Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1040 (2004) 53–61
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Preparation and characterization of a *p-tert*-butyl-calix[6]-1,4-benzocrown-4-bonded silica gel stationary phase for liquid chromatography

Lai-Sheng Li [a,b], Shi-Lu Da [a,*], Yu-Qi Feng [a], Min Liu [a]

[a] *Department of Chemistry, Wuhan University, 430072 Wuhan, China*
[b] *Analytical and Testing Center, Nanchang University, 330047 Nanchang, China*

Received 2 December 2003; received in revised form 27 March 2004; accepted 31 March 2004

Abstract

A *p-tert*-butyl-calix[6]-1,4-benzocrown-4-bonded silica gel stationary phase (CR6BS) was first prepared via 3-glycidoxypropyltrimethoxysilane as coupling reagent for high performance liquid chromatography. The structure of the new stationary phase was characterized by diffuse reflectance infrared fourier transform spectroscopy (DRIFT), elemental analysis and thermal analysis. The chromatographic performance of the bonded-stationary phase was evaluated by using neutral, acidic and basic solutes as probes. Meanwhile, comparative study of the new stationary phase with a *p-tert*-butyl-calix[6]arene-bonded silica gel stationary phase (C6BS, the parent) and ODS was done under the same chromatographic conditions. The results show that the new stationary phase has an excellent reversed-phase property, which is similar to C6BS and ODS. However, the selectivities for some aromatic compounds are different from the parent phase (C6BS) and ODS, especially the latter. In one hand, as hybrid of calixarene and crown ether, CR6BS with the oxygen atoms of ether-bridge can provide the complexation sites for the solutes, lacking of C6BS. On the other hand, the rigid conformation of CR6BS may be responsible to the different performance partially. CR6BS exhibits high selectivity in the separation of alkylated aromatics from their parents as compared with C6BS.

Keywords: Stationary phases, LC; Retention mechanism; *p-tert*-Butyl-calix[6]-1,4-benzocrown-4-bonded silica gel

1. Introduction

Much attention has been attracted to calixarenes [1,2] mainly in the field of supramolecular chemistry for the past twenty years, because it possesses excellent inclusion capability to many neutral molecules and ions. Calixarenes has also been added to mobile phases or immobilized onto matrices as stationary phases for gas chromatography (GC), high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) [3–6]. The results show that the recognition ability of the macrocycles could largely improve separation selectivities for many solutes, especially aromatics [7,8].

In 1993, Glennon and co-workers [9–11] first prepared silica-bonded calix[4]arene tetraester and used it to separate metal ions and amino acid esters in HPLC. Park et al. [12] reported the separation of some substituted aromatic positional isomers on a calix[6]arene-*p*-sulfonate-bonded silica stationary phase. The substituted aromatics, nucleosides, uracil derivatives, estradiol epimers and *cis*/*trans* isomers of proline-containing peptides on calix[*n*]arene-bonded ($n = 4, 5, 6, 8$) silica gel were successfully separated by Gebauer et al. [13–15]. Menyes et al. reported that a hexapropylether of *p-tert*-butyl-calix[6]arene linked covalently to silica, and was used to separate polycyclic aromatic hydrocarbons(PAHs) and fullerenes, and showed higher selectivity and lower consumption of solvent than conventional RP-C_{18} and the cyclodextrin-bonded phase [16]. In the past few years, our research group prepared *p-tert*-butyl-calix[*n*]arene-bonded ($n = 4, 6, 8$) silica gel stationary phases with coupling reagents by one-pot method [17,18] and investigated the chromatographic separation of

* Corresponding author. Tel.: +86-27-87684066; fax: +86-27-87647617.
E-mail addresses: dashilu@public.wh.hb.cn, lilaishengcn@163.com (L.-S. Li).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.03.067

some positional isomers, polycyclic aromatic hydrocarbons, nucleosides, bases and sulfonamide drugs [19]. The results show that calixarene-bonded stationary phases are excellent reversed-phase packings with including capability. The calixarene-bonded phase exhibits the promising application for HPLC.

Calixcrowns, the hybrid of calixarenes and ethers, are a novel family of calixarenes in which the phenolic oxygens are linked by poly (oxyethylene) chains intramolecularly. Due to their highly selective metal ion recognition, since 1983, the design, synthesis and complex assessment of these molecules have been very developed [20–23]. It has been shown that they can selectively recognize K^+ with respect to Na^+ even better than the natural valinomycin [24]. The remarkable selectivities for metal ions have led to their applications as ion-selective electrodes, ionphoric membranes, selective carrier mainly in the treatment of radioactive liquid wastes and so on [25,26]. Though numerous studies have been done, much less attention has been paid to its application for chromatography. Since 1995, a few applications of calixcrowns have been reported for GC [27,28]. In 1996, Arena and co-workers prepared 1,3-alternate calix[4]crown-bonded silica gel stationary phases via hydrosilanization and employed for the separation of K^+ and Cs^+ in HPLC [29]. The separation potential of calixcrowns for HPLC requires further to be explored.

In this paper, we first reported that a *p-tert*-butyl-calix[6]-1,4-benzocrown-4-bonded silica gel stationary phase (CR-6BS) which was prepared via 3-glycidoxypropyltrimethoxysilane as coupling reagent in the pretence of NaH and phase transfer catalyst for HPLC (shown Fig. 1). The structure of the new stationary phase was characterized by diffuse reflectance infrared fourier transform spectroscopy (DRIFT), elemental analysis and thermal analysis.

The goal of our work is mainly to evaluate the new calixcrown-bonded phase and investigate the chromatographic performance to vary with the introduction of the ether-bridge and the rigid conformation. So, the comparison studies of CR6BS with a reported *p-tert*-butyl-calix[6]arene-bonded stationary phase (C6BS, the parent) [17] and ODS were carried out under the same chromatographic conditions by using different solutes, such as basic, acidic and neutral compounds as probes. The retention mechanism was proposed.

2. Experimental

2.1. Apparatus

Elemental analysis was performed with a MOD-1106 elemental analyzer (Italy). A Model 710 instrument (Nicolet Analytical Instruments) was used for Fourier transform infrared (FTIR) spectroanalysis. A Shimaduz DT-40 thermal analyzer was used for thermogravimetric analysis. The liquid chromatographic system was composed of a P200 II pump, a UV200 II variable wavelength UV-detector attached Echrom 98 chromatographic data system (Dalian Elite company, Dalian, China), and a Rheodyne Model 7125 injector with 20 μl loop.

2.2. Chemicals

Silica (Kromasil, particle size 5 μm, pore size 100 Å, surface area 310 $m^2\,g^{-1}$, pore volume 0.9 $ml\,g^{-1}$) and ODS (Kromasil C_{18}, 5 μm particle size, the bonded amount 1.054 $mmol\,g^{-1}$) were purchased from Akzo Nobel (Sweden). 3-Glycidoxypropyl-trimethoxysilane was purchased from Wuhan University Chemical Plant (Wuhan, China). *p-tert*-Butyl-calix[6]-1,4-benzocrown-4 [30] and *p-tert*-butyl-calix[6]arene-bonded stationary phase (C6BS) [17] were synthesized according to reported procedures. Other reagents were obtained from various commercial sources and were analytical grade unless indicated. Water was doubly distilled water.

2.3. Preparation of CR6BS

The preparation scheme of the new stationary phase was shown in Fig. 1. The two-step procedure is as follow: The silica gel was activated according to conventional methods by hydrochloric acid to remove metal ion and maximize the number of silanol groups on the surface.

A mixture of 5.0 ml 3-glycidoxypropyltrimethoxysilane and 5.0 g activated silica gel in 50 ml freshly distilled dry toluene was stirred and heated at reflux under streaming dry nitrogen gas with 0.1 ml triethylamine as catalyst for 6 h. The bonded silica gel was filtered, washed with toluene and acetone. 5.93 g of 3-glycidoxypropyl-bonded stationary phase (GBS, the spacer-bonded silica gel), which to be used as precursor of following reaction, was obtained.

A mixture of 1.0 g *p-tert*-butyl-calix[6]-1,4-benzocrown-4 and 0.05 g NaH in 50 ml anhydrous toluene was heated with stirring under an inert atmosphere at 80 °C for 30 min. Subsequently, 3.0 g GBS and 0.5 g tetrabutylammonium bromide were added to the suspension and heated immediately to reflux for 24 h. The bonded-material was filtered and washed in sequence with toluene, acetone, doubly distilled water, dimethylformamide and acetone. After dried at 120 °C, 3.18 g the *p-tert*-butyl-calix[6]-1,4-benzocrown-4-bonded stationary phase (CR6BS) was dried. Weight gain was by 27.56% to bare silica gel in all.

2.4. Chromatographic procedure

The bonded phases (CR6BS, GBS, C6BS and ODS) were, respectively, packed into stainless-steel columns (150 mm × 4.6 mm i.d.) by using the balanced-density slurry technique. The mobile phases were methanol–water or phosphate buffers unless indicated. The flow rates were generally set at 0.8 $ml\,min^{-1}$. The samples were dissolved in methanol or mobile phases and kept in a refrigerator (in the dark). The wavelength of detection was at 254 or 270 nm. The

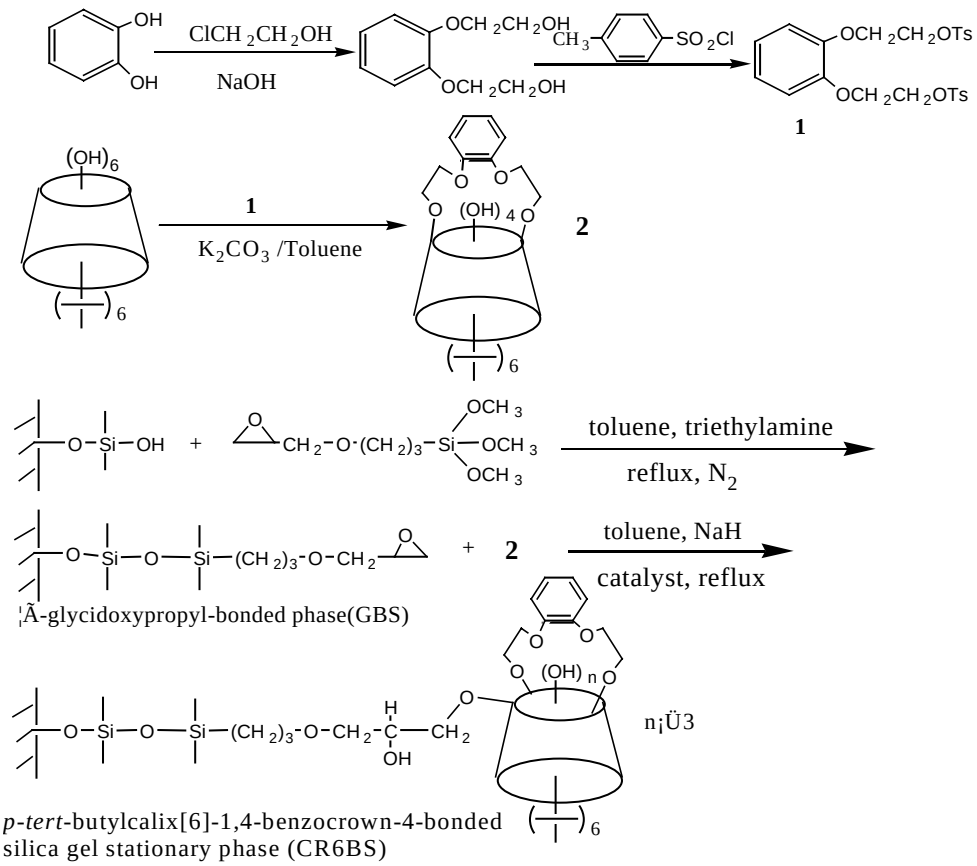

Fig. 1. The preparation of *p-tert*-butyl-calix[6]-1,4-benzocrown-4-bonded silica gel stationary phase (CR6BS).

concentration of samples was from 20 to 200 μg ml^{-1}. Typically, 5 μl of sample solutions were injected. The aqueous solution of 0.05 mol l^{-1} sodium nitrate was used as probe to determine void time. All measurements were carried out at ambient temperature (25 ± 2 °C)and repeated at least twice.

3. Results and discussion

3.1. Preparation of the bonded phases

Though preparation of the calixarene-bonded silica gel has been studied extensively, unfortunately, the most synthesizing methods were patents [14,16,31]. The reported methods mainly include: hydrosilation addition, thiolene addition and condensation reaction of the coupling reagents [10,12,13,17–19]. The addition reaction of *p*-allyl-calixarenes with triethoxysilane under catalysis with hexachloroplatinic acid was a usual method to prepare the calixarene-bonded phases [19]. In our laboratory, Xu et al. [17] prepared the *p-tert*-butyl-calix[6]arene-bonded phase via breaking ring reaction of 3-glycidoxypropyltrimethoxysilane as coupling reagent and perchloric acid as catalyst. The bonded amount of the stationary phase was less than 0.06 mmol g^{-1}, which could not exhibit ideal separation for the solutes to be tested because of lower bonded amount and column efficiency. The phenolic hydroxyl groups of the calix[6]arene were suppressed by $HClO_4$ as catalyst, leading to lower bonded amount. The new approach used includes two steps, the precursor, 3-glycidoxypropyl-bonded stationary phase (GBS), was first synthesized, then the calixcrown-bonded phases (CR6BS) were prepared by using phenolic sodium of *p-tert*-butyl-calix[6]-1,4-benzocrown-4 in the presence of phase transfer catalyst to cleave the epoxy groups of GBS. On one hand, the facile method can avoid the complicate manipulation and rigorous reaction of the hydrosilation addition. On the other hand, the active nucleophile, sodium of the calix[6]arene with the aid of phase-transfer catalyst can lead to higher bonded amount.

3.2. The structural characterization of CR6BS

The suspended state NMR is effective tool to characterize the structure of ligand on the surface of silica gel. In our laboratory, Xiao [18,32] reported that several calixarene-bonded phases were measured by ^{29}Si and ^{13}C CP-MAS (cross polarization/magic angle spinning) NMR. However, it is expensive. In general, diffuse reflectance infrared fourier transform spectroscopy (DRIFT), elemental analysis and thermal analysis can provide enough information for the phases.

The results of elemental analysis were shown in Table 1. The diffuse reflectance infrared fourier transform spec-

Table 1
The results of elemental analysis for the bonded stationary phases

Bonded phases	C (%)	H (%)	Bonded amounts (mmol g^{-1})
CR6BS	16.02	2.52	0.075
C6BS	15.75	2.48	0.083
GBS (the spacer)	9.18	1.58	0.956
ODS (Kromasil C_{18})	19.0	3.16	1.054

troscopy (DRIFT) with silica as blank was shown in Fig. 2.

Diffuse reflectance infrared fourier transform spectroscopy (DRIFT) is a method of FTIR to solid samples. The spectrum of DRIFT shows the disappearance of a strong absorption band at 3600–3700 cm^{-1}, which is characteristic of the residual Si–OH stretching frequency after subtraction of bare silica. Peaks at 2949.8, 2888.76 cm^{-1} are assigned to C–H stretching frequency. The characteristic absorption band of the benzene rings should appear at 1643.3, 1541.96, 1476.2 cm^{-1}, and peak at 1357.61 cm^{-1} is assigned to C-H bending frequency of the butyl group. Peaks at 798.5, 691.89 cm^{-1} should be the C–H out planar bending frequency of the benzene rings. The C–O stretching frequency of the ether-bridge almost overlaps with the Si–O appearing for a broad bond at 1065 cm^{-1}. The IR spectra indicate that the calix[6]crown ligand existed on silica gel.

Additionally, the thermal analysis of CR6BS shows that lose weight occurred mainly in the rang of temperature from 300 to 600 °C, which was over GBS (250–500 °C). It indicates that the new packing is possessed of high heat and chemical stability. Thermal analysis (from 30 to 750 °C, temperature rate 10 °C min^{-1}) shows that the CR6BS loses weight by 28.75% which is consistent with the gain weight of CR6BS preparation. The results indicate that the calix[6]crown was successfully immobilized to the spacer silica gel. The bonded amount of CR6BS was found to be 0.075 mmol g^{-1} according to the carbon content (Table 1).

3.3. Column efficiency and stability of CR6BS

The column efficiency of CR6BS was determined by using methanol-water (60:40, (v/v)) as mobile phase and biphenyl as solute probe at a flow rate of 0.8 ml min^{-1}. In this condition, the retention time of biphenyl is 9.78 min and the theoretical plate number was 21,000 m^{-1}. The column has alternately been eluted with methanol–water (60:40) and methanol–0.02 mol l^{-1} H_3PO_4 for a week. The retention time of biphenyl on CR6BS was 9.78 ± 0.2 min ($n > 20$). The column efficiency almost does not changed. The results show that the new packing is stable and repeatable in chromatographic procedure even if using acidic mobile phases. The long-time stability of CR6BS will be further investigated.

From the above test, CR6BS exhibited high efficiency for neutral solutes, such as biphenyl and so on. However, as can be noticed in Figs. 6–8, several polar solutes on CR6BS and C6BS displayed broad peaks slightly, which might be due to that the residual silanol groups partially led to weak ion exchange and various mechanisms without the capped process.

3.4. Separation of PAHs and alkylbenzenes

Many empirical tests [33–35] were used to evaluate hydrophobicity of stationary phases for RP-C_{18}. Though there exists no universally accepted chromatographic test for particular packing, especially, the calixarene-bonded phases, the alkylbenzene homogues and PAHs were often used as probes to investigate hydrophobicity of the new packing.

As can be seen in Fig. 3, the good linear relationship between log k values and n (the number of methylene groups in alkylbenzenes) on CR6BS was observed. This indicates the new material exhibits an excellent reversed-phase property, which is similar to conventional ODS. As can also noticed in Fig. 3, that the methanol content (70%) in mobile phase on ODS was more than those (60%) on CR6BS and

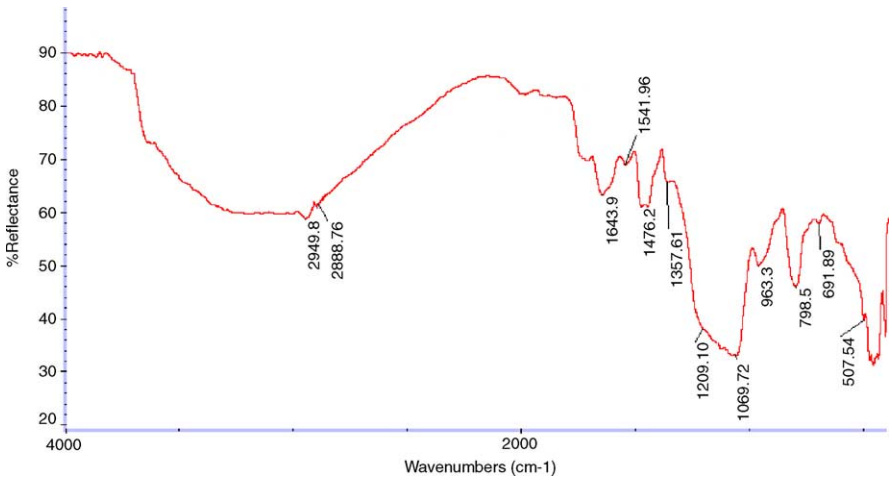

Fig. 2. The diffuse reflectance infrared fourier transform spectroscopy (DRIFT) of *p-tert*-butyl-calix[6]-1,4-benzocrown-4-bonded stationary phase (CR6BS).

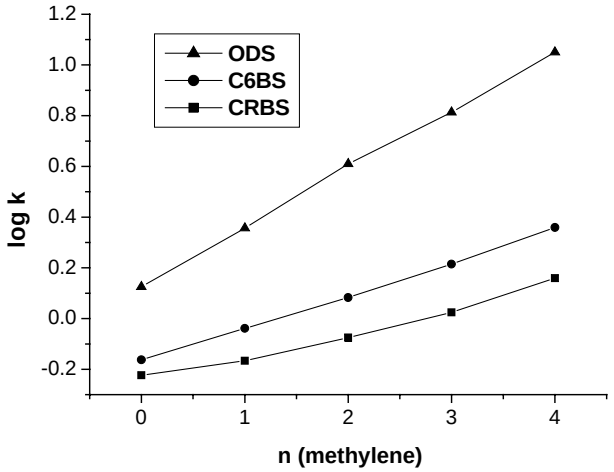

Fig. 3. The plot of log k vs. the number (n) of methylene groups in alkylbenzenes. Mobile phases: methanol-water (60:40 for CR6BS, 60:40 for C6BS, 70:30 for ODS (v/v)); flow rates: 0.8 ml min^{-1}; UV: 254 nm.

C6BS. It was because under the same condition butylbenzene on ODS exhibited too strong to be eluted. The slopes of three curves were in the order ODS > C6BS > CR6BS, which are correlated to their hydrophobicities. Table 1 shows the CR6BS was similar to C6BS in the carbon loads and bonded amounts. Meanwhile, the two packings have similar structures except for the ether-bridge. Obviously, it can be concluded that the introduction of the ether-bridge led to decrease the hydrophobicity and the selectivity of CR6BS for the methylene groups.

Fig. 4 showed that the separation of nine PAHs could be achieved on CR6BS and C6BS within 25 min., which indicate that the two packings exhibit typical reversed-phase property. It can also be noticed that C6BS has higher selectivity for alkylbenzenes than CR6BS. The reason can be explained as follows: On one hand, the ether-bridge can increase the polarity of CR6BS and decrease its hydrophobicity. On the other hand, relative rigid conformation of CR6BS weakens the efficient interaction between the *p-tert*-butyl clusters of CR6BS and the alkyl chains of the solutes. Thus, the selectivity of CR6BS for alkylbenzenes decreases (Table 2).

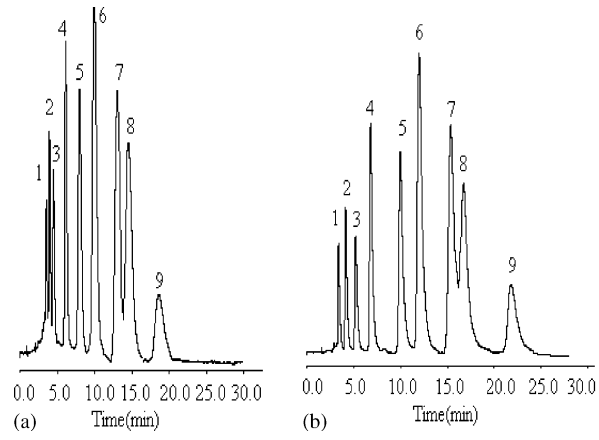

Fig. 4. The chromatograms of PAHs on CR6BS (a) and C6BS (b). Mobile phases: methanol–water (60:40 (v/v)); flow rates: 0.8 ml min^{-1}; UV: 254 nm. Peaks: (1) benzene; (2) toluene; (3) xylene; (4) naphthalene; (5) biphenyl; (6) fluorene; (7) phenanthrene; (8) anthracene; (9) fluoranthene.

Table 2
The retention factors (k) of PAHs on CR6BS, C6BS and ODS

Solutes	CR6BS[a]	C6BS[a]	ODS[b]
	Retention factors (k)		
Benzene	0.762	1.122	1.333
Toluene	0.973	1.620	2.272
Ethylbenzene	1.214	2.306	4.072
Propylbenzene	1.595	3.427	6.506
Butylbenzene	2.203	5.266	11.228
Naphthalene	2.067	3.276	5.128
Anthracene	5.988	9.535	12.154

[a] Methanol–water (60:40 (v/v)), 0.8 ml min^{-1}.
[b] Methanol–water (70:30 (v/v)).

Table 3 show that the selectivities of CR6BS, C6BS and ODS for methylene and phenyl groups. The solute probes are benzene/toluene/ethylbenzene/propylbenzene/butylbenzene ($n = 0, 1, 2, 3, 4$) and benzene/naphthalene/anthracene ($n = 1, 2, 3$), respectively [31]. It can been found from the slopes of linear equations (Table 3) that the selectivity of CR6BS for methylene groups is lower than that of C6BS, while its selectivity for phenyl groups is almost equal to latter. The results imply that the different sites of the bonded phases attribute to the selectivities for different kinds of solutes. For example, the *p-tert*-butyl cluster is mainly related to the selectivity for alkylbenzenes, while the hydrophobic moiety of the calix[6]arene ligand for PAHs. C6BS can provide a flexible *p-tert*-butyl cluster, which facilitates to recognize alkyl chains in alkylbenezenes, while lacking of CR6BS. However, the cone conformation of CR6BS improves the recognition for PAHs, which can partially make up for its weaker hydrophobicity. Fig. 4 illustrates that CR6BS has lower resolution to alkylbenzenes and better recognition for PAHs with rapid speed as compared with C6BS.

As you known, the hydrophobicity of propylbenzene is close to that of naphthalene. So, it is difficult to separate the pair of solutes. As can be observed in Fig. 5, the baseline

Table 3
The selectivities of CR6BS, C6BS and ODS for methylene and phenyl groups

Bonded phases	Groups	Equations of linear regression[a]	R[a]
CR6BS	Methylene	$\ln k = 0.2618n - 0.2932$	0.9972
	Phenyl	$\ln k = 1.0308n - 1.3136$	0.9998
C6BS	Methylene	$\ln k = 0.3842n + 0.0969$	0.9992
	Phenyl	$\ln k = 1.0699n - 0.9543$	0.9999
ODS	Methylene	$\ln k = 0.5312n + 0.2985$	0.9995
	Phenyl	$\ln k = 1.1051n - 0.7372$	0.9985

[a] Regressed according to the data of Table 2; n is the number of methylene or phenyl groups.

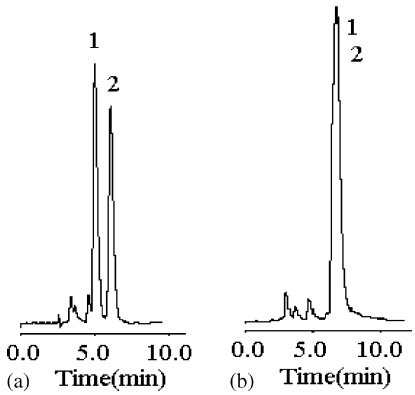

Fig. 5. The chromatograms of propylbenzene and naphthalene on CR6BS (a) and C6BS (b). Mobile: methanol–water (60:40 (v/v)); flow rates: 0.8 ml min^{-1}. Peaks: (1) propylbenzene; (2) naphthalene.

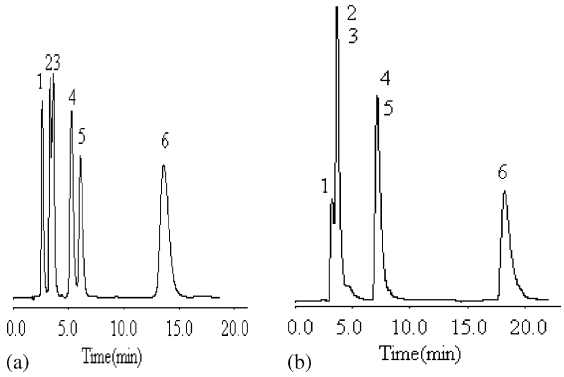

Fig. 6. The chromatograms of PAHs and *azo*-PAHs on CR6BS (a) and C6BS (b). Mobile phases: methanol–water (60:40 (v/v)); flow rates: 0.8 ml min^{-1}. Peaks: (1) pyridine; (2) quinoline; (3) benzene; (4) acridine; (5) naphthalene; (6) anthracene.

separation of propylbenzene and naphthalene on CR6BS can easily be achieved, instead of C6BS. Obviously, the above two factors are responsible for the chromatographic performance. As can also be notice in Table 2, the retention of propylbenzene was more than that of naphthalene, which is because that the octadecyl group of ODS can provide effective interaction with the propyl group of the analyte. Thus, the elution order of the above pair of solutes on ODS was different from both CR6BS and C6BS.

Other investigation is 'ionic induce fit'. Many experiments showed that calixcrowns have selective binding property with alkaline metal ions [24,29]. However, the obvious retention varieties of PAHs on CR6BS could not be observed by using methanol–phosphate buffers containing alkaline metal ions, such as K^+, Na^+ as mobile phases. This is related to the immobilization means, in which CR6BS was prepared via the lower rim bonded to silica, while the reported calixcrown phase employed the upper rim [29].

Obviously, the introduction of the ether-bridge can partially change the hydrophobicity and selectivity of CR6BS for the analytes as compared with its parent (C6BS).

3.5. The separation of PAHs and azo-PAHs

Six solutes, namely, benzene/naphthalene/anthracene/pyridine/quinoline/acridine were used as probes further to investigate the chromatographic property of CR6BS. Theses compounds possess the similar parent rings, such as benzene and pyridine. As can be noticed in Fig. 6, quinoline (double ring) was eluted nearby to benzene (single ring), and acridine (triple ring) was next to naphthalene (double ring). This is correlated to the decrease in hydrophobicities of the solutes due to introduction of the heterocyle with nitrogen and indicates the natural hydrophobic moiety of CR6BS.

As a rule, the hydrophobicity of *azo*-PAHs is different from that of PAHs, and C6BS with stronger hydrophobicity should provide better separation of the above solutes in comparison with CR6BS. However, the latter is superior to the former in this case (Fig. 6). Based on the fact, it can be assumed that the recognition of the bonded phases for the analytes was related to their conformation partially. For example, CR6BS has relative rigid cone conformation and definite mouth sizes of the cavities, which facilitate to recognize the pairs of solutes, such as acridine and naphthalene, quinoline and benzene, etc. In contrast to this, the conformational transition of C6BS led to poorly resolve to the above the pairs of solutes (Fig. 6). Meanwhile, it can also be observed that the three *azo*-PAHs on ODS can be eluted nearby benzene with poor resolution.

3.6. The separation of position isomers

The separation of several aromatic position isomers provides an example for difference between CR6BS and C6BS. As can be seen in Table 4, the elution orders for most solutes on the two packings were similar, which indicates that the same moieties of the calix[6]arenas result in the similar chromatographic selectivities for the isomers. Making a comparison between CR6BS and a *p-tert*-butyl-calix[8]arene-bonded phase with 0.071 mmol g^{-1} bond amount and similar spacer [36,37], it can also be found that the *k* values of the isomers on CR6BS (shown in Table 4) were small, which are mainly related to the sizes of the cavities.

However, it can also be noticed in Table 4, the retention of several solutes, such as benzenediol, methylphenol, methylaniline on CR6BS were greater those on C6BS. This behavior is in part contrary to the bonded amounts (Table 1). Obviously, the chromatographic performance of CR6BS is dependent on not only the moiety of calix[6]arene, but also the ether-bridge. The explanation should be that the additional hydrogen bond interaction between the hydrogen-donor (OH, NH_2) of the isomers and the oxygen atoms of the ether-bridge on CR6BS existed. The synergistic effects between the moiety and the ether-bridge of CR6BS increase the retention of the analytes.

The interaction of the ether-bridge with the isomers can also be observed (Table 4). For example,

Table 4
The elution order and the retention factors (k) of aromatic positional isomers on CR6BS and C6BS

Solutes	Retention factors (k)											
	CR6BS				C6BS				C8BS[a]			
Benzenediol[b]	*pmo*[c]	0.554	0.796	0.841	*pmo*	0.134	0.162	0.206	*pmo*	1.81	3.04	3.66
Benzenediamine[b]	*mpo*	0.479	0.492	0.643	*mpo*	0.557	0.864	1.478	*mpo*	0.22	1.25	1.79
Methylphenol[b]	*mpo*	1.665	1.681	1.842	*omp*	0.873	0.886	0.908	*omp*	3.20	3.92	9.56
Methylaniline[b]	*omp*	1.213	1.314	1.330	*omp*	0.842	0.864	0.886	*omp*	5.47	6.33	6.59
Phthalic acid[d]	*pmo*	0.593	0.995	5.036	*mpo*	0.447	0.491	1.566	*mpo*	0.93	1.36	4.61
Naphthol[e]	$\beta\alpha$	2.974	3.611		$\beta\alpha$	4.087	4.877		$\beta\alpha$	8.43	10.12	
Naphthylamine[e]	$\beta\alpha$	2.387	2.437		$\alpha\beta$	3.276	3.474		$\alpha\beta$	6.64	6.87	

[a] From literature [37].
[b] Mobile phases: methanol–water (40:60 (v/v)).
[c] The elution order.
[d] Mobile phases: methanol–0.02 mol l^{-1} KH_2PO_4 (50:50 (v/v), pH 3.5).
[e] Mobile phases: methanol–0.02 mol l^{-1} H_3PO_4 (70:30 (v/v)); 0.8 ml min^{-1}.

ortho-methylphenol on C6BS was first eluted, and inversely, the *ortho*-isomer was finally eluted on CR6BS. The different elution orders of naphthylamine on the two packings can be found. Apparently, when the interaction between the aromatic ring of the isomer and the moiety of the calix-crown took place, the hydrogen bond formation of the polar groups (OH, NH_2) with the ether-bridge occurred on in this case. The retention of phthalic acid on CR6BS was much stronger than those of the other isomers (Table 4 and Fig. 7). This phenomenon might be correlated to the chelation of phthalic acid with the oxygen atom of the ether-bridge [38].

3.7. The separation of N-substituted anilines

Many experimental results showed that the substituted anilines and their quaternary ammoniums were main guests of the calixarenes in solutions [39]. So, aniline, *N*-methylaniline, *N*,*N*-dimethyl-aniline and diphenylamine were used as probes further to investigate the property of new stationary phase for basic solutes.

The similar chromatograms of *N*-substituted anilines on CR6BS and C6BS can be observed (shown in Fig. 8). The elution orders of the analytes were also similar to those of ODS. The better separation and peak shapes of the anilines can, respectively, be observed on CR6BS and C6BS using the mobile phases at pH 5.5, which illustrated that the packings have weaker ion-exchange capacities and are suitable for basic solutes. The results indicate that hydrophobic interaction plays significant role in the separation of the *N*-alkyl or phenyl anilines.

According to the k and $\alpha_{1,2}$ values of anilines shown in Table 5, hydrogen bonded interaction between the anilines and CR6BS or C6BS existed besides hydrophobic interaction. For example, the $\alpha_{1,2}$ values of the analytes on ODS changed slightly from 2.357 to 2.772. In contrast to this, obvious changes of the $\alpha_{1,2}$ can be found from 1.362 to 3.711 on the two calix[6]arene-bonded columns. This is because the separation of the above anilines on ODS based on only hydrophobic interaction. However, other interactions can also contribute to the above chromatographic process on both CR6BS and C6BS. The results mainly ascribe to the hydrogen bond interaction between the anilines and the two calix[6]arene ligands. *N*,*N*′-dimethylaniline is ternary amine and does not contain N–H, lacking of hydrogen bond. Thus the k values of *N*,*N*′-dimethylaniline on the two calix[6]arene-bonded columns increase relative to *N*-methylaniline in a small degree, which led to the selectiv-

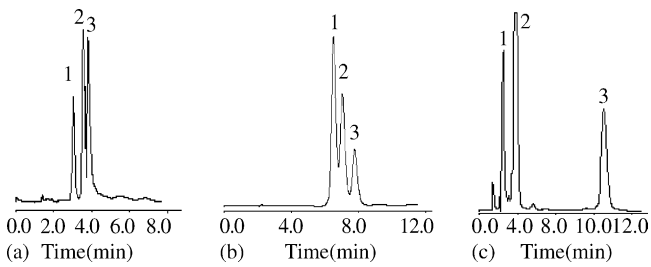

Fig. 7. The chromatograms of aromatic positional isomers on CR6BS. Mobile phases: (a) methanol-water (40:60 (v/v)); (b) methanol–water (40:60 (v/v)); (c) methanol–0.02 mol l^{-1} H_3PO_4 (70:30 (v/v)); flow rates: 0.8 ml min^{-1}. Peaks: (a) *p*-, *m*-, *o*-benzenediol; (b) *m*-, *p*-, *o*-nitroaniline; (c) *p*-, *m*-, *o*-phthalic acid.

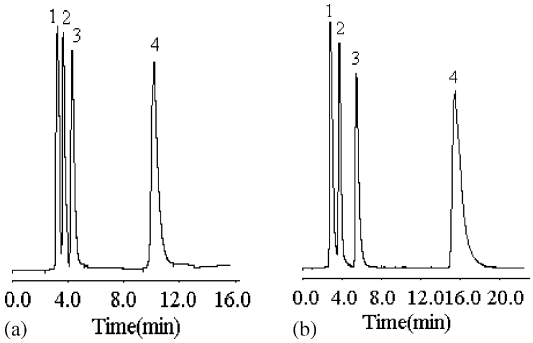

Fig. 8. The chromatograms of the *N*-substituted anilines on CR6BS (a) and C6BS (b). Mobile phases: methanol-0.02 mol l^{-1} KH_2PO_4 (55:45 (v/v), pH 5.5); flow rates: 0.8 ml min^{-1}; UV: 270 nm. Peaks: (1) aniline; (2) *N*-methylaniline; (3) *N*,*N*′-dimethylaniline; (4) diphenylamine.

Table 5
The retention factors (k) and the separation factors ($\alpha_{1,2}$) of the *N*-substituted anilines on CR6BS, C6BS and ODS

Solutes	CR6Bs[a]		C6BS[a]		ODS[b]	
	k	$\alpha_{1,2}$	k	$\alpha_{1,2}$	k	$\alpha_{1,2}$
Aniline	0.610		0.744		0.806	
		1.362		1.745		2.447
N-Methylaniline	0.831		1.298		1.972	
		1.381		1.806		2.772
N,*N*′-Dimethylaniline	1.148		2.344		5.467	
		3.652		3.711		2.357
Diphenylamine	4.196		8.698		12.883	

[a] Mobile phases: methanol–0.02 mol l^{-1} KH_2PO_4 (55:45 (v/v), pH 5.5), 0.8 ml min^{-1}, UV: 270 nm.
[b] Mobile phase: methanol–0.02 mol l^{-1} KH_2PO_4 (60:40 (v/v), pH 5.5).

ity (α) of CR6BS and C6BS for the anilines changed largely as compared with ODS.

Typical inclusion interaction and obvious difference between CR6BS and C6BS can not be discerned in the separation of the anilines. It is more likely due to the small cavities (7.6 Å) and the same as parents, which play important roles in this case, respectively.

4. Conclusion

A *p-tert*-butyl-calix[6]-1,4-benzocrown-4-bonded silica gel stationary phase (CR6BS) was first prepared and its structure was characterized. The chromatographic performance of CR6BS was evaluated by using different solutes as probes in comparison with a reported *p-tert*-butyl-calix[6]arene-bonded silica gel stationary phase (C6BS, the parent) and ODS under the same chromatographic conditions. Based on the chromatographic data, the new stationary phase has an excellent reversed-phase property, which was similar to C6BS and ODS. As a hybrid of calixarene and crown ether, the chromatographic performance of CR6BS is dependent on both the moiety of the calix[6]arene and the ether-bridge. The low flexibility conformation can facilitate to recognize PAHs from *azo*-PAHs or alkylbenzenes and is unfavorable of the separation within alkylbenzenes. The hydrophobic interaction, hydrogen bond interaction, favorable conformation and synergistic effect existed between the cavities and the ether-bridge altogether attribute to the chromatographic character of CR6BS.

Acknowledgements

The authors gratefully acknowledge National Nature Science Foundation of China (Grant: 20275029), the Excellent Young Teachers Program of MOE, P.R.C.

References

[1] C.D. Gutsche, I. Alam, Tetrahedron 44 (1988) 4689.
[2] E. Nomura, H. Taniguchi, K. Kawaguchi, Y. Otsuji, J. Org. Chem. 58 (1993) 4709.
[3] X.H. Lai, L. Li, C.Y. Wu, Chromatographia 50 (1999) 82.
[4] O.I. Kalchenko, J. Lipkowski, V.I. Kalchenko, M.A. Vysotzky, L.N. Markovsky, J. Chromatogr. Sci. 36 (1998) 269.
[5] T. Sokolieâ, U. Menyes, U. Roth, T. Jira, J. Chromatogr. A 948 (2002) 309.
[6] Y. Tanaka, Y. Kishimoto, K. Otsuka, S. Terabe, J. Chromatogr. A 817 (1998) 49.
[7] R. Mibradt, V. Böhmer, Calixarenes, Kluwer Academic Publishers, Dordrecht, NL, 2001, p. 663.
[8] T. Zhao, X. Hu, J.K. Cheng, X. Lu, Anal. Chim. Acta 358 (1998) 263.
[9] J.D. Glennon, K. O'Connor, S. Srijaranai, K. Manley, S.J. Harris, M.A. McKervey, Anal. Lett. 26 (1993) 153.
[10] J.D. Glennon, E. Horne, K. O'Connor, G.A. Kearney, S.J. Harris, M.A. McKervey, Anal. Proc. 31 (1994) 33.
[11] R. Brindle, K. Albert, S.J. Harris, C. Tröltzsch, E. Horne, J.D. Glennon, J. Chromatogr. A 731 (1996) 41.
[12] Y.K. Lee, Y.K. Ryu, J.W. Ryu, B.E. Kim, J.H. Park, Chromatographia 46 (1997) 507.
[13] S. Friebe, S. Gebauer, G.J. Krauss, J. Chromatogr. Sci. 33 (1995) 281.
[14] S. Gebauer, S. Friebe, G. Gübitz, G.J. Krauss, J. Chromatogr. Sci. 36 (1998) 383.
[15] S. Gebauer, S. Friebe, G. Scherer, G. Gübitz, G.J. Krauss, J. Chromatogr. Sci. 36 (1998) 388.
[16] U. Menyes, U. Roth, C. Tröltzsch, Eur. Patent 786661, A2, 1997.
[17] W. Xu, J.S. Li, Y.Q. Feng, S.L. Da, Y.Y. Chen, X.Z. Xiao, Chromatographia 48 (1998) 245.
[18] X.Z. Xiao, Y.Q. Feng, S.L. Da, Y. Zhang, Anal. Lett. 33 (2000) 3355.
[19] Y.X. Xiao, X.Z. Xiao, Y.Q. Feng, Z.H. Wang, S.L. Da, Talanta 56 (2002) 1141.
[20] C. Alfieri, E. Dradi, A. Pochini, R. Ungaro, G.D. Andreetti, J. Chem. Commun. (1983) 1075.
[21] A. Casnati, A. Pochini, R. Ungaro, F. Ugozzoli, F. Arnaud, S. Fanni, M.J. Schwing, R.J.M. Egberink, F. Dejong, D.N. Reinhoudt, J. Am. Chem. Soc. 117 (1995) 2767.
[22] P. Alan, Marchand, H.S. Chong, M. Takhi, T.D. Power, Tetrahedron 56 (2000) 3121.
[23] S. Memon, G. Uysal, M. Yilmaz, React. Funct. Polym. 47 (2001) 165.
[24] A. Casnati, A. Pochini, R. Ungaro, C. Bocchi, F. Ugozzoli, R.J.M. Egerink, H. Struijk, R. Lugetenberg, F. Dejong, D.N. Reinhoudt, Chem. Eur. J. 2 (1996) 436.
[25] V. Lamare, J.F. Dozol, S. Fuangswasdi, F. Arnaud-Neu, P. Thuery, M. Nierlich, Z. Asfari, J. Vicens, J. Chem. Soc. Perkin Trans. 2 (1999) 271.
[26] A. Arduini, A. Casnati, L. Dodi, A. Pochini, R. Ungaro, J. Chem. Soc. Chem. Commun. (1990) 1597.
[27] L. Lin, C.Y. Wu, Z.Q. Yan, X.Q. Yan, X.L. Su, H.M. Han, Chromatographia 47 (1998) 689.
[28] L.F. Zhang, L. Chen, X.R. Lu, C.Y. Wu, Y.Y. Chen, J. Chromatogr. A 840 (1999) 225.
[29] G. Arena, A. Casnati, A. Contino, L. Mirone, D. Sciotto, R. Ungaro, J. Chem. Soc. Chem. Commun. (1996) 2277.
[30] H.B. Li, Y.Y. Chen, React. Funct. Polym. 55 (2003) 171.
[31] T. Sokolieβ, U. Menyes, U. Roth, T. Jira, J. Chromatogr. A 898 (2000) 35.
[32] X.Z. Xiao, Y.Q. Feng, S.L. Da, Y. Zhang, Chromatographia 49 (1999) 643.

[33] K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Araki, N. Tanaka, J. Chromatogr. Sci. 27 (1989) 721.
[34] A.P. Goldberg, Anal. Chem. 54 (1982) 342.
[35] T. Sokolieβ, J. Schönherr, U. Menyes, U. Roth, T. Jira, J. Chromatogr. A 1021 (2003) 71.
[36] L.S. Li, M. Liu, S.L. Da, Y.Q. Feng, Talanta 62 (2004) 643.
[37] L.S. Li, M. Liu, S.L. Da, Q. Feng, Anal. Sci., 20(3), 2004, in press.
[38] Y.L. Hu, Y.Q. Feng, S.L. Da, J. Liq. Chrom. Rel. Technol. 24 (2001) 957.
[39] S. Shinkai, K. Araki, T. Matsuda, N. Nishiyama, H. Ikeda, L. Takasu, M. Iwamoto, J. Am. Chem. Soc. 112 (1990) 9053.