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Preparation of a new *1,3-alternate*-calix[4]arene-bonded HPLC stationary phase for the separation of phenols, aromatic amines and drugs

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ABSTRACT

We have synthesized the *1,3-alternate* 25,27-dioctyloxy-26,28-bis-[3-aminopropyloxy]-calix[4]arene and then immobilized onto γ-chloropropylsilica gel (CPS). The high-performance liquid chromatographic behavior of some aromatic hydrocarbons, phenolic compounds, aromatic amines and drug compounds was studied on this *1,3-alternate*-calix[4]arene-bonded silica gel stationary phase (CIMS). The effect of organic modifier content and pH of the mobile phase on retention and selectivity of these compounds were investigated. According to chromatographic data, it can be concluded that the selectivity of CIMS for analytes ascribes to various interactions between CIMS and the analytes, such as hydrophobic interaction, hydrogen bonding interaction, π–π interaction and inclusion interaction.

1. Introduction

The differences in many commercially available stationary phases are due to differences in their hydrophobic, ionic and polar properties [1]. Until now, there exists no universally accepted chromatographic test to choose an appropriate packing material for a particular separation problem [2]. In reversed-phase chromatography, many descriptors can give certain information to estimate the chromatographic behavior of the stationary phases; i.e. the type of the bonded ligand and its bondage to the surface, the surface coverage, the surface area and the support material are used to explain the differing properties of the chromatographic materials [3]. Nevertheless, the use of empirically based test mixtures is often inevitable because phases behave different than expected by their chemical and physical parameters. Test runs can provide information concerning the hydrophobic (hydrophobic retention capacity, hydrophobic selectivity, steric selectivity) and polar properties (silanol group activity, polar selectivity, ion exchange selectivity, complexation capacity) [4] of a column.

Calixarene skeleton represents one of the most important macrocyclic host molecule in supramolecular chemistry [5,6], together with crown ethers [7] and cyclodextrins [8]. Calixarenes are prepared by the base-catalyzed reaction of formaldehyde with phenol derivatives. They have a number of selective factors in configuration such as cavity-size, conformation and substituent. These characteristics of configuration lead to the formation of typical host–guest complexes between calixarenes and numerous compounds, and result in widely varying applications in ion-selective membranes and electrodes [9–14], electrophoresis [15–19] and chromatography [20–25].

In the field of liquid chromatography, calixarenes as stationary phases have attracted many researchers' attention during the last few years. Calixarenes are often used as mobile phase additives for HPLC and chemically bonded stationary phase for both GC and HPLC. Calixarene-bonded stationary phases are preferable to the use of calixarene additives because the UV detection of analytes is prevented by strong absorbance of calixarenes. Additionally, poor solubility of most calixarenes precludes their applications as additives in aqueous eluents. In the high-performance liquid chromatography, Calix[*n*]arene based stationary phases were used for separation of many inorganic and organic compounds, e.g. metal ions [26], aromatic positional isomers [27–30], steroids [24], amino acids [31], nucleosides [32], and water-soluble vitamins [33]. Several previous works have shown that calixarene-bonded stationary phases in a *cone* or *1,3-alternate* conformation are excellent in reversed-phase chromatography and exhibit promising application in HPLC. Barc et al. [34] prepared the *1,3-alternate* 25,27-bis-(pentafluorobenzyloxy)-26,28-bis-(3-propyloxy)-calix[4]arene-bonded silica gel as stationary phase and used for separation of acidic, basic and neutral molecules. Moreover, Magdelana et al. [35] synthesized the *1,3-alternate* 25,27-dibenzoiloxy-26,28-bis-[3-propyloxy]-calix[4]arene-bonded silica gel as a new type of HPLC stationary phase and HPLC results revealed that a new stationary phase is chemically stable and

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can be successfully used for separation of positional isomers of aromatic compounds [35]. In 2006, Dink and co-workers [36] prepared six calixarene-bonded silica gel stationary phases and their chromatographic performance was investigated by using PAHs, aromatic positional isomers and *E*- and *Z*-ethyl 3-(4-acetylphenyl) acrylate isomers as probes. 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.

In this paper, we report the synthesis of a new calix[4]arene-bonded silica gel stationary phase (CIMS) and the chromatographic separation of some aromatic hydrocarbons, phenolic compounds, aromatic amines and drug compounds on this new stationary phase. The chromatographic behaviour of some analytes on CIMS was compared with that of ODS under similar conditions in reversed-phase mode. The influence of several mobile parameters on the chromatographic behaviour of the solutes was investigated.

2. Experimental

2.1. Apparatus and materials

Elemental analyses were performed on a Leco CHNS-932 analyzer. ^{1}H NMR spectra were recorded with a Varian 400 MHz spectrometer in $CDCl_3$. FT-IR spectra was recorded with a PerkinElmer spectrum 100. High-performance liquid chromatography (HPLC) Agilent 1200 Series were carried out using a 1200 model quaternary pump, a G1315B model Diode Array and Multiple Wavelength UV–vis detector, a 1200 model Standard and preparative autosampler, a G1316A model thermostated column compartment, a 1200 model vacuum degasser, and an Agilent Chemstation B.02.01-SR2 Tatch data processor. Thermal gravimetric analysis (TGA) was carried out with Seteram thermogravimetric analyzer. Analysis was performed from room temperature to 900 °C at heating rate of 10 °C min^{-1} in argon atmosphere with a gas flow rate of 20 mL min^{-1}.

All analytes used in this study were of analytical grade and were obtained from Sigma–Aldrich (Steinheim, Germany). Silica gel (particle size 5 μm, pore size 100 Å and specific surface area 300–400 m^2 g^{-1}) were obtained from Merck (Darmstadt, Germany). ODS column (Zorbax C18, 5 μm particle size, specific surface area 180 m^2 g^{-1}, 80 Å pore size, carbon load 10%, 15 cm × 4.6 mm) was purchased from Agilent technology (Santa Clara, United States). A phosphate buffer (0.02 mol L^{-1}, pH 3.5–7.5) was prepared from KH_2PO_4 (Merck) with ultra high quality pure water, and filtered through a 0.45 mm Nylon filter before use. HPLC-grade methanol (MeOH) and acetonitrile (MeCN) were purchased from Merck (Darmstadt, Germany). HPLC water was obtained by passing boiled deionized water through a Milli-Q system.

2.2. Synthesis of 1,3-alternate 25,27-dioctyloxy-26,28-bis-[3-aminopropyloxy]-calix[4]arene (**4**)

25,26,27,28-Tetrahydroxy-calix[4]arene **1** was prepared according to the reported procedures [37]. According to Lysander's procedure [38], compound **1** was converted to 25,27-bis[(3-phthalimidopropyl)oxy]calix[4]arene **2** by adding of *N*-(3-bromopropyl) phthalimide and K_2CO_3 in dry acetonitrile. Yield 80%; m.p. 302–304 °C.

To give *1,3-alternate* dioctyloxy-bis(3-phthalimidopropoxy) calix[4]arene **3**, 25,27-bis[(3-phthalimidopropyl)oxy] calix[4]arene **2** (2.50 mmol), octyl iodide (15.0 mmol) and Cs_2CO_3 (9.80 mmol) in dry MeCN (100 mL) was refluxed for 72 h. Then the solvent was removed under reduced pressure. Subsequently, the residue was dissolved in CH_2Cl_2 (100 mL) and washed with 1N NH_4Cl (3 × 50 mL) and water (2 × 50 mL). The organic phase was separated, dried with $MgSO_4$, and evaporated to afford a solid, which was crystallized from MeOH to give **3** as a pure white powder. Yield 65%; m.p. 157–160 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66–7.81 (m, 8H, Pht-H), 6.98–7.02 (m,8H, ArH_m), 6.81 (t, J = 7.4 Hz, 2H, ArH_p), 6.73 (t, J = 7.4 Hz, 2H, ArH_p), 3.69 (8H, $ArCH_2Ar$), 3.61 (t, J = 7.2 Hz, 4H, $OCH_2(CH_2)_2N$), 3.51 (t, J = 7.4 Hz, 4H, $OCH_2CH_2CH_2N$), 3.46 (t, J = 7.2 Hz, 4H, $OCH_2C_7H_{15}$), 1.74 (p, J = 7.2 Hz, 4H, $CH_2CH_2CH_2N$), 1.19–1.41 (m, 24H, $OCH_2(CH_2)_6CH_3$), 0.92 (t, J = 7.2 Hz, 6H, CH_2CH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ 14.38, 22.96, 26.17, 29.62, 29.91, 30.02, 32.14, 35.52, 37.52, 68.89, 71.49, 122.19, 122.52, 123.33, 123.47, 129.70, 129.93, 132.41, 133.96, 133.99, 134.08, 134.15, 156.47, 156.94, 168.43. Anal. calcd for $C_{66}H_{77}O_8N_2$: C, 77.26; H, 7.51; N, 2.73. Found: C, 77.95; H, 7.29; N, 2.73.

1,3-Alternate 25,27-dioctyloxy-26,28-bis-[3-aminopropyloxy]-calix[4]arene **4** was obtained by removing phthalimido groups in product **3**. For this, a solution of compound **3** (0.23 mmol) and $NH_2NH_2{\cdot}H_2O$ (4.5 mmol) in ethanol (30 mL) was stirred at 110–120 °C for 8 h. Then the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (30 mL) and washed with a solution of NH_4OH (pH ~9) (3 × 15 mL). The organic phase was separated, dried with $MgSO_4$, and evaporated to afford **4** as a pure solid. Yield 92%; m.p. 124–127 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.04–6.99 (m, 8 H, ArH*m*), 6.78 (t, J = 7.2 Hz, 2H, ArH_p), 6.72 (t, J = 7.2 Hz, 2H, ArH_p), 3.70 (s, 8 H, $ArCH_2Ar$), 3.63, 3.48 (2× t, 8 H, J = 6.6 Hz ve J = 7.4 Hz, $OCH_2CH_2CH_2N$ and $OCH_2C_7H_{15}$), 2.67 (t, 4 H, J = 6.6 Hz, $CH_2CH_2CH_2N$), 1.7–1.65 (m, 4 H, $CH_2CH_2CH_2N$), 1.42–1.22 (m, 24 H, $OCH_2(CH_2)_6CH_3$), 0.92 (t, 6 H, J = 7.2 Hz, CH_2CH_3). ^{13}C NMR (400 MHz): δ 14.34, 14.37, 22.96, 26.15, 29.64, 29.80, 29.91, 32.14, 34.15, 37.53, 40.02, 70.35, 71.44, 122.03, 122.13, 129.80, 130.00, 133.84, 134.28, 156.87, 156.97. Anal. calcd for $C_{50}H_{70}O_4N_2$: C, 78.74; H, 9.18; N, 3.67. Found: C, 78.95; H, 9.26; N, 3.56.

2.3. Preparation of stationary phase (CIMS)

Scheme 2 shows the synthesis of a new calix[4]arene-bonded silica gel stationary phase. Details of the bonding procedure were as follows. The silica gel was immersed in 50 mL hydrochloric acid of 6 M. This mixture was allowed to reflux with stirring for 8 h to remove contaminants, followed by washing with distilled water until acid free and immersing in distilled water for approximately 1 h to clean and hydrolyze the surface [39,40]. Then it was filtered and dried under vacuum at 70 °C for 8 h.

To prepare the chloro-functionalized silica gel (CPS), 5 g of activated silica gel was dispersed into toluene (50 mL) in a flask of total volume 100 mL, and then 3-chloropropyltrimethoxysilane (5 mL) was gradually added into the solution with continuous stirring. The mixture was refluxed for 12 h. The final product was filtered off, washed with toluene, methanol and dried under vacuum at 70 °C for 8 h. Finally, γ-chloropropylsilica gel (CPS) was obtained and used as a precursor in the following reaction.

Compound **4** (0.8 g) was dissolved in dry acetonitrile (50 mL) and γ-chloropropylsilica gel (CPS) was added. The mixture was refluxed under nitrogen atmosphere for 72 h. Progress of the reaction was controlled by TGA analysis. After the reaction was completed, the calix[4]arene-bonded silica gel (CIMS) was filtered, washed with toluene, acetonitrile, acetone in turn, and then dried at 100 °C under vacuum for 8 h.

2.4. Chromatographic procedure

Stainless steel columns (150 mm × 4.6 mm I.D.) were packed with modified calix[4]arene-silica gels according to a slurry packing procedure by using methanol as the displacing agent for 5 h. Ana-

Table 1
Elemental analysis of the bonded phases.

Bonded phase	C %	H %	N %	Bonded amount/mmol g^{-1}
CPS	6.60	1.47	–	1.100
CIMS	16.92	2.23	0.64	0.235

lytes were dissolved in methanol at the concentration in range of 0.25–0.5 mg mL^{-1} and 20 μL of the solution were injected onto the chromatographic column. The mobile phases were metanol-water and acetonitrile phosphate buffers (0.02 mol L^{-1}, pH 3.5–7.5). The pH value of this buffer was adjusted with H_3PO_4 to 3.5 or with NaOH to 7.5. The retention time of the aqueous solution of sodium nitrate was used as the void time marker for the calculation of the capacity factor. All measurements were carried out at ambient temperature (25 ± 2 °C) and repeated at least twice.

3. Results and discussion

3.1. Preparation and characterization of the calix[4]arene-bonded stationary phase (CIMS)

The new calix[4]arene-bonded stationary phase (CIMS) was prepared using *1,3-alternate* 25,27-dioctyloxy-26,28-bis-[3-aminopropyloxy]-calix[4]arene as organic ligand and γ-chloropropylsilica gel (CPS) as shown in Schemes 1 and 2.

The prepared stationary phase (CIMS) was characterized by elemental analysis, FT-IR, thermal gravimetric analysis and chromatographic performance. The results of elemental analysis showed that the carbon contents of CPS and CIMS were 6.60 and 16.92%, respectively (Table 1). Depending on the contents of carbon, the bonded calixarene amount onto CIMS was found to be approximately 0.235 mmol g^{-1}. Also, from the FT-IR results, it was observed that compound **4** immobilized onto CPS due to 3440 cm^{-1}, 1480–1650 cm^{-1}, and 1100 cm^{-1} bands corresponded to Si–OH, benzene rings (C–H) and Si–O–Si, respectively.

The thermal analysis of CIMS has been investigated by TG analysis. Fig. 1 shows TG curves of CIMS and CPS materials. The total losses at temperature range of 25–900 °C are 9.1 and 21.9% for CPS and CIMS, respectively, due to the breakage of the calixarene units anchored on the silica-gel surface together with the condensation of remaining silanol groups to produce siloxane. Thermogravimetric results showed a direct relationship of the loss of mass to the amount of the calixarene molecules anchored on the silica-gel surfaces.

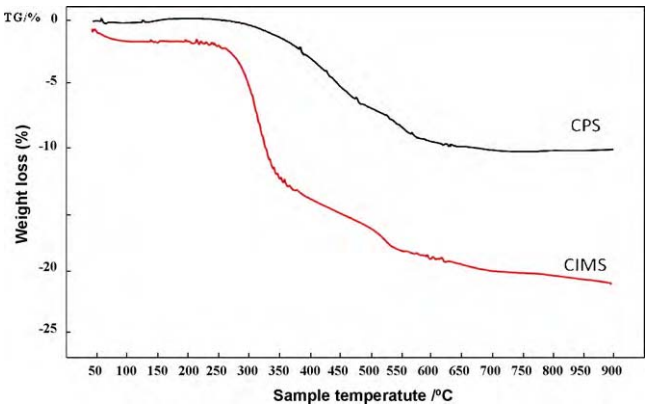


Fig. 1. Thermogravimetric curves of CPS and CIMS stationary phase.

Table 2
The retention factors (*k*) of some aromatic hydrocarbons on CPS, CIMS and ODS (methanol–H_2O, 75:25, v/v).

Solutes	CPS	CIMS	ODS
Quinoline	2.40	2.80	2.54
Toluene	2.85	3.63	5.18
Benzophenone	3.20	5.62	2.97
9,10-Phenanthroquinone	3.82	7.79	4.19
Dibenzyl	4.15	11.28	14.70
Anthracene	4.40	16.14	14.74

3.2. The chromatographic performance of CIMS stationary phase

The chromatographic performance of CIMS stationary phase was evaluated by using some aromatic hydrocarbons, phenolic compounds, aromatic amines and drug compounds. The effect of organic modifier content and pH of the mobile phase on retention and selectivity of some analytes was investigated and their chromatographic behaviors on CIMS stationary phase was compared with that on ODS.

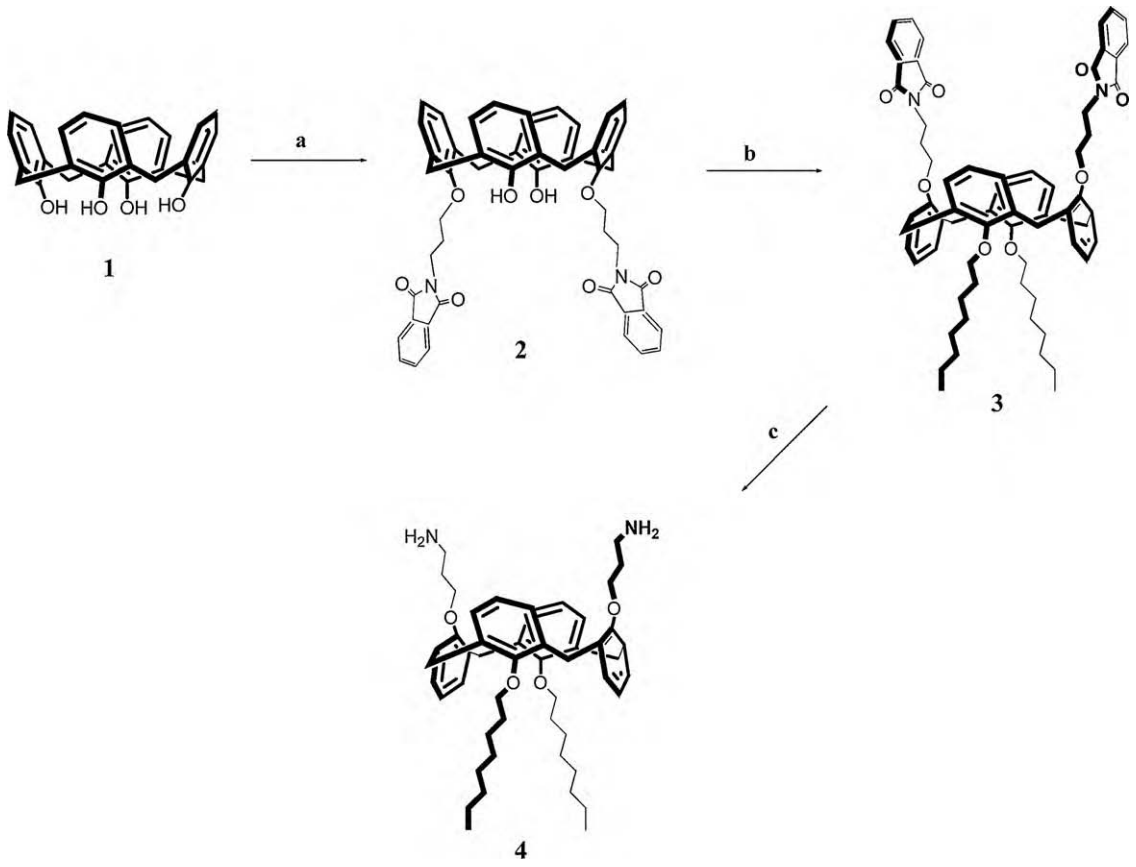
3.2.1. Separation of some aromatic hydrocarbons

The retention factors (*k*) of some aromatic hydrocarbons on CPS, CIMS and ODS were shown in Table 2. The retention values of aromatic hydrocarbons on CIMS are much higher than that on CPS and ODS. The results indicate that the *1,3-alternate*-calix[4]arene derivative was successfully immobilized to silica gel using a spacer. The chromatograms of aromatic hydrocarbons on CIMS and ODS were shown in Fig. 2. It can be seen from Fig. 2 that the elution order on CIMS was different from that on ODS. This implies that hydrophobic interaction is not the only factor in the separations. For example, the retention of toluen or dibenzyl as analyte on CIMS were weaker than those on ODS. However, the retention values of quinoline, benzophenone and 9,10-phenanthroquinone on CIMS exceeded those on ODS. This case cannot be explained only with the hydrophobic interaction. It ascribes partially to π–π and hydrogen bonding interactions. Moreover, the obvious difference in the selectivity for the analytes between CIMS and ODS can also be observed. For example, while dibenzyl and anthracene were eluted together on ODS column, these analytes were eluted separately on CIMS column.

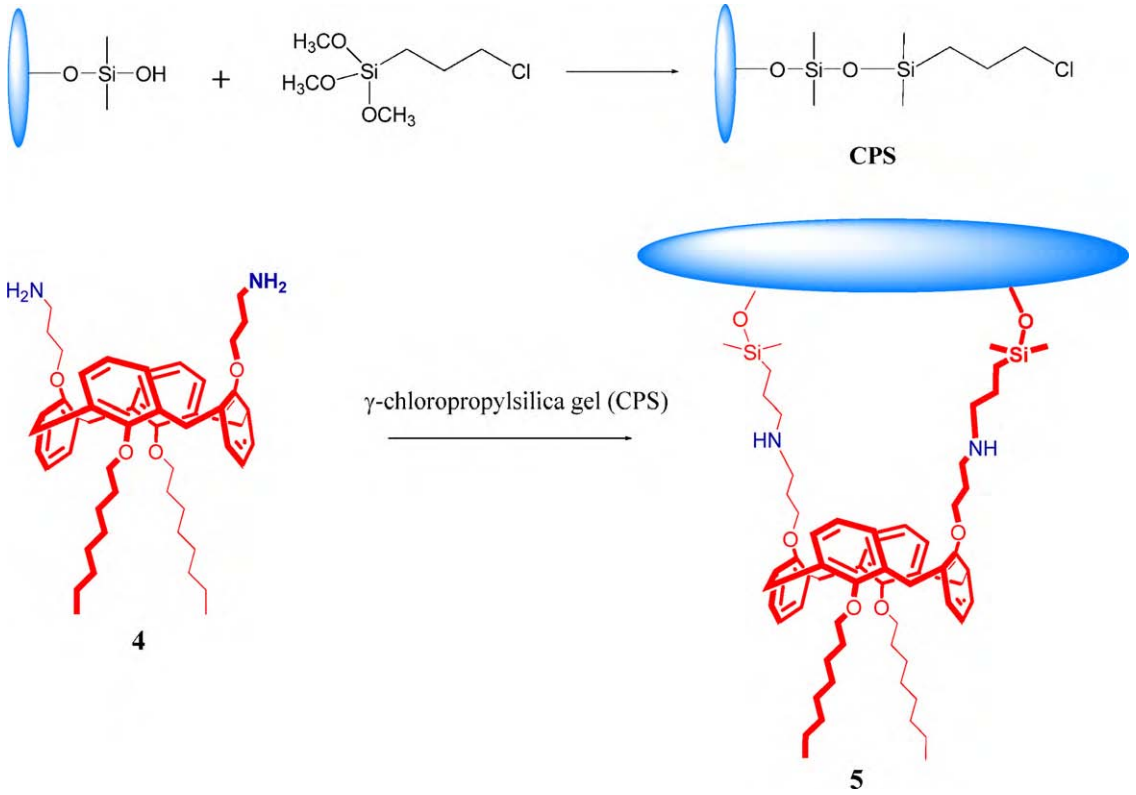
3.2.2. Separation of the aromatic amines

Reversed-phase liquid chromatography is widely used for separation and quantitative analysis of aromatic amines. In order to further study the chromatographic property of CIMS for basic solutes, aniline (pK_a = 4.63), 4,4-diaminodiphenylether (pK_a = 5.20), 4,4-diaminodiphenylmethane (pK_a = 5.08), 4-aminobiphenyl (pK_a = 4.27), 1-napthylamine (pK_a = 3.92), diphenylamine (pK_a = 0.9), 4-triphenylmethyl aniline (pK_a = 4.12) were used as probes. As shown in Fig. 3, the better separation of aromatic amines can be achieved on both CIMS and ODS. Nevertheless, the different *k* and $\alpha_{1,2}$ values of the analytes on the two columns can respectively be observed. As can be seen in Table 3, the $\alpha_{1,2}$ values (1.260–1.358) of solutes on ODS are different from the $\alpha_{1,2}$ values (1.124–1.459) on CIMS. The analytes were also eluted in the same order on the two columns. Aromatic amines gave comparatively stronger retention on CIMS columns than that on ODS, which means that there were the additional interactions with exception of the hydrophobic interaction between aromatic amines and CIMS. These additional interactions possibly included π–π interaction and hydrogen bonding interactions between the analytes and CIMS stationary phase.

In general, charged species are rapidly eluted, whereas the retention of neutral molecules increases due to hydrophobic interaction in reversed-phase chromatography. As can be seen in



Scheme 1. Synthesis of *1,3-alternate* 25,27-dioctyloxy-26,28-bis-[3-aminopropyloxy]-calix[4]arene [4]: (a) *N*-(3-bromopropyl) phthalimide, MeCN, K_2CO_3, reflux for 24 h; (b) octyl iodide, Cs_2CO_3, MeCN, reflux for 72 h; (c) $NH_2NH_2{\cdot}H_2O$, ethanol, reflux for 8 h.



Scheme 2. Immobilization of compound **4** onto CPS.

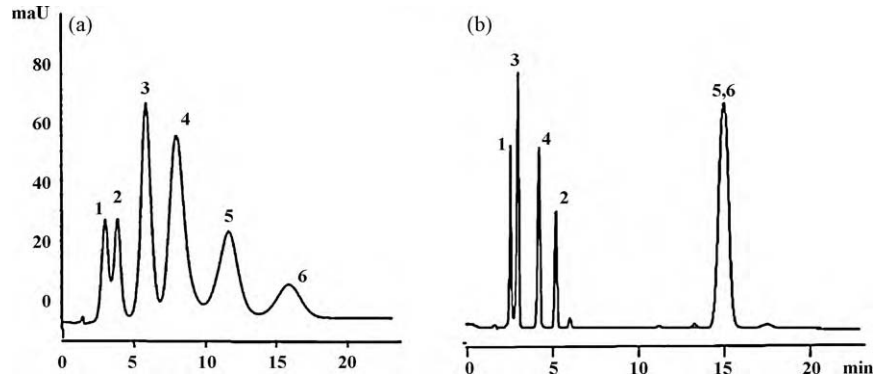


Fig. 2. The chromatograms of some aromatic hydrocarbons on CIMS (a) and ODS (b). *Chromatographic conditions*: mobile phase: methanol–H_2O (75:25, v/v); flow rate 0.8 mL min^{-1}, detection UV at 254 nm, temperature 25 °C. Analytes: (1) quinoline, (2) toluene, (3) benzophenone, (4) 9,10-phenanthroquinone, (5) dibenzyl, (6) anthracene.

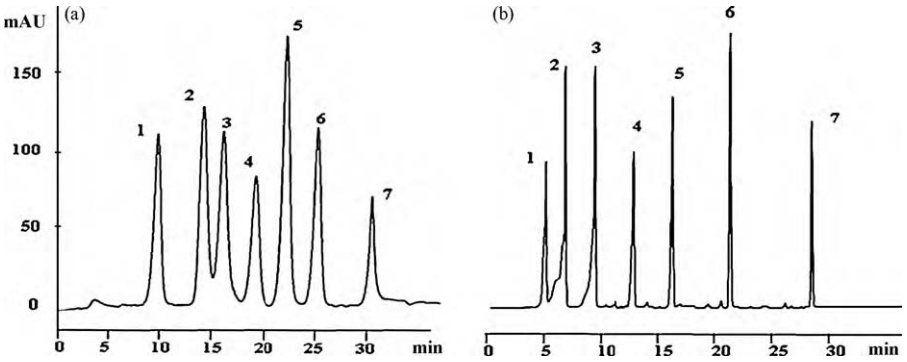


Fig. 3. The chromatograms of the aromatic amines on CIMS (a) and on ODS (b). Mobile phases: acetonitrile (A)–0.02 M KH_2PO_4, pH 6.5 (B) (gradient elution: 0 min 20% A and 80% B; 35 min 80% A and 20% B), flow rates: 1 mL min^{-1}, UV: 254 nm. Analytes: (1) aniline, (2) 4,4-diaminodiphenylether, (3) 4,4-diaminodiphenylmethane, (4) 4-aminobiphenyl, (5) 1-napthylamine, (6) diphenylamine, (7), 4-triphenylmethyl aniline.

Fig. 4, the retention of 4,4-diaminodiphenylether (pK_a = 5.20) and 4,4-diaminodiphenylmethane (pK_a = 5.08) with the strong basic character as well as other aromatic amines on CIMS were dependent on the pH of mobile phases. The phenomenon ascribed to the protonation and deprotonation of the amino groups with changing the pH in mobile phases. This can be attributed to the fact that the protonated solutes at lower pH deprotonate and change to the neutral form gradually with increasing pH, leading to the stronger hydrophobic interaction between the solutes and the new stationary phase. It was further evidence of reversed-phase chromatography in the separation of aromatic amines using CIMS as a stationary phase.

Table 3
The retention factors (k) and the separation factors ($\alpha_{1,2}$) of some aromatic amines on CIMS and ODS.

Solutes	CIMS		ODS	
	k	$\alpha_{1,2}$	k	$\alpha_{1,2}$
Aniline	9.657		5.315	
		1.459		1.325
4,4-Diaminodiphenylether	14.090		7.044	
		1.150		1.354
4,4-Diaminodiphenylmethane	16.210		9.651	
		1.184		1.358
4-Aminobiphenyl	19.198		12.958	
		1.150		1.260
1-Napthylamine	22.082		16.331	
		1.124		1.313
Diphenylamine	24.829		21.446	
		1.208		1.326
4-Triphenylmethyl aniline	30.003		28.444	

Mobile phases: acetonitrile (A)–0.02 M KH_2PO_4, pH 6.5 (B) (gradient elution: 0 min 20% A and 80% B; 35 min 80% A and 20% B), 1 mL min^{-1}, 254 nm.

3.2.3. Separation of phenolic compounds

Three phenolic compounds (phenol, 1,4-dihydroxybenzol and 1,2,3-trihydroxybenzol) were used as probes for further investigation of the chromatographic characteristic of the new CIMS stationary phase. These phenolic compounds contain the number of hydroxyl groups from 1 to 3. As can be seen in Fig. 5a, when more hydroxyl groups were attached to benzene ring, the molecule became a stronger polarity, weaker hydrophobicity, and

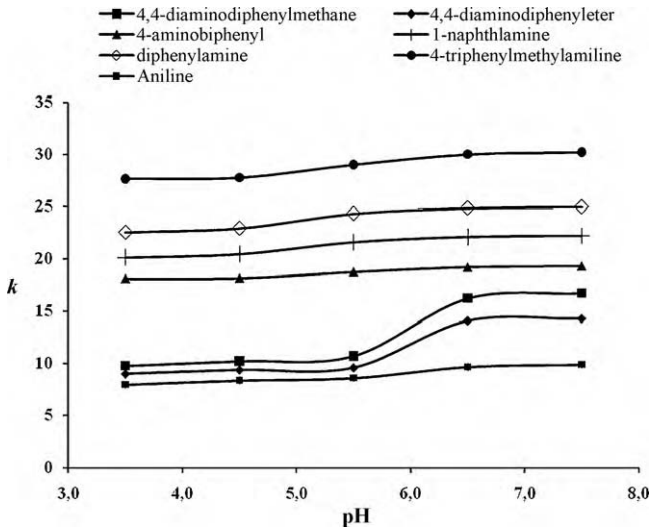


Fig. 4. Influence of the pH of mobile phases on the k values of the aromatic amines on CIMS. Mobile phases: acetonitrile (A)–0.02 M KH_2PO_4 (B) (gradient elution: 0 min 20% A and 80% B; 35 min 80% A and 20% B), 1 mL min^{-1}.

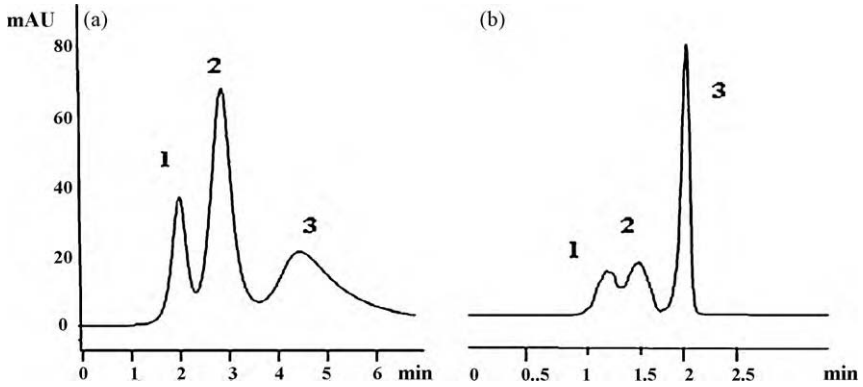


Fig. 5. The chromatogram of phenolic compounds on CIMS (a) and on ODS (b). Mobile phases: acetonitrile–0.02 M KH_2PO_4 (55:45, v/v, pH 6.5); flow rates: 1 mL min^{-1}, UV: 254 nm. Analytes: (1) phenol, (2) 1,4-dihydroxybenzol, (3) 1,2,3-trihydroxybenzol.

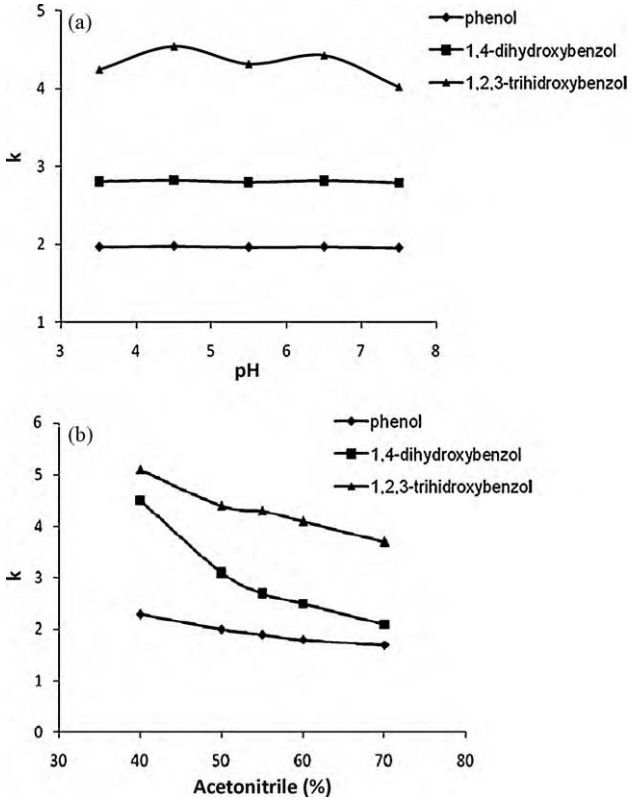


Fig. 6. Effect of the pH (a) and the acetonitrile content (b) of mobile phases on the *k* values of the phenols on CIMS. Mobile phase: (a) acetonitrile–0.02 M KH_2PO_4 (55:45, v/v); (b) acetonitrile–0.02 M KH_2PO_4 (pH 6.5).

longer retention time. The better separation of these solutes can be achieved on the novel CIMS column than on the ODS one (Fig. 5b). Three phenolic compounds were successfully resolved on new CIMS stationary phase. Moreover, the retention values of the analytes were greater than on ODS. Obviously, the strong retention mainly results from hydrogen bond and π–π interaction between the moiety of calix[4]arene and the phenolic compounds besides hydrophobic interaction.

The influence of variation in pH of the mobile phase on retention and peak symmetry was investigated in the range of pH 3.5–7.5 for phenolic compounds (Fig. 6a). As a rule, when the pH of mobile phases varied, the retention of the acidic or basic compounds should undergo considerable changes. In our study, the results showed that the retention of the phenolic compounds on CIMS was slightly dependent on the pH of the mobile phases. As seen in Fig. 6a, whereas the plots of phenol and 1,4-dihydroxybenzol indicated a flat curve in the pH range from 3.5 to 7.5. The plot of 1,2,3-trihydroxybenzol showed little changes with changes in pH of the mobile phase.

Fig. 6b illustrates that the retention of the phenolic compounds decrease with the increasing methanol content as organic modifier in mobile phases. In reversed-phase chromatography, increase of organic modifier content in the mobile phase leads to decrease in the retention time of analytes. These results suggest that CIMS phase behaves as a reversed-phase material, and hydrophobic interaction is one of the important factors playing role in the retention of the analytes.

3.2.4. Separation of some drug compounds

Non-steroidal anti-inflammatory drugs (NSAIDs) are acidic molecules possessing diverse structural features and thus their separations typically cause problems in RP-HPLC analysis [41,42]. We used ester derivatives of selected four drug compounds in this study (Fig. 7). CIMS stationary phase was used for separation of them

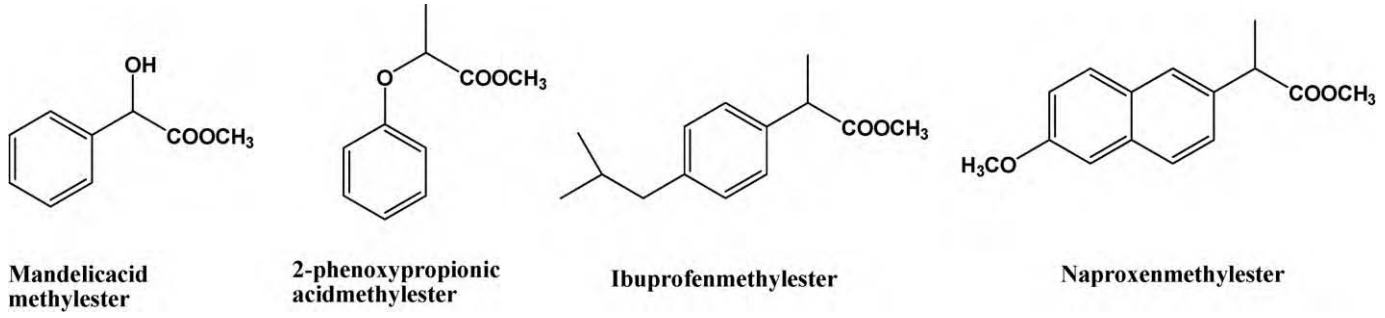


Fig. 7. Structures of drug compounds used in this study.

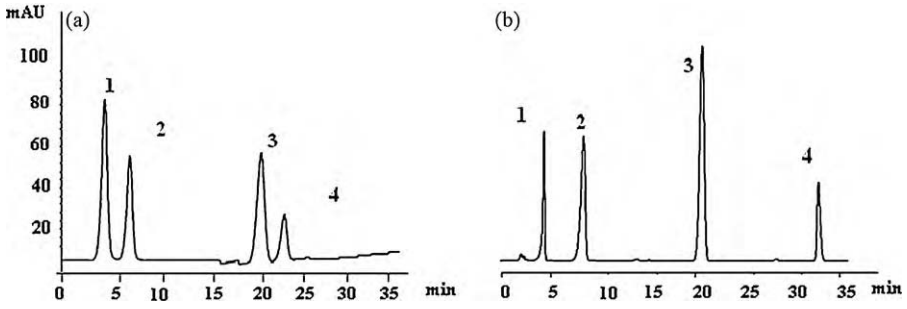


Fig. 8. The chromatograms of some drug compounds on CIMS (a) and on ODS (b). Mobile phases: acetonitrile (A)–0.02 M KH_2PO_4, pH 6.5 (B) (gradient elution: 0–10 min 40% A and 60% B; from 40–60% A within 10–35 min), flow rates: 1 mL min^{-1}, UV: 254 nm. Analytes: (1) mandelic acid methyl ester, (2) 2-phenoxypropionic acid methyl ester, (3) naproxen methyl ester, (4) ibuprofen methyl ester.

and compared with ODS column. As seen in Fig. 8, it can also be observed in the chromatograms that both CIMS and ODS columns exhibited higher selectivity for drug compounds. The elution order of drug compounds was same on CIMS and ODS column. The retention values of drug compounds on CIMS were smaller than those on ODS under the same chromatographic conditions. It is obvious that hydrophobic interaction was the predominant factor for the retention behavior of drug compounds on the two packings.

4. Conclusion

As a new stationary phase, the *1,3-alternate*-calix[4]arene derivative was synthesized and immobilized onto silica gel using a spacer. The chromatographic performance of this new column material was investigated by using some aromatic hydrocarbons, aromatic amines, some phenolic compounds and drug compounds. Their retention behaviors were compared with those on ODS. These results showed that CIMS can behave as a reversed-phase material with weaker hydrophobicity as compared with ODS. Some aromatic hydrocarbons and phenolic compounds on CIMS were successfully separated. The new stationary phase can provide various sites for the analytes, such as hydrophobic interaction, hydrogen bonding interaction, dipole–dipole interaction. Further studies on the retention mechanism and application of the new stationary phase will be carried out soon.

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