially protonated (the pK_b values for *p*-, *o*-, *m*-aminophenols are 8.50, 9.28 and 9.83, respectively [32]). The migration order was $p<o<m$ isomer, which was in accordance with their charge-to-mass ratio order (the electropherogram was not shown). Fig. 5A shows the electropherogram of the positional isomers of aminophenols with a running buffer at pH 5.0 containing 5.0 m*M* *p*-(quaternary ammonium) calix[4]arene. Compared to that without the calixarene, two differences were observed: the separation for the three isomers was further improved and the migration order was completely reversed: $m<o<p$ isomers. The reversed migration order was not surprising since the EOF was reversed with the calixarene and aminophenols were protonated at pH 5.0. But it was not expected that the enhancement of the separation selectivity for aminophenols was not large like that for benzenediols. We assumed that the repulsive force between protonated aminophenols and cationic *p*-(quaternary ammonium) calix[4]arene retarded the solute entrance into the cavity of *p*-(quaternary ammonium) calix[4]-arene, and thus weakened the interaction between them. It seemed that it was the charge of the solute rather than the modifier that mainly dominated the separation selectivity for protonated aminophenols under the conditions of the relatively low pH and modifier concentration.

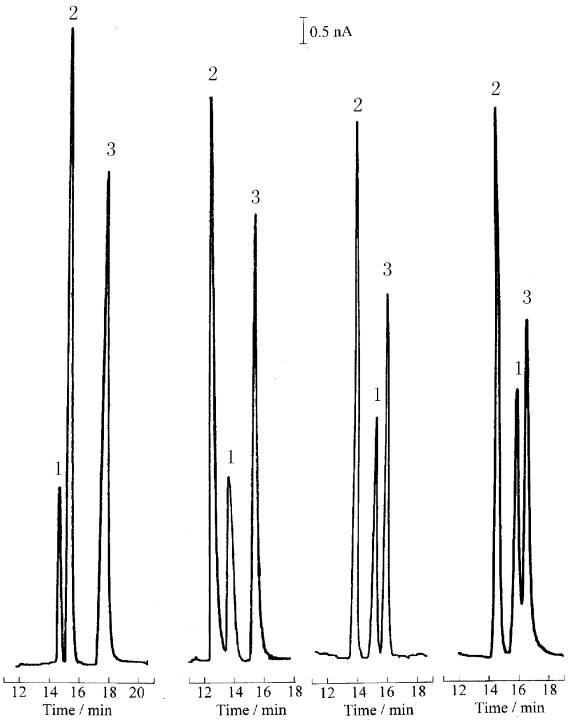

Fig. 5. Electropherograms of aminophenols. Peaks: 1=*o*-aminophenol, 2=*m*-aminophenol, 3=*p*-aminophenol. Conditions: solutes, 0.2 m*M*; *p*-(quaternary ammonium) calix [4]arene, (A) 5.0 m*M*, (B) 4.0 m*M*, (C) 6.0 m*M* and (D) 8.0 m*M*; 30.0 m*M* phosphate buffer, pH 5.0 (A) and 6.2 (B, C, D). Other conditions as in Fig. 2.

In order to verify the above hypothesis, the pH of the running buffer was raised to 6.2 (when the pH was more than 7.0, the speed of EOF was so fast that aminophenols could not be separated even with a high concentration of *p*-(quaternary ammonium) calix[4]arene). It was found that the migration speed of *m*-aminophenol slowed down and fell behind *o*-aminophenol. Moreover, with increasing the concentration of *p*-(quaternary ammonium) calix[4]-arene, this difference became bigger and bigger (Fig. 5B–D). When the concentration reached 6.0 m*M*, a nearly baseline separation was obtained with the migration order of $o<m<p$ isomers (Fig. 5C). These facts indicated that the interaction of *m*-aminophenol with *p*-(quaternary ammonium) calix[4]arene increased with the decrease of the protonation degree of *m*-aminophenol, and thereby the repulsive force between them, as the pH of the running buffer increased. The change of the buffer pH from 5.0 to 6.2 did not significantly affect the migration of *o*- or *p*-aminophenol. This was attributed to their minor changes of protonation degree because of relatively low pK_b values, and the still existing strong repulsion with the calixarene as the buffer pH changed within the small range. Zhao et al. reported that the migration order of aminophenols was the same as that of benzenediols when anionic *p*-sulfonic calix[4]arene was used as modifier [25], which might be due to the relatively strong interaction between the protonated solutes and the anionic calixarene.

3.3. Molecular modeling interpretation of the interaction mechanism

The above results demonstrated that *p*-(quaternary ammonium) calix[4]arene could enhance the selectivity of CE for the separation of benzenediols and

aminophenols, and suggested that the interaction occurred in the cavity of *p*-(quaternary ammonium) calix[4]arene. In order to further interpret the interaction mechanism and the elution orders of the solutes in CE, molecular modeling studies, which have been widely used in the calixarene field [33] and also in CE by Sun et al. [23] were carried out. Fig. 6 shows the molecular modeling depictions of the lowest energy complexes of *p*-(quaternary ammonium) calix[4]arene with benzenediols and protonated aminophenols. It is well known that XH/π (X=C, N, O etc.) interaction plays an important role in the molecular recognition [34]. The molecular modeling results confirmed the effect of this type of interaction on the formation of inclusion complexes between *p*-(quaternary ammonium) calix[4]arene and the solutes. As shown in Fig. 6, the methyl groups of *p*-(quaternary ammonium) calix[4]arene are very close to the benzene rings of benzenediols and aminophenols. The distances between some hydro-

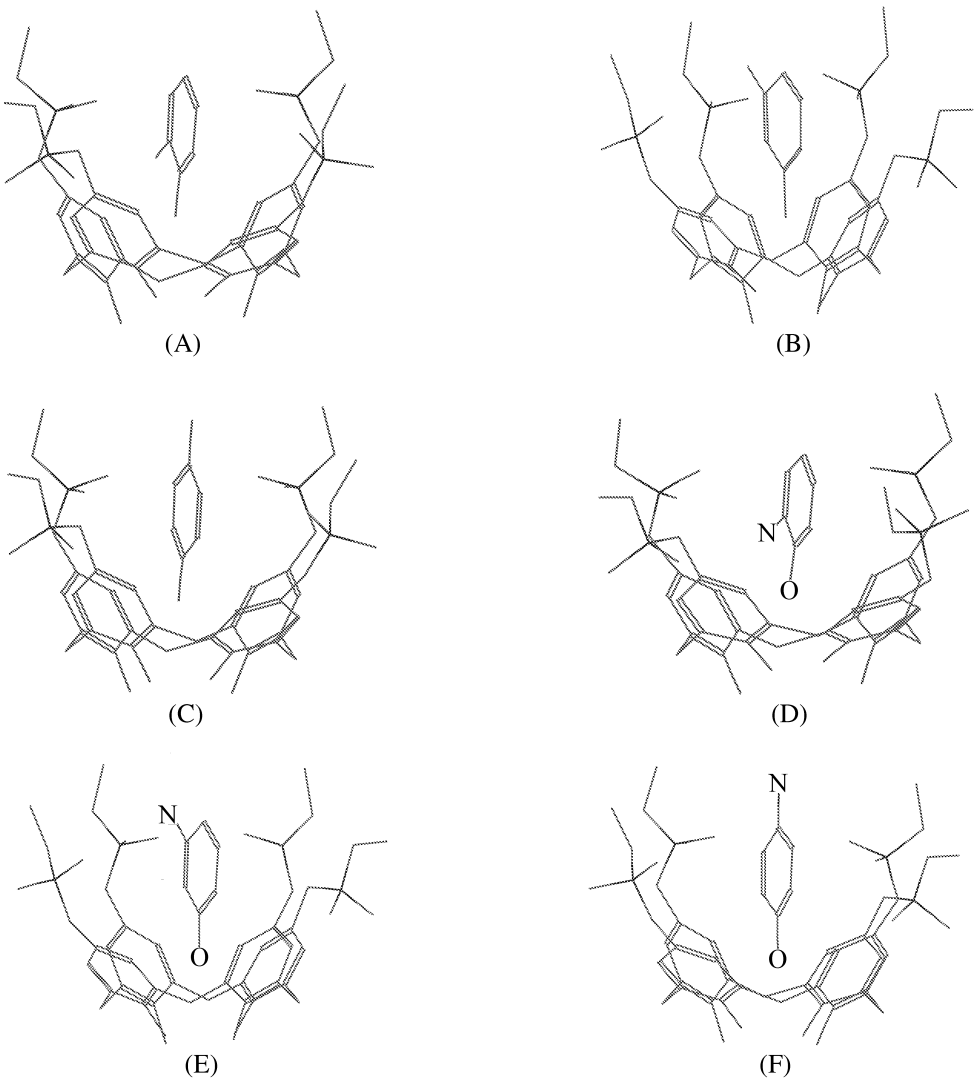

Fig. 6. Molecular modeling depictions of the lowest energy complexes of (A) *o*-benzenediol, (B) *m*-benzenediol, (C) *p*-benzenediol, (D) *o*-aminophenol, (E) *m*-aminophenol and (F) *p*-aminophenol with *p*-(quaternary ammonium) calix[4]arene (side view).

gen atoms of the methyl groups and carbon atoms of the guest's benzene ring are in the range of 2.72–2.80 Å. It was also found that the hydroxyl groups of the guests, which directed inside the calixarene cavity, were very close to two of the benzene rings of *p*-(quaternary ammonium) calix[4]arene. The distances between the hydrogen atoms of hydroxyl groups and the carbon atoms of the benzene ring (closest to the hydroxyl group) of the calixarene were in the range of 2.31–2.50 Å. These distances were less than the sum of the Van der Waals radii of hydrogen and carbon atoms (2.92 Å), which suggested the presence of CH/π and OH/π interactions [33]. These kinds of interactions contributed much to the formation of inclusion complexes between *p*-(quaternary ammonium) calix[4]arene and benzenediols or aminophenols

The interaction energy, which is defined by the difference between the energy of the inclusion complex and the total energy of the original free ligand (solute) and site (calixarene), was obtained by molecular modeling calculation to illustrate the interaction strength for the different isomers. The energy orders were *p*-benzenediol (−22.18 kcal/mol)>*m*-benzenediol (−23.37 kcal/mol)>*o*-benzenediol (−26.27 kcal/mol), *o*-aminophenol (−48.72 kcal/mol)>*m*-aminophenol (−55.65 kcal/mol)>*p*-aminophenol (−59.19 kcal/mol). The more negative the interaction energy, the more stable the inclusion complex will be. So, the stability orders of the calixarene–solute complexes were in accordance with the solutes elution orders in CE. Overall, the molecular modeling method can be used to understand the interaction mechanism of the calixarene with the solutes and its different selectivity for them in CE separation.

3.4. Character of electrochemical detection

In the UV detection mode, calixarenes produce relatively large background due to their strong UV absorption and make the electrophoretic baseline noisy [18,23,25]. The CV experiments for *p*-(quaternary ammonium) calix[4]arene showed that no redox wave existed in a scanning potential range from 0 to 1.0 V (vs. SCE) (the figure was not shown), illustrating that the modifier was not electroactive within this potential range. This is a bonus to the use of the calixarene in modifying electrophoresis selectivity by ED. Both benzenediols and aminophenols possess high electroactivity, and hence, a high detection sensitivity can be obtained. The results are summed up in Table 1. The detection limits for benzenediols and aminophenols with ED were about two orders of magnitude lower than those reported with UV detection [25]. High detection sensitivity is beneficial to the studies of intermolecular interaction because the permitted concentration of introduced solute is very low, and thus, only a low concentration of modifier is needed.

4. Conclusions

A cationic *p*-(quaternary ammonium) calix[4]-arene, which was synthesized for the first time, can be used as a modifier in CE with some features different from the anionic ones previously used to

Table 1
Detection limits of benzenediols and aminophenols with ED[a]

Solute	Migration time (min)	Detection limit	
		Concentration (μmol/l)	Mass (pg)
p-Benzenediol	14.50	0.80	0.50
m-Benzenediol	16.17	4.12	2.28
o-Benzenediol	17.03	1.48	0.74
m-Aminophenol	14.82	2.64	1.57
o-Aminophenol	15.49	0.75	0.40
p-Aminophenol	17.85	1.16	0.55

[a] Concentration detection limit was estimated at signal-to-noise ratio of 3. Mass detection limit was calculated based on migration time and concentration detection limit. Experimental conditions for benzenediols as in Fig. 4D, and for aminophenols as in Fig. 5A.

enhance the separation selectivity for benzenediol and aminophenol isomers. The interaction mechanism of the calixarene with the solutes and the explanation to its different selectivity for them were given by using molecular modeling. ED, which unlike UV detection does not suffer from the strong background absorption of calixarenes, was demonstrated to be a powerful tool for studying electrophoretically the interaction between calixarenes and electroactive guest molecules. We speculate that *p*-(quaternary ammonium) calix[4]arene is more suitable for the separation of anionic solutes because it charges positively, and moreover, can reverse the direction of EOF.

Acknowledgements

This project (grant No. 29835110) was financially supported by the National Natural Science Foundation of China.

References

[1] R.M.C. Sutton, K.L. Sutton, A.M. Stalcup, Electrophoresis 18 (1997) 2297.
[2] K. Verleysen, J. Vandijck, M. Schelfautand, P. Sandra, J. High Resolut. Chromatogr. 21 (1998) 323.
[3] C. Desiderio, S. Fanali, J. Chromatogr. A 818 (1998) 281.
[4] D.W. Armstrong, U.B. Nair, Electrophoresis 18 (1997) 2331.
[5] S. Hu, E.Q. Fu, P.C.H. Li, J. Chromatogr. A 844 (1999) 439.
[6] W.-H. Chen, C.-Y. Liu, J. Chromatogr. A 848 (1999) 401.
[7] S. Fanali, J. Chromatogr. A 792 (1997) 227.
[8] J.H.T. Luong, A.L. Nguyen, J. Chromatogr. A 792 (1997) 431.
[9] G. Vigh, A.D. Sokolowski, Electrophoresis 19 (1997) 2305.
[10] M. Fillet, P. Hubert, J. Crommen, Electrophoresis 19 (1998) 2834.
[11] B. Chankvetadze, J. Chromatogr. A 792 (1997) 269.
[12] J.B. Vincent, D.M. Kirkby, T.V. Nguyen, G. Vigh, Anal. Chem. 69 (1997) 4419.
[13] S. Shinkai, T. Arimura, K. Araki, H. Kawabata, J. Chem. Soc. Perkin Trans. 1 (1989) 2039.
[14] C.D. Gutsche, I. Alam, Tetrahedron 44 (1988) 4689.
[15] S. Shinkai, S. Mori, H. Koreishi, T. Tsubaki, O. Manabe, J. Am. Chem. Soc. 108 (1986) 4314.
[16] S. Shinkai, K. Araki, O. Manabe, J. Am. Chem. Soc. 110 (1988) 7214.
[17] S. Shinkai, T. Arimura, H. Satoh, O. Manabe, J. Chem. Soc. Chem. Commun. (1987) 1495.
[18] D. Shohat, E. Grushka, Anal. Chem. 66 (1994) 747.
[19] K. Baechmann, A. Bazzanella, I. Haag, K.-Y. Han, R. Arncke, V. Boehmer, W. Vogt, Anal. Chem. 67 (1995) 1722.
[20] K. Baechmann, A. Bazzanella, B. Goettlicher, I. Haag, K.-Y. Han, R. Arncke, V. Boehmer, GIT Spez. Chromatogr. 15 (1995) 96.
[21] A. Bazzanella, H. Moerbel, K. Baechmann, R. Mibradt, V. Boehmer, W. Vogt, J. Chromatogr. A 792 (1997) 143.
[22] A. Bazzanella, K. Baechmann, R. Mibradt, V. Boehmer, W. Vogt, Electrophoresis 20 (1999) 92.
[23] S. Sun, M.J. Sepaniak, J.-S. Wang, C.D. Gutsche, Anal. Chem. 69 (1997) 344.
[24] M.S. Peña, Y.L. Zhang, I.M. Warner, J. Chromatogr. A 816 (1998) 243.
[25] T. Zhao, X. Hu, J. Cheng, X. Lu, Anal. Chim. Acta 353 (1998) 263.
[26] L. Arce, A. Segura Carretero, A. Rios, C. Cruces, A. Fernandez, M. Valcarcel, J. Chromatogr. A 816 (1998) 243.
[27] C.D. Gutsche, K.C. Nam, J. Am. Chem. Soc. 110 (1988) 6153.
[28] D.-K. Xu, L. Hua, H.-Y. Chen, Chem. J. Chin. Univ. 17 (1996) 707.
[29] D.-K. Xu, L. Hua, H.-Y. Chen, Anal. Chim. Acta 375 (1996) 90.
[30] W.-C. Yang, A.-M. Yu, Y.-Q. Dai, H.-Y. Chen, J. Chromatogr. A 867 (2000) 261.
[31] C.D. Gutsche, B. Dhawan, J.A. Levine, K.H. No, L.J. Bauer, Tetrahedron 39 (1983) 409.
[32] A.D. John, Lange's Handbook of Chemistry, 13th Edition, McGraw-Hill, 1985.
[33] X.D. Yu, L. Lin, H.M. Han, C.Y. Wu, Wuhan Univ. J. Nat. Sci. (Engl. Ed.) 4 (1999) 463.
[34] M. Nishio, Y. Umezara, M. Hirote, Y. Takeuchi, Tetrahedron 51 (1995) 8665.