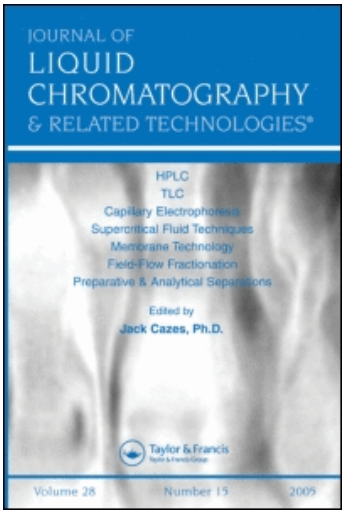

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P-sulfonic Calix[4]Arene as Running Buffer Additive in Electrokinetic Chromatography

T. Zhao[a]; X. -B. Hu[a]; J. -K. Cheng[a]; X. -R. Lu[a]
[a] Department of Chemistry, Wuhan University, Wuhan, China

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P-SULFONIC CALIX[4]ARENE AS RUNNING BUFFER ADDITIVE IN ELECTROKINETIC CHROMATOGRAPHY

Tao Zhao, Xu-Bo Hu, Jie-Ke Cheng,* Xue-Ran Lu

Department of Chemistry
Wuhan University
Wuhan 430072, China

ABSTRACT

The application of a new water-soluble calixarene, *p*-sulfonic calix[4]arene, as running buffer additive in electrokinetic chromatography was first investigated. Full separation of eight phenols was obtained within 8.5 min using only 5 mM *p*-sulfonic calix[4]arene as additive. Efficiency up to 8.8×10^5 plates/m was achieved. The influences of running buffer, additive concentration, field strength, temperature, and injection on mobility, elution range, migration of the solutes, resolution, and efficiency were reported, and the interaction of phenols with the calixarene was also discussed.

INTRODUCTION

The application of macrocyclic compounds as capillary electrophoresis (CE) running buffer additives has been shown to impart unique selectivity in separations of structurally similar solutes. Typical examples of macrocyclic reagents are cyclodextrins (CDs) and crown ethers. CDs have been proven to be extremely useful reagents as running buffer additives, with many applications in conventional CE,[1,2] micellar electrokinetic capillary chromatography (MECC),[3,4] and electrokinetic chromatography (EKC).[5]

Crown ethers have also been effectively employed as CE running buffer additives.[6,7] Both these classes of additives having cavities can form a variety of host-guest-type complexes with the solutes. But CDs can only form complexes with organic molecules and crown ethers can only do so with ions.

Recently, a class of new macrocyclic compounds, calixarenes, have been demonstrated as CE reagents.[8-10] Calixarenes are macrocyclic molecules made up of phenolic units meta linked by methylene bridges. They posses basket-shaped cavities.[11] Since Gutsche[12] and Shinkai[13,14] have found that water-soluble calixarenes can form a variety of host-guest-type complexes with organic guests in water, and Ungaro[15] and Chang[16] have found that calixaryl esters show high alkali metal ion affinity, the calixarenes were counted as the third generation of supermolecules, after CDs and crown ethers. The calixarenes have several advantages over the traditional additives. First, as indicated above, the calixarenes can form complexes with both organic and ionic molecules. Second, the size of the calixarene cavity is more flexible. Finally, calixarene can be easily synthesized and derivatized. Although the calixarenes have these advantages, little work has been done to investigate the utility of calixarenes in CE. In 1994, Shohat and Grushaka[8] first studied the effects of calixarene in CE. Bachmann and co-worksers[9] utilized resorcarenes to separate polycylic aromatic hydrocarbons (PAHs). Sun et al.[10] also studied the separation of PAHs employing *p*-(carboxyethyl)calix[n]arenes as running buffer additives in capillary electrokinetic chromatography.

In the present work, a new water-soluble calixarene, *p*-sulfonic calix[4]arene (see Fig.1), was applied. Retention behaviors of phenols in EKC employing *p*-sulfonic calix[4]arene as running buffer additive were first investigated. The effects tha. experiment parameters such as running buffer, additive concentration, field strength, temperature, and injection exert on mobility, elution range, migration of the solutes, resolution, and efficiency were reported, and the interaction of phenols with the calixarene was also discussed.

EXPERIMENTAL

Apparatus

A Spectra PHORESIS 1000 instrument with a intel 386 computer (Thermo Separation Products, USA) was employed. Analysis was performed in an uncoated capillary (43cm x 50μm i.d., effective length 35 cm, Yongnian Optical Fiber Factory, China). A 0.22μm cellulose membrane filter (Shanghai Institute of Medical Engineering, Shanghai) was used.

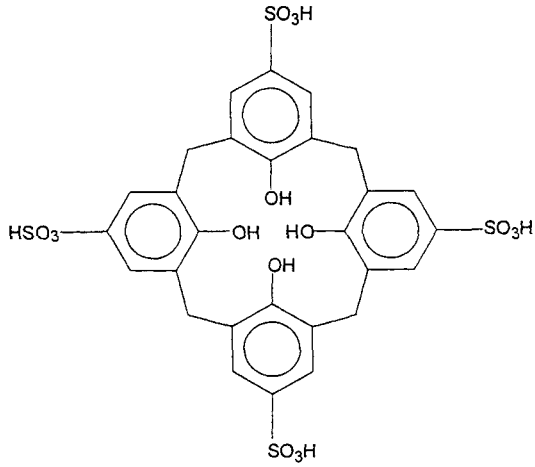

Figure 1. Structure of *p*-sulfonic calix[4]arene.

Reagents

Four different buffer solutions were prepared: (A) pH 5.0 phosphate buffer, using 0.05 M disodium hydrogen phosphate adjusted to the desired pH with 0.05 M sodium dihydrogen phosphate; (B) pH 4.0 phosphate buffer, using 0.05 M sodium dihydrogen phosphate adjusted with phosphoric acid; (C) pH 5.0 phosphate-borate buffer, using 0.04 M sodium dihydrogen phosphate and 0.02 M sodium tetraborate adjusted with phosphoric acid; and (D) pH 5.0 acetate buffer, using 0.05 M sodium acetate adjusted with acetic acid. The *p*-sulfonic calix[4]arene was synthesized as described by Shinkai.[17] Weighed amount of the calixarene was dissolved in buffer solution and ultrasonicated for 5 min, yielding calixarene concentration of 2.5-10.0 mM.

Benzenediol isomers, nitrophenol isomers, and phenol (Shanghai Reagent Factory, Shanghai) were used as they were dissolved in a methanol-water (1:1) mixture. p-Aminophenol (Shanghai Reagent Factory, Shanghai) was prepared in the methanol-water (1:1) mixture containing 1.0 mg/mL vitamin C (Northeast Medicine Factory, Shenyang). The concentrations of standard phenols solutions were in the range 0.33-1.47 mg/mL.

Water was doubly distilled. All reagents were of A.R. purity grade. Buffer and phenols solutions were filtered through a 0.22μm cellulose membrane.

Table 1

Effect of Running Buffer on Separation of Phenols*

		Buffer A	Buffer B	Buffer C	Buffer D
p-Aminophenol	t_r (min)	3.50	4.30	4.39	3.37
	k'	-0.194	-0.173	-0.165	-0.191
p-Benzenediol	t_r (min)	5.18	7.70	6.15	4.86
	k'	0.036	0.017	0.025	0.016
m-Benzenediol	t_r (min)	5.18	7.85	6.20	4.89
	k'	0.036	0.023	0.030	0.020
Phenol	t_r (min)	5.18	8.00	6.37	5.00
	k'	0.036	0.028	0.046	0.033
p-Nitrophenol	t_r (min)	5.18	8.00	6.52	5.00
	k'	0.036	0.028	0.059	0.033
o-Benzenediol	t_r (min)	5.18	8.00	7.01	4.93
	k'	0.036	0.028	0.102	0.024
m-Nitrophenol	t_r (min)	5.80	9.57	7.44	5.53
	k'	0.109	0.079	0.137	0.092
o-Nitrophenol	t_r (min)	8.48	23.50	12.84	7.80
	k'	0.359	0.260	0.456	0.300
EOF	t_0 (min)	4.89	7.27	5.89	4.73
Calixarene	t_{cal} (min)	-8.11	-3.16	-8.10	-6.66

* Conditions: fused-silica capillary (43cm x 50 μm i.d., effective length 35 cm), 5 mM *p*-sulfonic calix[4]arene, 25°C, 14kv, hydrodynamic injection 1s, detection 275 nm.

Procedure

A region of the capillary polyimide coating was removed to generate a transparent detection "window." At the start of each day and whenever the running buffer was changed, the capillary was rinsed with 1M NaOH, 0.1M NaOH and water each for 5 min, then it was rinsed with buffer for 10 min and preequilibrated by putting voltage on capillary for 10 min.

After each run, the capillary was rinsed with the running buffer for 5 min. The maximum absorptivity of *p*-sulfonic calix[4]arene was at 215 nm. Detection at 275 nm was performed and the wavelength was founded to be more favorable for UV detection of the phenols. Hydrodynamic injection and electrokinetic injection were used.

RESULTS AND DISCUSSION

Effect of Running Buffer

Table 1 provides information on the retention characteristics of the four running buffers for the phenols. The capacity factor, k', was determined by the equation [18]:

$$k' = \frac{t_r - t_0}{t_0(1 - \frac{t_r}{t_{cal}})}$$

t_0 is the effective retention time of an unretained solute in calixarene, marked by methanol; t_{cal} is the effective retention time of the calixarene, estimated from injections of the *p*-sulfonic calix[4]arene; t_r is the retention time of the solute.

At the pH of the running buffer, the *p*-sulfonic calix[4]arene was negatively charged (the pKa value of the calixarene is 3.26).[11] To determine t_{cal}, it was necessary to reverse the polarity of the system, since the calixarene actually has an opposing mobility that is larger than that of EOF. As seen in Table 1, the migration time of *p*-aminophenol was less than t_0. It could be attributed to a cathodic mobility due to partial protonation, which led to the negative value for k'.

At the four running buffers, the best separation was obtained with the buffer C. The k' of phenols were larger than that with other buffers. It was thought that the addition of borate would improve the selectivity of the system, since borate ions are known to form complexes with phenols.[19] But the complexation was much weaker than that of phenols with the calixarene because the separation of phenols in the buffer without calixarene was very poor (Fig.2).

The effect of change in pH was also shown. When pH was decreased, the decrease of EOF resulted in an increase of the mobility (to anode) of calixarene, and the k' value reduced. For example, the k' of *m*-nitrophenol was reduced from 0.109 to 0.079.

Effect of p-Sulfonic Calix[4]arene Concentration

The effect of p-sulfonic calix[4]arene concentration on the separation of phenols were studied (Fig. 2 and 3). Figure 2 shows that there was a poor separation without the calixarene in the running buffer. As the calixarene added

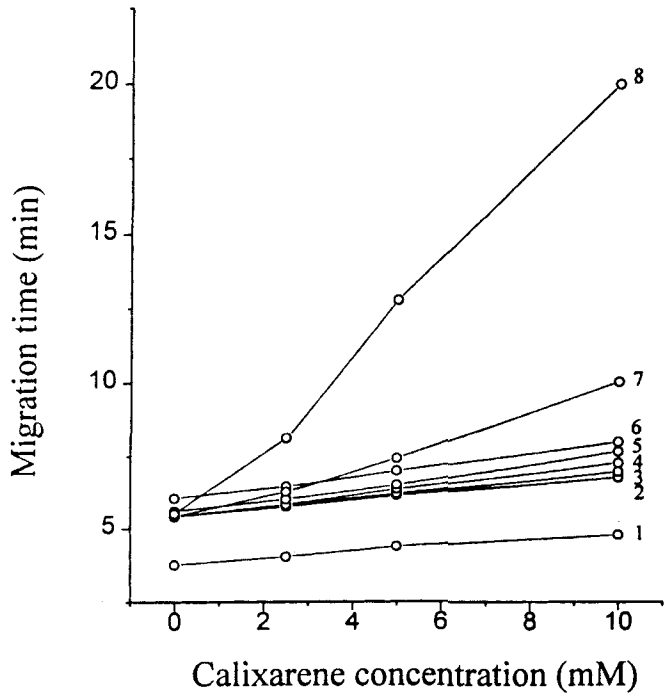

Figure 2. Effect of p-sulfonic calix[4]arene concentration on migration times of phenols. Conditions: fused-silica capillary (43cm×50μm i.d., effective length 35cm), 40 mM phosphate- 20 mM borate buffer (pH 5.0), 25°C, 14kv, hydrodynamic injection 1s, detection 275 nm. 1, p-aminophenol; 2, p-benzenediol; 3, m-benzenediol; 4, phenol; 5, p-nitrophenol; 6, o-benzenediol; 7, m-nitrophenol; 8, o-nitrophenol.

and its concentration increased, the migration time of solutes became long and an increasing trend in the resolution can be also observed. Figure 3 shows plots of k' versus the calixarene concentration for some phenols. It can be seen that the k' values are almost linearly correlated with the concentration.

At the concentration higher than 5 mM *p*-sulfonic calix[4]arene, fairly successful separation of phenols could be obtained (but there was only a partial separation between *p*- and *m*-benezediol).

The addition of the *p*-sulfonic calix[4]arene in the running buffer was limited by its UV absorption. At the high concentration (over 10.0 mM), the baseline was noisy although a suitable wavelength was operated.

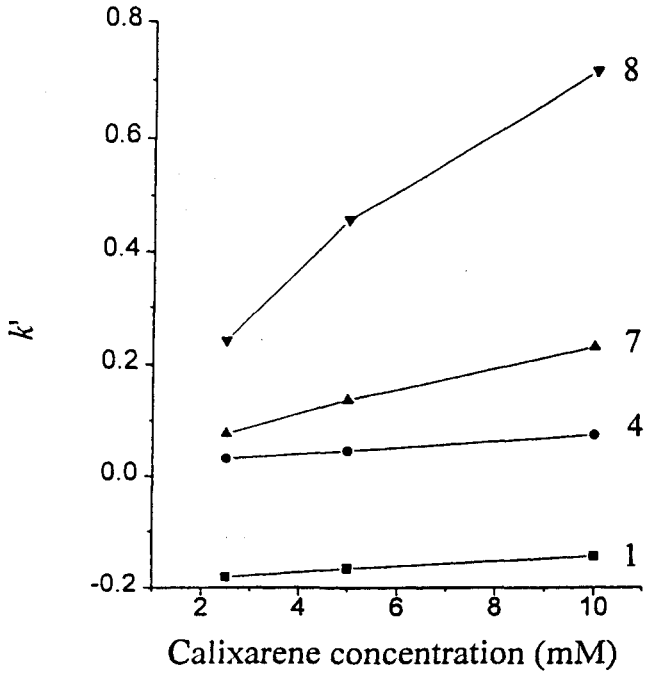

Figure 3. Effect of p-sulfonic calix[4]arene concentration on capacity factors of some phenols. Conditions as in Figure 2.

Protecting Agent

p-Aminophenol was easily oxidized without protecting agent.[20] Vitamin C was added to the *p*-aminophenol solution as protecting agent in our experiment. Although there was UV absorption, vitamin C wouldn't influence the separation because at the pH of the running buffers, vitamin C was negatively charged and its migration time (over 20 min) was longer than that of all phenols.

Effect of Voltage

In Figure 4, the dependence of efficiency on voltage is shown for three phenols. Optimum result was obtained for applying a voltage of 18 kv, and the theoretical plate numbers were in the range of $0.8\text{-}4.6\text{x}10^5$ per meter. Higher voltage led to peak broadening due to increased Joule heating.

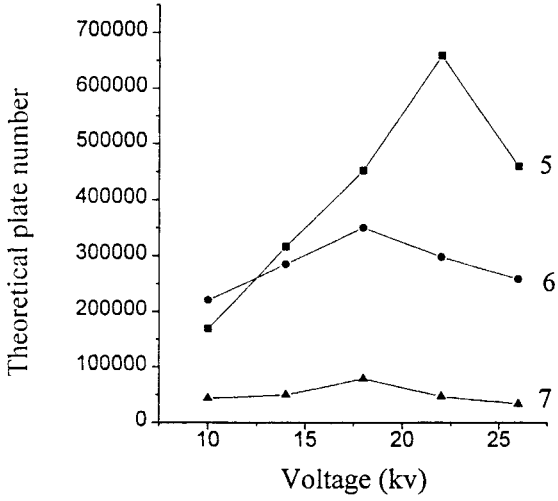

Figure 4. Effect of voltage on efficiency. Conditions: 5 mM p-sulfonic calix[4]arene, other conditions as in Figure 2.

Peaks of *o*-benezediol and *m*-nitrophenol partially overlapped when voltage was over 22 kv. When voltage was low, separation times became long and axial diffusion of solutes in CE tend to dominate, which led to decreased efficiency. At 10 kv, phenol and *p*-nitrophenol were poorly resolved.

Effect of Temperature

Temperature effect on separation was studied in the range 15-45°C. Migration times of solutes decreased with the increasing temperature. Like the effect of voltage, higher temperature led to low efficiency due to the increasing current levels and the associated Joule heating. When temperature was over 35°C, peaks of *p*-, *m*-benzenediol and phenol; *o*-benzenediol and *m*-nitrophenol seriously overlapped. Much lower temperature also influenced the separation. It could be seen that phenol and *p*-nitrophenol were poorly separated at 15°C.

Effect of Injection

The effects of electrokinetic injection and hydrodynamic injection were investigated (Figure 5A and B). Fig 5A shows the dependence of resolutions between *p*-benzenediol and *m*-benzenediol and *m*-benezenediol and phenol on

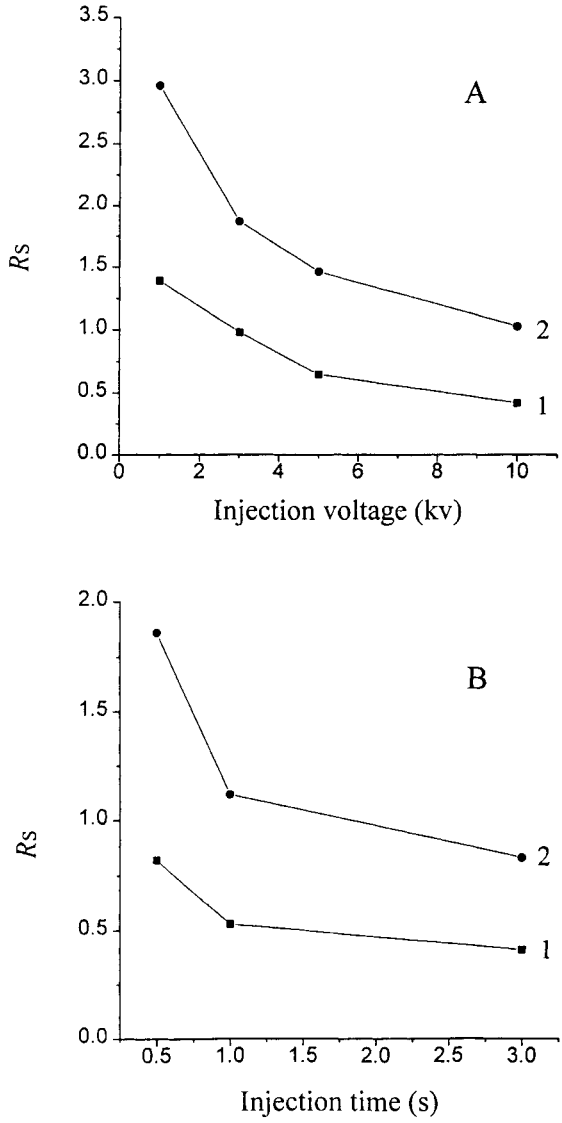

Figure 5. Effect of (A) injection voltage in electrokinetic injection and (B) injection time in hydrodynamic injection on resolution of (1) p-benzenediol and m-benzenediol and (2) m-benzenediol and phenol. Conditions: (A) 18kv, 25°C, electrokinetic injection 1s, other conditions as in Figure 4. (B) hydrodynamic injection, other conditions as in (A).

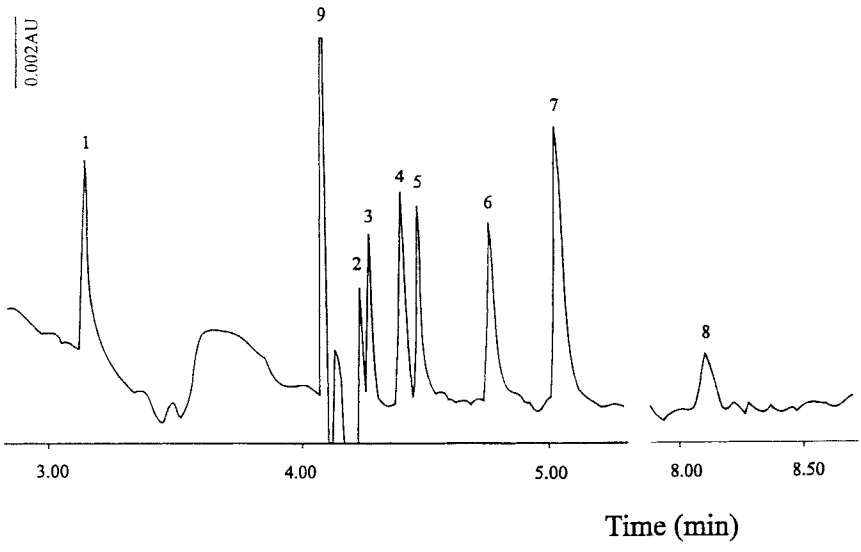

Figure 6. Electrophergram of eight phenols. Conditions: electrokinetic injection 1kv,1s, other conditions as in Figure 5. 1, p-aminophenol; 2, p-benzenediol; 3, m-benzenediol; 4, phenol; 5, p-nitrophenol; 6, o-benzenediol; 7, m-nitrophenol; 8, o-nitrophenol; 9, methanol.

the injection voltage in electrokinetic injection. It could be seen that the resolutions reduced with the increasing injection voltage. Figure 5B shows the effect of injection time on the separation in the hydrodynamic injection. Like electrokinetic injection, long injection time led to the poor separation. From Figure 5, it can be seen that the resolution in electrokinetic injection was greater than that in hydrodynamic injection. In our experiment, the phenols were dissolved in water-methanol mixture. The ionic strength of sample solutions were much lower than that of the running buffer. Sample stacking occurring in electrokinetic injection could improve the separation.[21,22] So the electrokinetic injection was more favorable for the separation than hydrodynamic injection. The baseline separation between *p*- and *m*-benzenediol was obtained when injection voltage was 1 kv in electrokinetic injection and the resolution between the two solutes was 1.4.

Separation of Phenols

Electropherogram of phenols is shown in Figure 6. The eight phenols can be fully separated within 8.5 min. The detection limits (calculated at to noise

Table 2

Reproducibility of Migration Times and Detection Limits of Phenols

	Average Migration Time (Min)	RSD (%)	Detection Limit (ng/μL)
p-Aminophenol	3.15	0.91	10.5
p-Benzenediol	4.31	0.98	8.3
m-Benzenediol	4.35	0.97	5.9
Phenol	4.47	1.25	14.7
p-Nitrophenol	4.56	1.20	4.0
o-Benzenediol	4.88	1.41	6.5
m-Nitrophenol	5.10	1.38	2.7
o-Nitrophenol	8.09	1.70	13.8

ratio of 2) and the reproducibilities of migration times of 5 injections were given in Table 2. RSD of migration times were in the range 0.9-1.7%. Efficiency up to $8.8x10^5$ plates/m (to *p*-benzenediol) was achieved and the efficiency was higher than that of methods with SDS[23] and CD[1] as buffer additives.

Capacity factors in our experiment are rather low (Table 1), but the relatively larger charge-to-mass ratio of calixarene produce a high electrophoretic mobility. Thus, the observed elution range was wider than that normally observed in MECC.

Sun et al.[10] reported that the best separations of PAHs were obtained with the calix[n]arenes (n=5,7) as the running buffer additives. Calix[4]arene cavity was too small for complexation and the large calix[8]arene cavity couldn't provide a suitable "snug" fit. In the present work, the calix[4]arene was successful in the separation of phenols. So, the calix[4]arene cavity was suitable for complexation with the solutes.

Separation selectivity in CE system involving CDs was reported to depend on the physiochemical and geometric characteristics of the solute guest and the CD host.[2,4,24] Hydrophobic interactions of the solute with the apolar cavity of the CD can act in concert with polar and hydrogen bonding interactions with hydroxyl groups on the "lip" of the CD. Similar interactions are expected to influence solute-calixarene complexation. In the electropherogram of phenols, the migration order of benezediols was p<m<o isomer, as the same as the nitrophenol isomers. It was thought that the migration orders of the two classes of isomers were dependent on the hydrophobic interactions of solute with the cavity of the basket-like calixarene. The interaction was influenced by the hydrophobicity and magnitude of the solute molecule. For nitrophenols, the

hydrophobicity of para isomer is less than that of meta isomer, which is even less than that of ortho isomer; the respective solubilities of *p*-, *m*- and *o*-nitrophenol are 1.7, 1.4 and 0.2g in 100g water.[25]

At the running buffer, the *p*-sulfonic calix[4]arene was negatively charged, and its electrophoretic mobility was in the direction opposite to the electroosmotic flow. So *p*-nitrophenol was eluted first, followed by *m*-nitrophenol, and then *o*-nitrophenol.

For benzenediols, the solubilities of meta and ortho isomers were identical with nitrophenols, but the solubilities of para isomer was less than meta and ortho isomers.[25] It seems that the meta and the ortho isomeric molecules were more fit for the size of the p-sulfonic calix[4]arene cavity than the para isomer, which may enhance the hydrophobic interactions of meta and ortho isomers with calixarene. So the meta and the ortho isomers elute after the para isomer. The migration order of benezediols was the same as for nitrophenols.

Nitrophenols can act as hydrogen bond acceptors, which enhance the interactions with calixarene. So nitrophenols elute late, following benezediols and phenol. As seen in Figure 6, *p*-aminophenol elutes first. It can probably be attributed to a cathodic mobility (see related discussion above).

CONCLUSIONS

p-Sulfonic calix[4]arene can be used as the running buffer additive in EKC. Although the capacity factors are rather low, the high charge-to-mass ratio of the calixarene produce a relatively wide elution range.

As the the calix[n]arenes (n=5,7) provided the best separation of PAHs, the calix[4]arene was successful in the separation of phenols and the full separation of eight phenols were observed using only 5 mM *p*-Sulfonic calix[4]arene as running buffer additive .

As a drawback, the calixarene can produce relatively large background in spectrophotometic detection due to its strong UV absorption, which led to a noisy baseline at the high calixarene concentration. This limitation will be investigated in our later work.

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