Journal of Chromatographic Science 2012;50:123–130
doi:10.1093/chromsci/bmr041

Article

Development of Three End-Capped Para-Benzoyl Calix[4,6, or 8]arene Bonded Stationary Phases for HPLC

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Three end-capped para-benzoyl calixarene bonded silica gel stationary phases are prepared and characterized by elemental analysis, infrared spectroscopy, and thermal analysis. The comparison and selectivity of these phases are investigated by using PAHs, disubstituted benezene, and naphthalene positional isomers as probes. Possible separation mechanism based on the different interactions between calixarenes and analytes are discussed. The results indicate that the separation for those analytes are influenced by the supramolecular interaction including π–π interaction, π-electron transfer interactions, space steric hindrance, and hydrogen bonding interaction on the calixarene columns. Importantly, the aromatic probes with polar groups such as –OH, $-NO_2$, and $-NH_2$ could regulate the selectivity of calixarene-bonded stationary phases.

Introduction

Calixarenes are macrocyclic molecules composed of phenol units linked by alkylidene. As a typical representative of the third-generation supermolecules after crown ethers and cyclodextrins, they have been increasingly attracting the attentions of scientists (1, 2) because of their excellent inclusion ability toward the neutral molecules and ions. In the field of chromatography, they have been extensively applied as the mobile phase additive (3–13), capillary inner-wall modifier (14–21), and the bonded stationary phases (22–48). The UV absorption of calixarenes is too high, which seriously decreases the detection sensitivity of the analytes, so calixarene-bonded stationary phases are preferable to the use of calixarene additives. Early in 1983, Mangia et al. (22) first prepared 4-tert-butylcalix[8]arene bonded stationary phase for gas-solid chromatography, and in 1993 Glennon et al. (23) first prepared a silica-bonded calix[4]-arene stationary phase for high-performance liquid chromatography. This created a precedent in this area. Thereafter, gas chromatography (GC) (29–31) and liquid chromatography (LC) (23–28, 32–48) calixarene-bonded stationary phases have been prepared.

To date, more and more applications of different calixarene-bonded stationary phases have been reported for the analyses of metal ions (23–25), aromatic positional isomers (34, 37, 39, 41, 42, 48), uracil derivatives and estradiol epimers (26), PAHs (33–37, 41, 44, 48), amino acid esters (24, 25), nucleosides (32–34), sulphonamides (41, 44, 51), and so on.

Lots of previous work has shown that LC calixarene-bonded silica stationary phases are excellent in reversed-phase separation performance and seem promising for future applications (32–48, 52). Recently, a *p*-tert-butylcalix[4]arene modified sol-gel column was developed for open-tubular capillary electrochromatography (21) and six LC calixarene-bonded stationary phases (48), and their separation performance and separation mechanism was investigated, assisted with a quantum chemistry calculation (48–50). In order to develop new calixarene stationary phases, the present work reported three end-capped para-benzoyl calix[n]arene ($n = 4, 6, 8$) bonded stationary phases (ec-BC[n]BS) ($n = 4, 6, 8$). Their chromatographic performance was investigated by using PAHs, positional isomers of substituted aromatics, and naphthalene as probes. Additionally, the comparison has been used to explore the differences and similarities between ec-BC[n]BS and ODS (ZorBax-SB-C_{18}). The separation mechanism was also proposed.

Experimental

Apparatus and reagents

The elemental analysis was performed with a Flash EA 1112 elemental analyzer. A Bruker Vector 22 instrument was used to obtain the infrared spectra. A Shimadzu DT-40 thermal analyzer was used for thermogravimetric (TG) analysis. Chromatographic analyses were performed using a Waters HPLC system with a 600 E pump and a 2996 diode array detector (Waters Co. Ltd., Milford, MA). The modified silica gels were slurry packed into stainless steel columns (150 mm × 4.6 mm, i.d.) using a packing machine (Kerui Tech. Co. Ltd., Dalian, China) under the pressure of 50 MPa. A ZorBax-SB-C_{18} column (Agilent, 150 mm × 3.9 mm, i.d., 5 μm) was used as a comparison with the homemade calixarene columns.

The source of the reagent of silica gel (with particle size of 5 μm, pore size of 90 Å, and specific surface area of 270 m^2/g), γ-glycidoxypropy1 trimethoxysilane (KH-560), and other chemicals and solvents, unless specified, are all from the same source with the previous document (48). Para-benzoyl calix[n]arene ($n = 4, 6, 8$) (BzCx[n], $n = 4, 6,$ or 8) were synthesized in accordance with the previously published procedures (53).

Synthesis of BzCx[n] (n = 4, 6, or 8)

Para-tert-butylcalix[n]arene and calix[n]arene were prepared in good yields in accordance with the previous literature (54–57). Figure 1 shows the specific synthesis steps of para-benzoyl calixarenes. The para-benzoyl calixarenes were prepared according to the literature (53) with a slight modification, by adding one equivalent of distilled benzoyl chloride to 0.24 equivalent of calix[4]arene (0.16 equivalent of calix[6]arene or 0.12 equivalent of calix[8]arene) and aluminum chloride (1.5 equivalent) in 50 mL dry nitrobenzene. The mixtures were stirred at 25°C under an atmosphere of nitrogen for 20 h. HCl (20 mL at

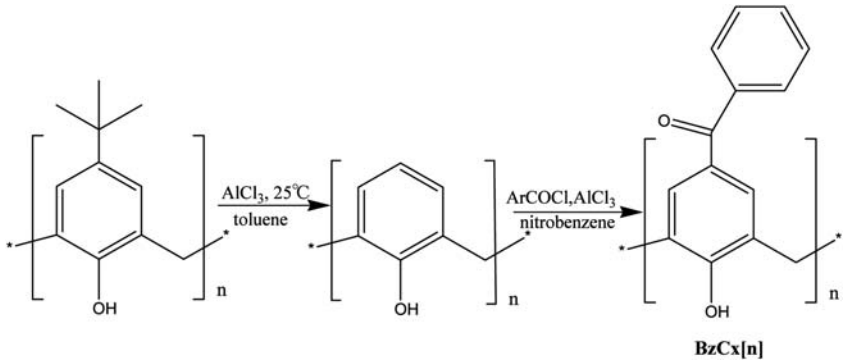

Figure 1. The Synthesis of Para-benzoyl calix[n]arene (BzCx[n] ($n = 4$, 6 or 8).

1.0 mol/L) was added to stop the interaction. The solvent was then removed by using steam distillation. The residues were dissolved in acetone. The mixture was filtered and the organic phases was evaporated. The crude BzCx[n] were purified by recrystallization (acetone). The purity of BzCx[n] was obtained more than 98% by using the area normalization method of HPLC [conditions: CN column, detection at 265 nm, MeOH–H_2O (20:80, v/v) mobile phase and 0.8 mL/min flow rate].

Preparation of three end-capped para-benzoyl calixarene-bonded stationary phases

The para-benzoyl calix[n]arene-bonded silica gel stationary phases (BC[n]BS, $n = 4$, 6, 8) without end-capping and end-capped BC[n]BS (ec-BC[n]BS, $n = 4$, 6, 8) (Figure 2) were prepared according to similar procedures (48) with a slight modification.

Silica gel was activated according to conventional methods (48). A mixture of 100 g silica gel and 100 mL hydrochloric acid (0.5 M) in 500 mL water was stirred for 24 h at room temperature, then refluxed for 10 h, filtered, then washed with water to pH = 7, and dried at 160°C for at least 10 h.

The γ-glycidoxypropyl-bonded silica gel (GBS) was prepared accorded to the previous work (48), and the mixture of KH-560, activated silica gel, toluene (freshly distilled), and triethylamine (used as a catalyst) was stirred at 80°C with dry N_2 protection for 8 h, then filtered by 1.5 μm filter, washed with toluene and acetone in turn, dried at 100°C under vacuum for 8 h. Finally, γ-glycidoxypropyl-bonded silica gel (GBS) was obtained.

A mixture of 2.0 g calixarene (BzCx[4], BzCx[6] or BzCx[8]) and 0.6 g NaH in 60 mL freshly distilled toluene was refluxed at 80°C with dry N_2 protection for 30 min, and then the supernatant liquid was transferred to a 100-mL three-neck flask, and then 5.0 g GBS and 60 mL dry toluene was added. This mixture was again refluxed with the catalyst for 48 h. The products, BC[4]BS, BC[6]BS, and BC[8]BS, were formed, respectively. A quarter of the BC[n]BS reaction solution was transferred out, filtered, washed, and dried as the preparation of GBS described earlier. The remaining BC[n]BS mixture was added with 10 mL trimethylchlorosilane, refluxed at 80°C with dry N_2 protection for another 6 h, and then filtered by 1.5 μm filter, washed in sequence with toluene, dichloromethane, doubly distilled water, and acetone for three times, to insure the calixarene that does not bond with the silica gel as it is washed off. Finally, it was dried at 100°C under vacuum for 8 h (58, 59) and ec-BC[n]BS ($n = 4$, 6, 8) was prepared.

Chromatographic procedures

The bonded phases, ec-BC[n]BS ($n = 4$, 6, 8), were respectively packed into 150 mm × 4.6 mm i.d. stainless steel columns according to a slurry packing procedure (48) by using methanol as the displacing agent (50 MPa, 2 h). The mobile phase used was ACN–H_2O, MeOH–H_2O, or MeOH in a 0.01 mol/L water solution of phosphate buffer solution (pH 4).

The analytes were dissolved in the mobile phase at a concentration in the range of 5–100 μg/mL, and 20 μL of the solution was injected onto the chromatographic column. In order to obtain the column efficiency and capacity factors of the probes, the dead times (t_0) were determined by injecting 0.05 M sodium nitrate ($NaNO_3$) at UV detection 210 nm in a MeOH–H_2O (70:30, v/v) as the mobile phase. All measurements were performed in triplicate.

Results and Discussion

Characterization of the calixarene-bonded stationary phases

The para-benzoyl calix[n]arene-bonded silica gel stationary phase (ec-BC[n]BS, $n = 4$, 6, 8) was obtained by the interaction of para-benzoyl calix[n]arene with GBS by the breaking ring reaction method (36, 48, 51).

The bonded amounts of organic ligand and their thermal stability were obtained and listed in Table I. GBS without BzCx[n] is an intermediate product of the bonded stationary phase. Its carbon content is lower than the other bonded silica gel stationary phases. According to the carbon content of the bonded silica gel stationary phases, the bonded amounts of BzCx[n] on the silica gel were calculated by subtracting that of GBS. As is shown in Table I, as a result of the spatial hindrance of the bonded organic ligands during the reaction, the bonded amounts onto silica gel decreased with the increase of calix[n]arene size (from 4 to, 6, 8).

The thermal stability of the bonded phases has been investigated by TG analysis. It can be seen from Table I, the temperatures of weight loss and thermal decomposition were over

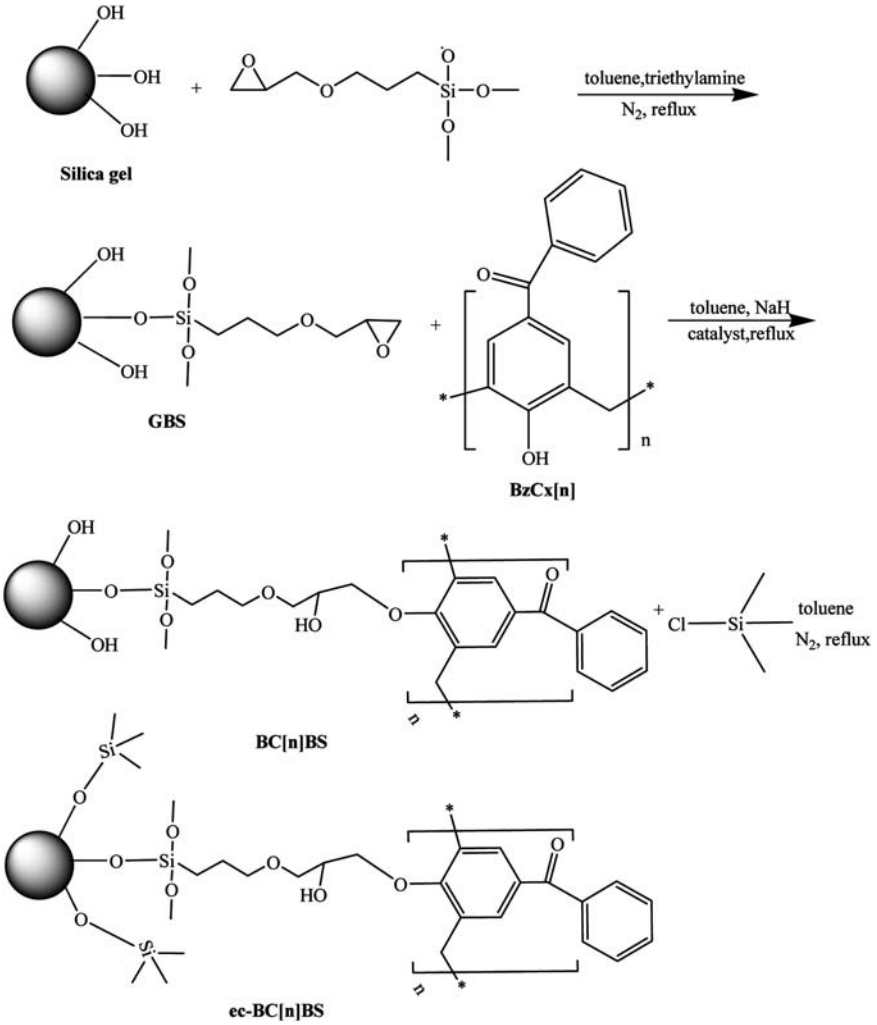

Figure 2. Preparation scheme of ec-BC[n]BS ($n = 4, 6, 8$).

Table I
Results of the Elemental Analysis and Thermal Analysis of the Bonded Phases

Bonded phase	By elemental analysis			By thermal analysis (TG)	
	C (%)	H (%)	Bonded amount (mmol BzCx[n]/g silica gel)	Weight loss (%)	Decomposition temperature (°C)
GBS	5.76	0.35	0.600*	9.21 (230–400°C)	246
BC[4]BS	12.37	0.68	0.098		
ec-BC[4]BS	13.30	0.83		14.22 (360–540°C)	400
BC[6]BS	12.90	0.70	0.071		
ec-BC[6]BS	14.57	0.97		15.45 (340–540°C)	380
BC[8]BS	13.52	0.79	0.058		
ec-BC[8]BS	14.96	0.95		16.03 (300–540°C)	350

*The bonded amount of γ-glycidoxypropyl trimethoxysilane (mmol/g silica gel).

300°C for three ec-BCBS[n]. It indicates that the new ec-BCBS[n] bonded phases possessed high thermal and chemical stability, which guarantee the longevity of columns, as well as the reproducibility of the method.

IR spectra (not shown here) were used to demonstrate the formation of the bonded phases. Their IR spectra show a weakened absorption band at 3450 cm^{-1}, which is characteristic of the stretching frequency of residual Si-OH and the residual phenolic hydroxyl group on the calixarenes. Peaks at 2945 ± 10 and 2875 ± 10 cm^{-1} are assigned to C–H stretching frequency. The peak at 1735 ± 10 cm^{-1} is assigned to the carbonyl group of the calixarene. Also, the characteristic absorption band of the benzene ring appears at 1450 ± 10 cm^{-1} and 1625 ± 10 cm^{-1}. The broad bond at 1110 cm^{-1} is assigned to the overlaps of C–O of the ether-bridge and the Si–O. All IR spectra indicate that BzCx[n] are bonded onto silica gel through KH-560.

Efficiency and stability of ec-BC[n]BS column

The dead times were determined by $NaNO_3$ using MeOH–water (70:30, v/v) as a mobile phase at 1.0 mL/min and UV detection at 210 nm. The effective theoretical plate numbers for ec-BC[n]BS column using biphenyl (20 μg/mL) as a solute probe were 8300, 8700, and 8100 plates/m, respectively.

The columns have alternately been eluted with ACN–water, MeOH–water, and MeOH–KH_2PO_4 (0.01 mol/L) phosphate

buffer (pH 4) for 3 months (20 injections for each mobile phase per day). The results demonstrate that the column efficiency reduction was less than 3%, and the relative standard deviations (RSDs) of the retention times of biphenyl were less than 2.0% ($n = 10$). This indicates that these ec-BC[n]BS columns are stable and repeatable.

HPLC separations on the ec-BC[n]BS columns

In order to evaluate the characteristic of three new ec-BC[n]BS columns, the HPLC separations of the seven PAHs, aromatic positional isomers, and naphthalene isomers were carried out. Their capacity factors k′ and resolutions (R) were calculated and listed in Tables II, III, and IV; their separation mechanism is also discussed therein.

The separation of PAHs

In this section, the separations (Figure 3) for seven PAHs on three ec-BC[n]BS and ODS columns were carried out under the same chromatographic conditions. As can be seen, the elution orders of PAHs on ec-BC[n]BS columns are the same as that on the ODS column, indicating the ec-BC[n]BS and ODS have similar hydrophobic interactions with PAHs.

Comparing the values of the capacity factor k′ (see Table II) of acenaphthene, anthracene, pyrene, and chrysene (except anthracene on the ec-BC[6]BS column) on the ec-BC[n]BS columns, it was found that the k′ increased gradually from ec-BC[4]BS, ec-BC[6]BS to ec-BC[8]BS, these might be attributed to the growth in number of benzene rings of the bonded phases, which leads to the stronger π−π interactions with the PAH.

Table II
k′ and R Values of PAHs on Various Columns

Bonded Phase		Benzene	Toluene	Biphenyl	Acenaphthene	Anthracene	Pyrene	Chrysene
ec-BC[4]BS	k′	1.63	1.93	3.52	3.90	5.19	7.02	9.38
	α	1.18	1.82	1.11	1.33	1.35	1.34	
	R	0.91	3.63	0.91	3.06	3.07	3.07	
ec-BC[6]BS	k′	1.50	1.84	3.48	3.92	4.76	7.30	9.94
	α	1.23	1.89	1.13	1.21	1.53	1.36	
	R	1.27	4.76	1.04	2.79	2.77	3.06	
ec-BC[8]BS	k′	1.67	1.91	3.50	4.02	5.40	7.56	10.08
	α	1.14	1.83	1.15	1.34	1.40	1.33	
	R	0.69	3.45	0.97	2.04	2.44	2.38	
ODS*	k′	2.66	3.90	7.61	8.95	11.15	15.58	21.79
	α	1.47	1.95	1.18	1.25	1.40	1.40	
	R	3.85	7.43	2.14	3.21	5.13	5.79	

*ODS, Agilent ZorBax-SB-C_{18} column; mobile phase, ACN−water (60:40, v:v); flow rate, 1.0 mL/min; detection wavelength, 258 nm.

The separation of disubstituted benezene positional isomers

Five groups of disubstituted benezene positional isomers were separated on three ec-BC[n]BS columns. For each group, the HPLC condition was optimized, and better separation was obtained by comparing these results with their separation on an ODS column. Table III shows the k′ and R values, and Figure 4 shows the typical chromatograms for m, o, and p-benzenediol on ec-BC[n]BS and ODS columns.

As can been seen in Table III, when ec-BC[n]BS columns are compared with ODS columns, it was found that the $-NO_2$ and −OH substituted benezene have longer retention times on ec-BC[n]BS than that on ODS, which may be associated to π-electron transfer interactions of the calixarene and the analyte molecule. The cavity of calixarene is hydrophobic and abundant of π-electron, and the $-NO_2$ and −OH substituted groups are strong electron withdrawing group. Therefore the stronger π-electron transfer interactions between the guest molecule and the host calixarene enhance the analyte's retention.

For o-, m-, and p-nitrophenol, it can be noted that o-nitrophenol was last eluted out on both the ec-BC[n]BS and ODS column; however, m- or p-nitrophenol was last eluted out on p-tert-butyl-calix[4]arene and calix[4]arene-bonded stationary phases reported earlier (48). This implies that carbonyl group (−C = O) and benzene ring on the benzoyl group of ec-BC[n]BS plays additional roles on the separation mechanism for o-, m-, and p-nitrophenol. These additional interactions are possibly π−π and hydrogen bonding interaction.

Table III
k′ and R Values of the Aromatic Positional Isomers on Various Columns

Bonded Phase		Aminophenol*	Nitroaniline*	Nitrophenol†	Benzenediol†	Benzenediamine†
ec-BC[4]BS	Elution order	p m o	m p o	m p o	p m o	m p o
	k′	1.14 1.38 1.98	9.00 12.84 14.99	9.36 10.48 13.31	1.42 1.95 2.39	0.73 0.73 1.40
	α	1.21 1.48	1.43 1.17	1.12 1.27	1.37 1.23	1.00 1.93
	R	0.61 1.27	2.20 0.86	0.80 1.84	1.21 0.88	0.00 2.20
ec-BC[6]BS	Elution order	p m o	m p o	m p o	p m o	m p o
	k′	0.88 1.14 1.57	6.78 9.65 11.05	7.46 8.11 11.58	1.12 1.53 1.85	0.14 0.55 0.90
	α	1.30 1.38	1.42 1.15	1.09 1.43	1.37 1.21	3.93 1.64
	R	0.97 2.92	3.06 0.78	0.61 1.98	1.34 1.02	3.38 2.25
ec-BC[8]BS	Elution order	p m o	m o p	m p o	p m o	o p m
	k′	0.97 1.18 1.67	7.68 11.53 24.71	8.36 8.96 12.38	0.99 1.32 1.69	0.59 0.59 1.54
	α	1.22 1.42	1.50 2.14	1.07 1.38	1.33 1.28	1.00 2.61
	R	0.64 1.57	2.51 4.96	0.40 2.08	0.89 0.85	0.00 4.93
ODS‡	Elution order	p m o	m o p	p m o	p m o	m p o
	k′	0.56 0.75 1.22	3.19 5.35 6.52	2.54 4.53 5.50	0.67 0.96 1.40	0.32 0.53 5.50
	α	1.34 1.63	1.68 1.22	1.78 1.21	1.43 1.46	1.66 1.00
	R	0.96 2.05	4.81 2.24	2.21 4.55	0.72 1.04	0.76 0.00

* Mobile phase, MeOH−0.01 mol/L KH_2PO_4 (40:60, v:v).
† Mobile phase, MeOH−water (40:60, v:v).
‡ Agilent ZorBax-SB-C_{18}.

As shown in Table III, the elution order of nitroaniline isomers on ec-BC[4]BS and ec-BC[6]BS columns is m < p < o, which was consistent with the reported literatures (32, 48). With the proton-donor capability increasing from m- to p- and to o-nitroaniline, the hydrogen bonding interaction between protonated nitroaniline and the residual calixarene phenolic OH group and carbonyl group (−C = O) of ec-BC[4]BS and ec-BC[6]BS was enhanced, which leads to the following elution order: m < p < o. However, when using the ec-BC[8]BS column, the elution order becomes m < o < p, and the retention time of p-nitroaniline is greatly increased compared with ec-BC[4]BS and ec-BC[6]BS, which may be associated with the guest-host interactions of the calixarene cavity and the analyte molecule. The supramolecular inclusion may include π−π interaction, hydrogen bonding interactions between $-NO_2$ of p-nitroaniline and −OH of ec-BC[8]BS, and between $-NH_2$ of p-nitroaniline and −C = O of ec-BC[8]BS.

From Table III, the elution order of aminophenol and benzenediol is the same when on both the ec-BC[n]BS column and the ODS column. However, the elution orders of benzenediamine on ec-BC[4]BS and ec-BC[6]BS are different from that on the ec-BC[8]BS or ODS, indicating that there are more complicated interactions, which influence the k′ and R values, as well as separation selectivity.

Table IV
k′ and R Values of Naphthalene Derivatives on Various Columns

Bonded Phase		Naphthol*	Naphthylamine†	Dimethylnaphthalene‡
ec-BC[4]BS	Elution order	β α	α β	2,6- 2,3- 2,7-
	k′	11.14 13.26	17.58 19.06	6.64 6.64 6.64
	α	1.18	1.08	1.00 1.00
	R	1.08	0.76	0.00, 0.00
ec-BC[6]BS	Elution order	β α	α β	2,6- 2,3- 2,7-
	k′	8.68 10.39	14.35 15.23	5.29 5.29 5.29
	α	1.20	1.06	1.00 1.00
	R	1.51	0.57	0.00 0.00
ec-BC[8]BS	Elution order	β α	α β	2,6- 2,3- 2,7-
	k′	8.30 9.78	16.14 19.17	5.91 5.91 5.91
	α	1.18	1.07	1.00 1.00
	R	1.05	0.34	0.00 0.00
ODS§	Elution order	α β	α β	2,3- 2,6- 2,7-
	k′	5.08 5.96	11.42 11.42	17.53 17.53 19.34
	α	1.17	1.00	1.00 1.10
	R	1.31	0.00	0.00 1.22

* Mobile phase, MeOH−water (50:50, v:v).
† Mobile phase, MeOH−0.01 mol/LKH_2PO_4 (40:60, v:v).
‡ Mobile phase, MeOH−water (70:30, v:v).
§ Agilent ZorBax-SB-C_{18}.

The separation of naphthalene derivatives on ec-BC[n]BS

The prepared para-benzoyl calixarene phases have more benzene rings comparing to the p-tert-butylcalix[4]arene-bonded and calix[4]arene-bonded stationary phases. As expected, they might have a stronger π−π interaction with naphthalene derivatives. The comparison of chromatographic behaviors on three ec-BC[n]BS and ODS columns is given in Table IV and Figure 5 (typical chromatograms).

From Table IV, the elution orders of naphthols on the calixarene column and the ODS column were opposite. The elution order of naphthols on the ODS column is α < β, which is in agreement with their pKa values (9.30 for α-naphthol and 9.57 for β-naphthol). This is because the separation of naphthol on the ODS column is based on hydrophobic interaction. However, when on the calixarene column, the elution order is β < α, which is consistent with the reported literatures (60, 61); this may be because α-naphthol is easy to ionize compared with β-naphthol; in addition, ionized oxygen anions of naphthalene increase the electron density of the naphthalene ring, so the π−π interaction between α-naphthol and the benzene ring of ec-BC[n]BS is stronger; thus, β-naphthol was first to be eluted out.

As shown in Table IV, the separation of naphthylamine on calixarene columns is better than that on the ODS column under the same conditions. The elution order of naphthylamine on calixarene columns was always α < β, which might be depended on their pKb values, 10.08 for α-naphthylamine and

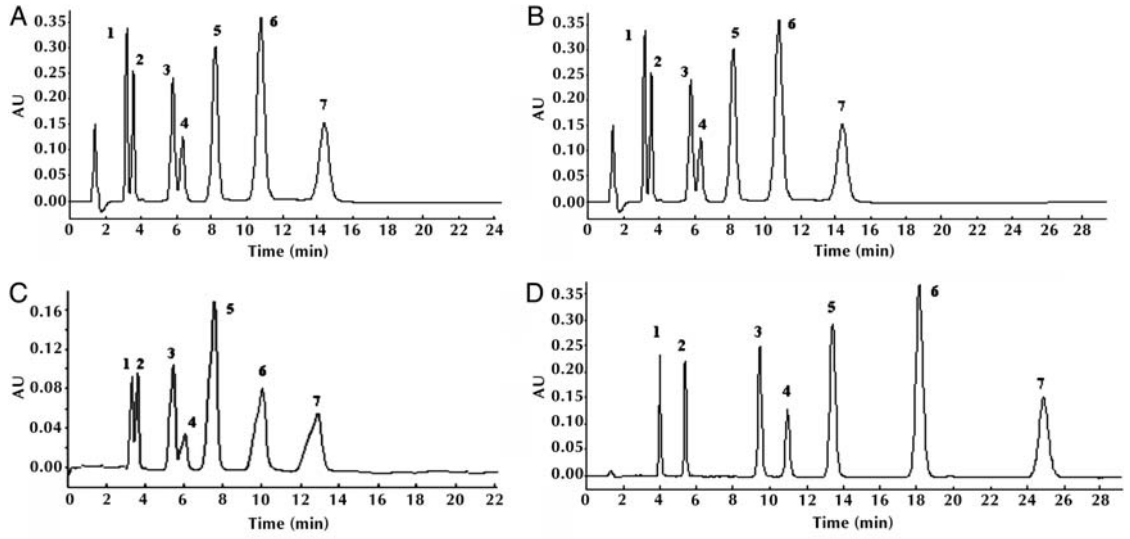

Figure 3. HPLC separations of PAHs on ec-BC[4]BS (A), ec-BC[6]BS (B), ec-BC[8]BS (C), and ODS (D) columns. Conditions: Mobile phase, ACN-water (60:40,v/v); flow rate, 1.0 mL/min; detection wavelength: 258 nm. Peaks: 1, benzene; 2, toluene; 3, biphenyl; 4, acenaphthene; 5, anthracene; 6, pyrene; 7, chrysene.

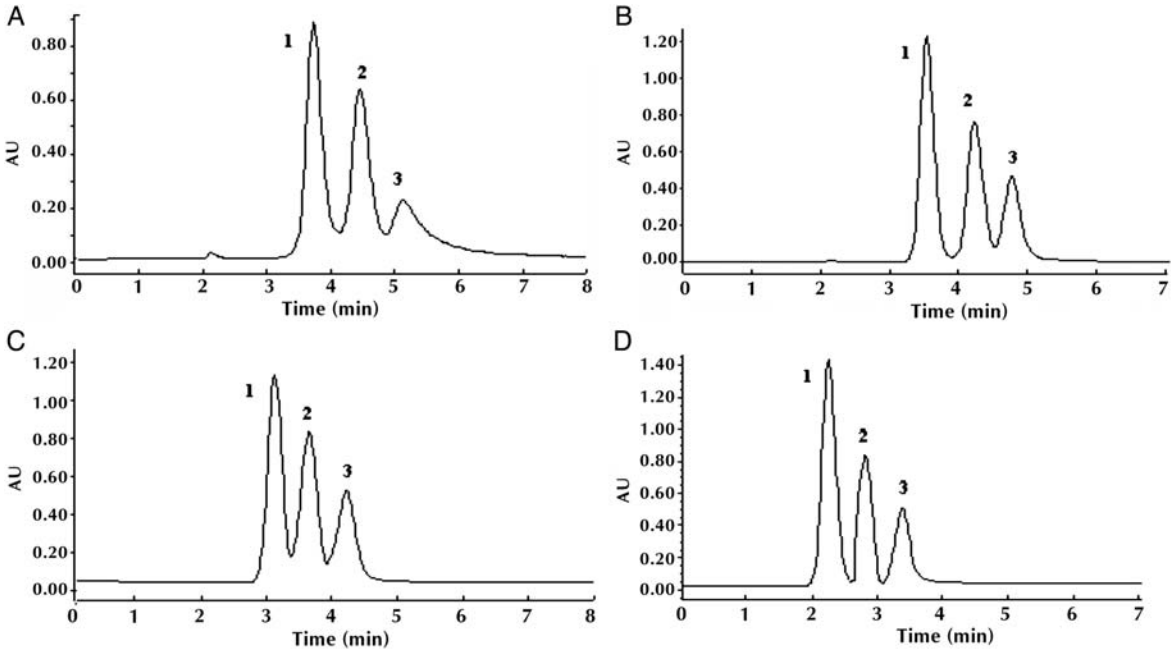

Figure 4. HPLC separations of benzenediol on ec-BC[4]BS (A), ec-BC[6]BS (B), ec-BC[8]BS (C), and ODS (D) columns. Conditions: Mobile phase, MeOH–water (40:60, v/v); flow rate, 0.8 mL/min; detection wavelength, 230 nm. Peaks:1, p-Benzenediol; 2, m-Benzenediol 3, o-Benzenediol.

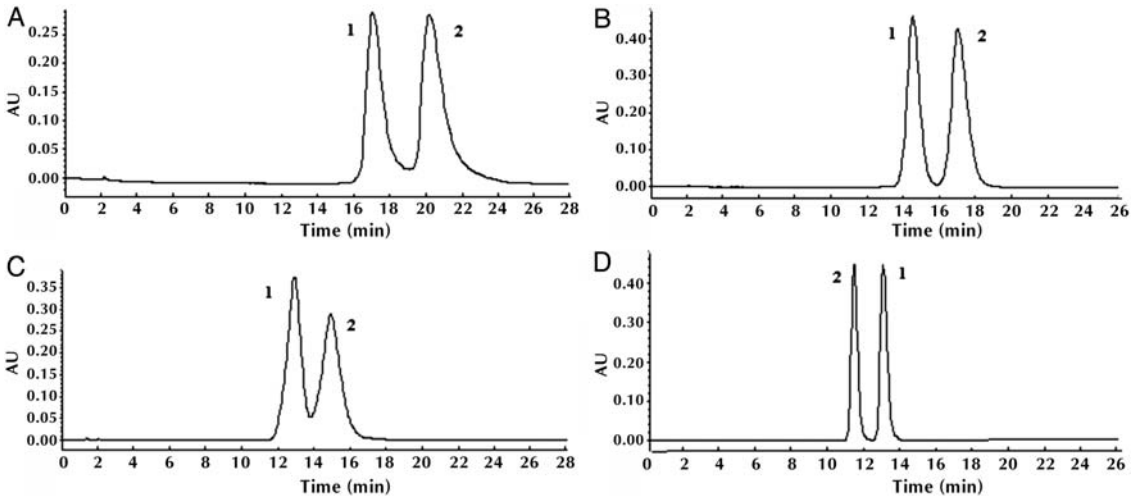

Figure 5. HPLC separations of naphthol on ec-BC[4]BS (A), ec-BC[6]BS (B), ec-BC[8]BS (C), and ODS (D) columns. Conditions: Mobile phase, MeOH–water (40:60, v/v); flow rate, 0.8 mL/min; detection wavelength, 275 nm. Peaks: 1; β-naphthol; 2, α-naphthol.

9.89 for β-naphthylamine. α-Naphthylamine is more easily protonated when in the weak acidity mobile phase. When naphthylamine protonated, the p–π conjugate interaction between p-electron on the N atom and the naphthalene ring, the degree of delocalization of naphthylamine reduced, thus the π-π interaction of α-naphthylamine and calixarene bonded stationary phase was weakened. Therefore α-naphthylamine was easier to be eluted out.

At the same time, it can be sees that naphthol and naphthylamine gave comparatively stronger retention on calixarene columns than that on ODS, which further confirmed that there were the additional interactions with exception of the hydrophobic interaction between naphthalene and calixarene.

For dimethylnaphthalene, three position isomers did not achieve separation on ec-BC[n]BS, and retention times were also less than that on ODS column. It implies that n–π interaction between ODS and naphthalene was stronger than π–π effect between ec-BC[n]BS and naphthalene.

From this discussion, it is noteworthy the aromatic isomers and naphthalene derivatives, which own polar groups such as –OH, $-NO_2$, and $-NH_2$, tend to obtain better separation and selectivity on calixarene-bonded phases by contrast.

Conclusions

The chromatographic behavior of seven PAHs, five disubstituted aromatic positional isomers, and three naphthalene derivatives on three ec-BC[n]BS were studied by using different compositions of the mobile phases, respectively. Their retention behavior were compared with those on ODS. The results shows that the end-capped para-Benzoyl calixarene-bonded stationary phase exhibits high selectivity for PAHs, and aromatic isomers with polar groups such as $-OH$, $-NO_2$, and $-NH_2$. The interaction including hydrophobic interaction, $\pi-\pi$ interaction, space steric hindrance, and hydrogen bonding interaction between calixarenes and probes manipulates their chromatographic behaviors on the calixarene columns.

Acknowledgements

The authors acknowledge the support of NSF of China (20875083, 20775073, 21077095), State Key Laboratory of Environmental Chemistry & Ecotoxicology (KF2008-22) and the Innovation Scientists & Technicians Troop Construction Projects of Zhengzhou City (10LJRC192). They also thank Dr. Julie Rimes for assistance with editing the text.

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