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New oxo-bridged calix[2]arene[2]triazine stationary phase for high performance liquid chromatography

Wenjie Zhao[a,b], Kai Hu[a], Caijuan Wang[a], Song Liang[a], Bailin Niu[c,*], Lijun He[b], Kui Lu[b], Baoxian Ye[a], Shusheng Zhang[a,*]

[a] Chemistry Department, Key Laboratory of Chemical Biology and Organic Chemistry of Henan, Zhengzhou University, Zhengzhou 450052, China
[b] School of Chemistry and Chemical Engineering, Henan University of Technology, Zhengzhou 450001, China
[c] School of Chemical and Energy Engineering, Zhengzhou University, Zhengzhou 450001, China

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ABSTRACT

A new oxo-bridged calix[2]arene[2]triazine bonded stationary phase (OCATS) for high performance liquid chromatography (HPLC) was prepared using 3-aminopropyltriethoxysilane as coupling reagent. The structure of new material was characterized by infrared spectroscopy, elemental analysis and thermogravimetric analysis. The chromatographic performance and retention mechanism of the new stationary phase were evaluated in reversed-phase mode compared with ODS using different solute probes including polycyclic aromatic hydrocarbons (PAHs), mono-substituted benzenes, disubstituted benzene isomers. The new OCATS stationary phase could provide various interactions for different solutes, such as hydrophobic, hydrogen bonding, π—π and inclusion interactions. The synergistic effects resulting from aromatic rings, bridging oxygen atoms and triazine nitrogen atoms and alkyl linkers in the new material improved the separation selectivity by multiple retention mechanisms. The retention behaviors of the analytes on OCATS column were explained with the assistance of quantum chemistry calculation results using DFT-B3LYP/STO-3G* base group. The OCATS column was successfully employed for the analysis of melamine in infant formula.

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1. Introduction

Evolution of molecular recognition promotes advances in other scientific fields, such as chemical sensor, mimic enzyme catalysis and chromatography. In fact, the selective recognition of host to guest molecules is very closely related to the selective separation in chromatography. According to the macrocyclic molecular recognition rules, the research on high selective host–guest interaction stationary phase has become an active branch in chromatography. As representatives of macrocyclic host molecules, crown ether, cyclodextrin and calixarene have been widely used to synthesize chromatographic stationary phases with special properties. Crown ether modified stationary phases have been successfully used to separate phenols and monosaccharides [1], isomers of cresol and xylenol [2], chiral amino acids and amino alcohols [3]. Stationary phases linked with cyclodextrin derivatives provided good resolution for a variety of enantiomers and positional isomers, such as *o*-, *m*-, *p*-nitroaniline[4], phenylpropionic acid [5], optical isomers of flavanones and flavanone glycosides [6]. Calixarene-bonded silica gels have been used to separate polycyclic aromatic hydrocarbons (PAHs) [7–11], aromatic positional isomers [7,11–15], water-soluble vitamins [16], sulphonamides [11,17,18], nucleosides [19] and so on. Based on the obtained chromatographic data, it was often concluded that the retention of solutes on the macrocycle stationary phase may involve a variety of interaction mechanisms including hydrophobic, π—π, hydrogen bonding, π-electron transfer and inclusion interactions in high performance chromatography (HPLC) [7–19]. As a result, the stationary phases utilizing a multiple retention mechanism have been proposed to achieve the desired improvement in the separation selectivity for specific solutes and offer more potential than classical reversed-phase chromatography. Therefore, design and synthesis of new functional macrocyclic host molecules and utilize them as selectors in chromatography separation have always been one of the driving forces promoting the major advances in supramolecular chemistry and chromatographic science.

* Corresponding authors at: Chemistry Department, Daxue Road 75, Zhengzhou 450052, China. Tel.: +86 371 67763224.
E-mail addresses: nbl@zzu.edu.cn (B. Niu), zsszz@126.com, zhangshusheng@371.net (S. Zhang).

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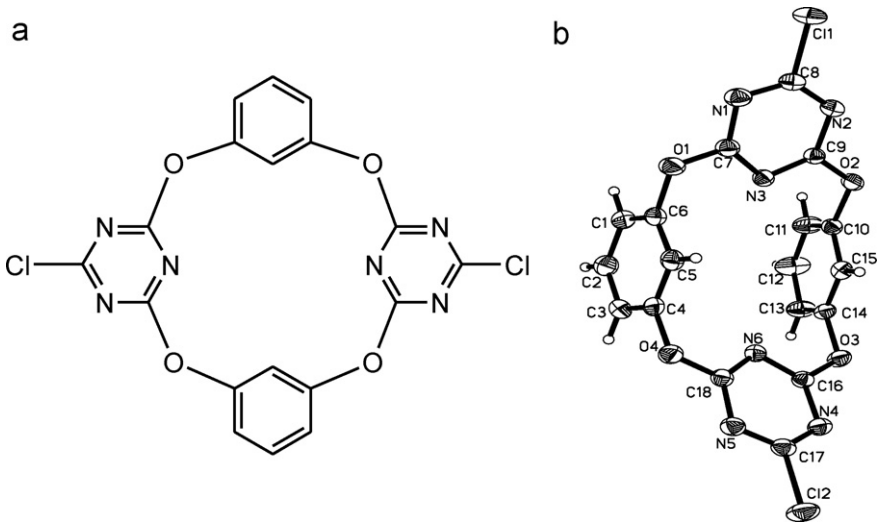

Fig. 1. Chemical and single-crystal structure of oxo-bridged calix[2]arene[2]triazine.

Along with the advances in the field of calixarenes, oxo-bridged calix[2]arene[2]triazine has emerged as a novel type of macrocyclic host molecule in supramolecular chemistry [20]. Being different from the conventional calixarenes in which the aromatic rings are linked by methylene units, oxo-bridged calix[2]arene[2]triazine assembles aromatic rings by oxygen atoms. The tetraoxacalix[2]arene[2]triazine adopts a 1,3-alternate conformation with two benzene rings nearly face-to-face parallel and two triazine rings tending to an edge-to-edge orientation in solid phase (Fig. 1). Viewed from molecular structure, the tetraoxacalix[2]arene[2]triazine is more like the combination of calixarene and crown ether. Since the oxygen atom featuring different electronic and steric properties from carbon can adopt covalent conjugation with their neighbouring aromatic rings, therefore tetraoxacalix[2]arene[2]triazine could exhibit unique structural features and versatile recognition properties in comparison to conventional calixarenes. For example, tetraoxacalix[2]arene[2]triazine has been reported to recognize halides through the formation of anion–π interaction [21]. The dihydroxylated tetraoxacalix[2]arene[2]triazine host molecule also could act as a hydrogen-bond donor to interact with 2,2′-bipyridine, 4,4′-bipyridine and 1,10-phenanthroline guests[22]. As a chromatographic ligand, oxo-bridged calix[2]arene[2]triazine has distinct interaction sites, such as aromatic rings (hydrophobic interaction and π—π interaction), bridging oxygen atoms and nitrogen atoms located on triazine rings (hydrogen bonding interaction) and cavity of the macrocycle (inclusion interaction). It was anticipated that the benzene rings and triazine rings, bridging oxygen atoms, the cavity, and the conjugated system composed of aromatic rings with oxygen atoms in this macrocyclic compound might serve to improve the selectivity of chromatographic separation. The present paper reported for the first time the preparation and application of silica bonded oxo-bridged calix[2]arene[2]triazine stationary phase for separation of several types of aromatic compounds including PAHs, mono-substituted benzenes, isomers of disubstituted benzene and melamine in HPLC. The influence of methanol concentrations on the chromatographic behavior of the solutes was also investigated. Meanwhile the quantum chemistry calculation method was introduced to provide an assistant support for the separation mechanism. As a consequence of its multiple active sites, the bonded material was a novel HPLC stationary phase with multiple retention mechanisms, while it was operated in a reverse phase elution mode.

2. Experimental

2.1. Apparatus and materials

Silica gel (with particle size of 5 μm, pore size of 100 Å and specific surface area of 300 m^2/g) was provided by Lanzhou Institute of Chemical Physics, Chinese Academy of Science (Lanzhou, China). 3-Aminopropyltriethoxysilane (KH-550) was purchased from Jingchun Chemical Reagent Co. Ltd. (Shanghai, China). Oxo-bridged calix[2]arene[2]triazine was synthesized according to published procedures [20]. Melamine (standard) was purchased from TCI Chemical Reagent Co. Ltd. (Japan). Unless specified otherwise, all chemicals and solvents were of analytical reagent grade and purchased from the Beijing Chemical Plant (Beijing, China). Methanol (MeOH) and acetonitrile (MeCN) were of HPLC grade and purchased from the Luzhong Reagent Plant of Shanghai (Shanghai, China). Water was purified using Milli-Q purification equipment.

Each group of the analytes used as probes was dissolved in acetonitrile to yield a stock solution of 500 μg/mL. The working solutions were prepared by diluting each stock solution in the mobile phase to form the desired concentrations of 10, 50 and 100 μg/mL, respectively.

Elemental analysis was performed with a Flash EA 1112 elemental analyzer. IR spectra were recorded with a Bruker Vector 22 instrument. Thermal gravimetric analysis (TGA) was carried out on a Shimadzu DT-40 thermal analyzer, the analysis was performed from 40 °C to 650 °C at heating rate of 10 °C/min in argon atmosphere with a gas flow rate of 20 mL min^{-1}. Single crystal X-ray diffraction data were collected on a Bruker SMART APEX2 X-ray diffractometer equipped with a normal focus Mo-target X-ray tube.

2.2. Preparation of oxo-bridged calix[2]arene[2]triazine-bonded stationary phases(OCATS)

2.2.1. Sililation of silica gel

Silica was immersed in hydrochloric acid/water solution (1:1, v/v) for 24 h and then washed with water and dried under vacuum at 120 °C for 8 h. In a round-bottomed flask equipped with a reflux condenser and a gas inlet valve, 6.0 g activated silica was dispersed in toluene. After the addition of 10 mL 3-aminopropyltriethoxysilane, the mixture was stirred and refluxed for 24 h under a nitrogen atmosphere before being cooled to room temperature. The suspension was filtered, washed successively with toluene, acetone, methanol and acetone/water (v/v, 1/1), and then dried at 100 °C under vacuum for 12 h. Finally,

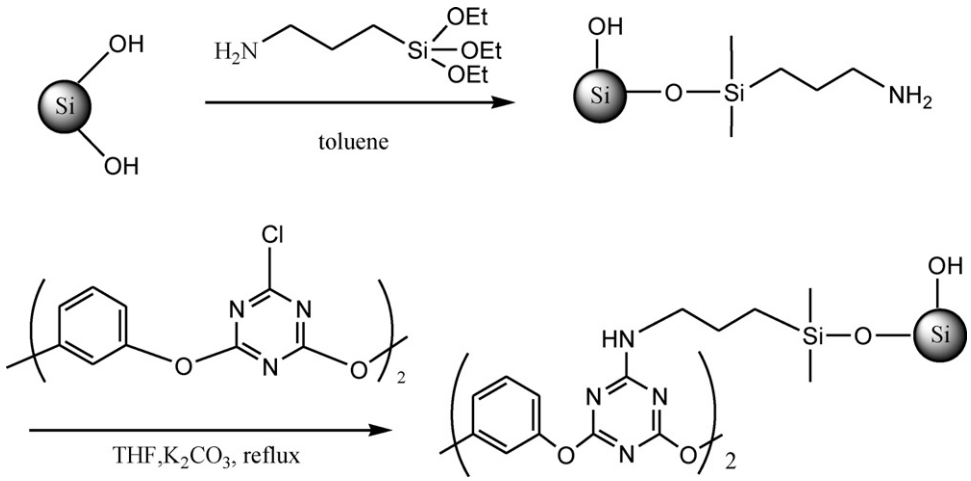

Fig. 2. Preparation scheme of oxo-bridged calix[2]arene[2]triazine-bonded silica gel stationary phase (OCATS).

3-aminopropyltriethoxyl-bonded silica gel (ABS) was obtained and used as a precursor in the following reaction.

2.2.2. Preparation of oxo-bridged calix[2]arene[2]-triazine-bonded silica stationary phase (OCATS)

Oxo-bridged calix[2]arene[2]triazine was synthesized according to the reported procedure [20]. The schematic diagram of the synthetic procedure for calix[2]arene[2]triazine-bonded silica gel stationary phases is shown in Fig. 2. Details of the bonding procedure are as follows. In a round-bottomed flask equipped with a magnetic stirrer and a gas inlet valve, a mixture of 3-aminopropyltriethoxyl-bonded silica gel (ABS) (3 g) and K_2CO_3(0.62 g) in anhydrous tetrahydrofuran (80 mL) was stirred for 0.5 h. Then tetraoxocalix[2]arene[2]triazine (1.2 g) was added. The mixture was then refluxed under nitrogen atmosphere for 24 h before being cooled to room temperature. The suspension was filtered, and washed successively with water, tetrahydrofuran and methanol. The oxo-bridged calix[2]arene[2]-triazines-bonded silica stationary phase (OCATS) was dried under vacuum at 90 °C for 12 h prior to packing or characterization by FTIR, EA and TGA.

2.3. Chromatographic evaluation

OCATS was slurry packed into a bare stainless steel tube column (150 mm × 4.6 mm i.d. Innosep scientific Co. Ltd., Zhengzhou, China) using tetrachloromethane as slurry solvent and methanol as propulsive solvent. A CGY-100B pneumatic pump (Bejing Fusiyuan Mechanical Processing Factory, Beijing, China) was used with packing pressure of 50 MPa.

Chromatographic tests were carried out using a Agilent 1200 series system equipped with a 1200 model quaternary pump, a G1314A model Multiple Wavelength UV-vis detector, a G1316A model thermostated column compartment, a 1322A model vacuum degasser and an Agilent Chemstation B.03.02 Patch data processor. An Eclipse XDB-C18 column (Agilent, 150 mm × 4.6 mm i.d., 5 μm) was used as a comparison with the home-made OCATS column. Mobile phase was filtered through a 0.45-μm nylon membrane filter and was degassed ultrasonically prior to use. The signal of the acetonitrile (or methanol) solvent in the UV detection was used as the marker of void time for the calculation of capacity factor.

3. Results and discussion

3.1. Preparation and characterizations

The dichloro-substituted oxo-bridged calix[2]arene[2]triazine was synthesized through an efficient fragment coupling approach starting from cyanuric chloride and resorcinol according to the reference[20]. The single-crystal (Fig. 1) obtained was in accord with reference [20], which could identify the structures of the macrocycle and provide accurate molecule model for quantum chemistry calculation. Since the novel calixaromatic comprise a potential reaction site on the larger rim of triazine ring, the efficient functionalizations of dichloro-substituted oxygen-bridged calix[2]arene[2]triazines on the larger rims through very convenient and straightforward nucleophilic substitution reactions of the chlorines by amines containing chelating groups, such as 8-hydroxyquinoline, 2,2′-bipyridylamine, naphthylamine has been reported [23]. Inspired by these results, we prepared the stationary phase of dichloro-substituted oxygen-bridged calix[2]arene[2]triazine on the larger rim through nucleophilic substitution reaction of the chlorines by amine of 3-aminopropyltriethoxyl-bonded silica gel (ABS) under the same reaction condition.

Examination of the elemental analysis of ABS and OCATS showed the successful immobilization of oxo-bridged calix[2]arene[2]triazine on silica surface. In ABS, the content of C, H and N was 5.91%, 1.33% and 1.88%, respectively. In OCATS, the content of C, H and N was 13.85%, 1.55% and 4.86%, respectively. The bonding amount of OCATS stationary phase was about 360 μmol g^{-1}, which was calculated from the carbon content.

The materials were also analyzed by FTIR (Fig. S1). Compared with ABS, the characteristic absorption bands of the benzene rings appeared at1596, 1545 and 1483 cm^{-1}, respectively. The C—O stretching frequency of the ether-bridge almost overlaps with the Si—O appearing for a broad bond at 1108 cm^{-1}. The IR spectra indicated that the organic ligands are bonded onto silica gel.

The thermo gravimetric curve can give information on the material thermal stability and also confirm the amount of the immobilized compound. The mass loss of OCATS phase occurred in the range from 250 to 500 °C and the decomposition temperature of phase is 421.5 °C, indicating the stationary phase has good thermal stability below 250 °C.

3.2. Chromatographic separations

3.2.1. Separation of PAHs

In this section, the chromatographic retention behaviors of six PAHs (benzene, biphenyl, fluorene, anthracene, pyrene and chrysene) were investigated on the OCATS stationary phase. Fig. 3 shows the typical chromatograms on both OCATS and ODS, and Table 1 listed their capacity factor (k) and log $K_{o/w}$ values (the log octanol–water partition coefficient). It can be seen from Fig. 3 and

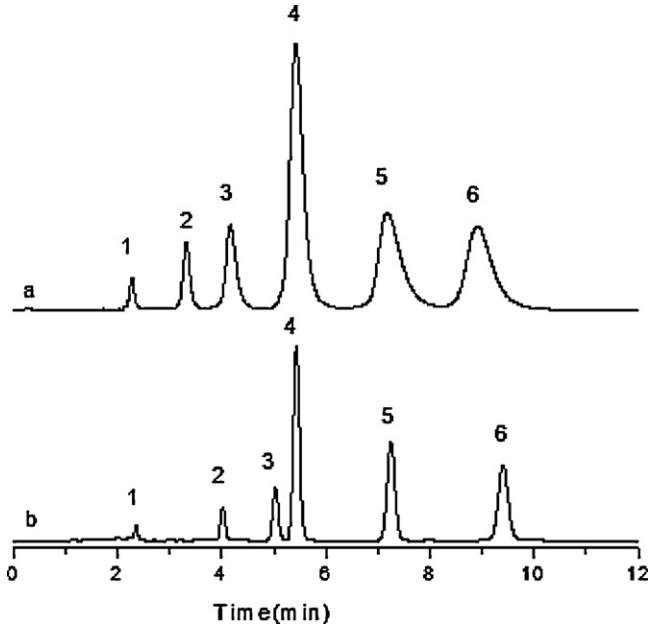

Fig. 3. Chromatograms of PAHs on OCATS(a) and ODS(b) Mobile phase, methanol–water (85/15, v/v); flow rates: 1.0 mL min^{-1}; detection wavelength, 254 nm. Peaks: 1, benzene; 2, biphenyl; 3, fluorine; 4, anthracene; 5, pyrene; 6, chrysene.

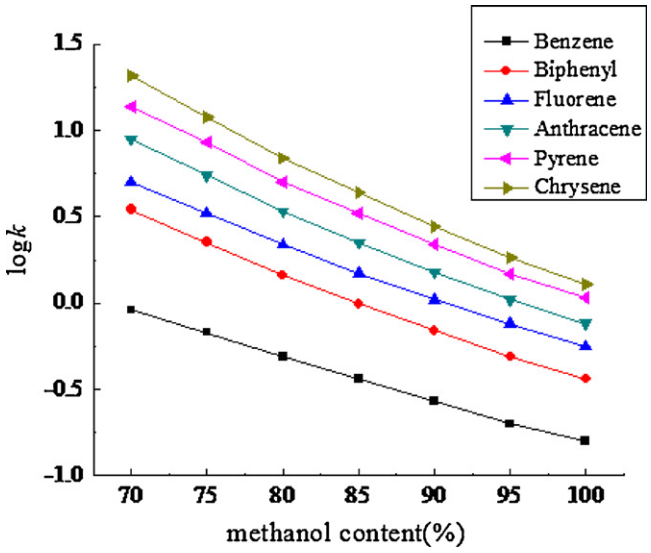

Fig. 4. Effect of methanol content on log k of PAHs on OCATS column mobile phase, different methanol contents; flow rates, 1.0 mL min^{-1}; detection wavelength, 254 nm.

Table 1 that the elution order of analytes on OCATS was the same as that on ODS, the k values increased with the increase of log $K_{o/w}$ values. Meanwhile, the k values of six PAHs linearly declined with increase of methanol content in the mobile phase (Fig. 4). These results were in accordance with reversed-phase separation mechanism, and indicated that hydrophobic interaction between the OCATS stationary phase and PAHs played an important role in the separation.

In addition, the k values of PAHs on OCATS are similar to those on ODS, though the latter has a higher carbon loading and bonded amount (more than 1000 μmol g^{-1}), which may attributed to the existence of the other interactions, such as π—π interaction, between the solutes and the stationary phase.

Table 1
The capacity factors (k) of PAHs and momo-substituted benzene on OCATS and ODS.

Analytes	Log $K_{o/w}$	Columns	
		OCATS k	ODS k
PAHs[a]			
Benzene	2.13	0.36	0.41
Biphenyl	4.01	0.99	1.41
Fluorene	4.18	1.49	2.01
Anthracene	4.45	2.25	2.26
Pyrene	4.88	3.30	3.34
Chrysene	5.81	4.33	4.63
Mono-substituted benzenes[b]			
Aniline	0.9	2.90	1.03
Acetophenone	1.58	4.11	2.27
Nitrobenzene	1.81	6.31	3.84
Toluene	2.73	7.35	10.89
Chlorobenzene	2.81	8.62	10.89
Bromobenzene	2.99	10.16	13.00
Iodobenzene	3.25	13.19	17.92

Log $K_{o/w}$ data taken from SRC PhysProp database.
[a] Mobile phase, methanol–water (85/15, v/v); detection wavelength, 254 nm.
[b] Mobile phase, acetonitrile–water (35/65, v/v); detection wavelength, 254 nm.

3.2.2. Separation of mono-substituted benzenes

Separation of seven mono-substituted benzenes also was carried out and the typical chromatograms are shown in Fig. 5. As can be seen from Fig. 5 and Table 1, the elution order of mono-substituted benzenes on OCATS column followed the order of hydrophobicity and log $K_{o/w}$ values of the solutes, which implied that the OCATS stationary phase featured reversed-phase separation mechanism. On one hand, the retention of nonpolar solutes (toluene, chlorobenzene, bromobenzene and iodobenzene) on OCATS stationary phase were weaker than those on ODS, which indicated that the hydrophobic interaction on OCATS was weaker than those on ODS, but the former had advantage in the rapid analysis of the above mentioned nonpolar aromatics. On the other hand,

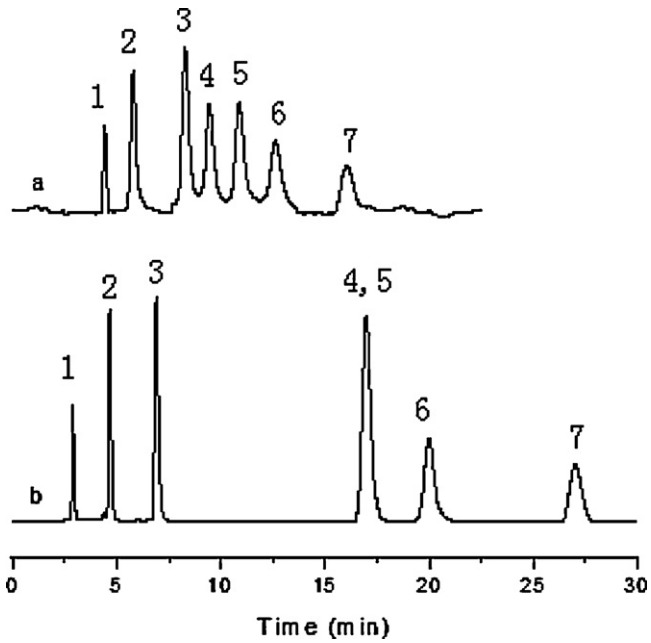

Fig. 5. Chromatograms of *mono*-substituted benzenes on OCATS (a) and ODS (b) mobile phase, acetonitrile–water (35/65, v/v); flow rates: 1.0 mL min^{-1}; detection wavelength, 254 nm. Peaks: 1, aniline; 2, acetophenone; 3, nitrobenzene; 4, toluene; 5, chlorobenzene; 6, bromobenzene; 7, iodobenzene.

the retentions of other solutes, such as aniline, acetophenone and nitrobenzene on OCATS were stronger than those on ODS. These might be attributed to the additional π—π and hydrogen bond interactions.

For toluene and chlorobenzene, their log $K_{o/w}$ values are 2.73 and 2.81, respectively. Therefore the hydrophobicity difference of toluene and chlorobenzene is so close that they coeluted on ODS. However, they can be separated completely on OCATS under the same elution condition (Fig. 5), and chlorobenzene exhibited stronger retention than toluene. The possible reason is that chlorobenzene has more aboundant π electrons caused by the conjugated effect of chlorine atom with benzene ring, which induced the stronger π—π interaction between the calixarene and chlorobenzene. The quantum chemistry calculation method performed on the Gaussian 03 series of programs by the basis set of DFT-B3LYP/STO-3G* was applied to investigate the separation mechanism. The geometries of the guest analytes, the host calixarene, and the host–guest complexes were optimized, to ensure the obtained structures at the lowest energy. Then, their Gibbs free energy change ΔG values (Table S1) on formation of a so-called calixarene-analyte supramolecule (two molecules as close as possible) were calculated. According the quantum chemistry calculation results, the more negative ΔG value, the more stable calixarene-analyte supramolecule, and the stronger interaction and retention. Some analyte-calix[2]arene[2]triazine supramolecule structures were optimized and shown in Fig. S2. It can be seen from Fig. S2a and b that toluene deviated from the cavity of calix[2]arene[2]triazine but chlorobenzene approached the calixarene central cavity. This result indicates that the chlorobenzene-calix[2]arene[2]triazine supramolecular was more stable than the toluene-calix[2]arene[2]triazine. Thus, the lower ΔG value for chlorobenzene-calix[2]arene[2]triazine than toluene-calix[2]arene[2]triazine was obtained (Table S1). The more stable supramolecular and lower ΔG value for chlorobenzene-calix[2]arene[2]triazine resulted in the stronger retention of chlorobenzene than toluene on OCATS column.

3.2.3. *Separation of positional isomers of disubstituted aromatics*

The separations for some disubstituted aromatic positional isomers with different polar and nonpolar character were individually investigated on OCATS. Retention capacity factors (k) of these isomers were calculated under the optimized chromatographic conditions, and listed in Table 2. First of all, hydrophobic interaction also played an important role in the separation of the isomers. For *ortho*-isomers of dihydroxybenzene, benzenediamine and aminophenol with an intramolecular hydrogen bonding, the stronger hydrophobicity than *meta*- and *para*-isomers made them last eluted on OCATS and ODS.

In contrast with our previous research of conventional calix[4]arene-bonded stationary phases (C4BS) [7], one goal of this work is mainly to evaluate the chromatographic performance of the new phase after the introduction of oxygen-bridge and the nitrogen atoms on triazines. In oxo-bridged calix[2]arene[2]triazine, the strong conjugation system due to oxygen atoms being conjugated with their adjacent aromatic rings make calix[2]arene[2]triazine have stronger delocalization π electrons than the conventional calixarenes. Thus, the stronger hydrogen bonding interaction and stronger π—π interaction than C4BS could be predicted. It can be noticed from Table 2 that retentions of dihydroxybenzene, benzenediamine, aminophenol, nitrophenol and nitroaniline on OCATS are stronger than those on C4BS and ODS. Obviously, the chromatographic performance of OCATS is dependent on not only the moiety of calix[2]arene[2]triazines, but also the oxygen-bridges and the triazine nitrogen atoms. The synergistic effects between the moiety and the ether-bridge of OCATS increase the retention of the analytes.

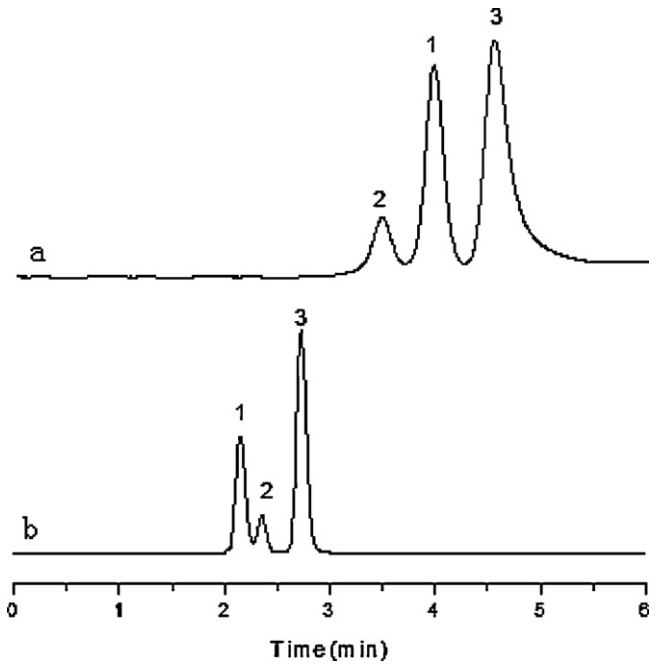

Fig. 6. Chromatograms of dihydroxybenzene on OCATS (a) and ODS (b) mobile phase, methanol–water (40/60, v/v); flow rates, 1.0 mL min^{-1}; detection wavelength, 254 nm. Peaks: 1, *p*-dihydroxybenzene; 2, *m*-dihydroxybenzene; 3, *o*-dihydroxybenzene.

As can be seen from Fig. 6 and Table 2, the elution order of *m*- and *p*-isomers of dihydroxybenzene was opposite to the pK_a value orders and log $K_{o/w}$ value orders. It seems unreasonable. Thus in the inclusion complexation mechanism it is proposed that the linear shape of the *p*-dihydroxybenzene facilitates its penetration more deeply into the cavities of the calix[2]arene[2]triazine. The inclusion interaction is also supported by the quantum chemistry calculation. As can be seen from Fig. S2c and d, *p*-dihydroxybenzene inclined to enter into the cavity of the calix[2]arene[2]triazine, whereas *m*-dihydroxybenzene deviated from the cavity of the calix[2]arene[2]triazine in the optimized complex structures. Meanwhile, the more negative ΔG value for *p*-dihydroxybenzene-calix[2]arene[2]triazine than that of *m*-dihydroxybenzene-calix[2]arene[2]triazine were also obtained by quantum chemistry calculation. These results made *p*-dihydroxybenzene the stronger retention than *m*-dihydroxybenzene on OCATS.

The retentions of the probes containing —NO_2 substituent at the phenyl ring, such as nitrophenol, nitroaniline and nitrotoluene were stronger than others. The results are similar to those obtained on the conventional calixarene stationary phases [13,15,24,25]. This behavior may be due to π-electron transfer interaction resulting from the electron-withdrawing effect of the nitro group of analyte. In comparison with *meta*-isomer of nitrophenol and nitroaniline, the nitro groups at *o*- or *p*-isomers providing an additional conjugated effect resulted in the corresponding compounds the higher proton–donor ability and more abundant delocalization π electrons, which could strengthen the hydrogen bonding interaction and π—π interaction. Furthermore, the strong conjugation system in oxo-bridged calix[2]arene[2]triazine enhance the π—π and π-electron transfer interactions. As can be seen from Table 2, it is obvious that nitrophenols and nitroanilines exhibited stronger retention than those on C4BS, which indicated that the stronger π-electron transfer interaction existed in OCATS caused by the large conjugation system in calix [2] arene [2] triazine. So, it is not surprise that *o*- and *p*-isomers of nirtophenol and nitroaniline represent stronger retention than the *m*-ones.

Table 2
The capacity factors (k) of disubstituted benzene positional isomers on OCATS, C4BS and ODS.

	Isomers	pk_a	Log $K_{o/w}$	OCATS k	C4BS k	ODS k
Dihydroxybenzene	*o*-	9.48	0.88	3.04	1.53	0.93
	m-	9.44	0.80	2.09	1.32	0.66
	p-	9.96	0.59	2.53	0.89	0.52
Aminophenol	*o*-	9.71	0.62	4.27	–	1.56
	m-	9.87	0.21	2.27	–	0.77
	p-	10.30	0.04	1.98	–	0.4
Nitrophenol	*o*-	7.23	1.79	19.6	3.24	3.91
	m-	8.36	2.00	15.7	3.79	2.87
	p-	7.15	1.91	27.1	3.79	2.41
Nitroaniline	*o*-	-0.28	1.85	19.4	7.26	2.65
	m-	2.45	1.37	13	4.26	1.31
	p-	1.11	1.47	15.6	6.18	0.86
Benzenediamine	*o*-	4.47	0.15	2.93	0.46	0.99
	m-	4.88	−0.33	2.18	0.28	0.54
	p-	6.08	−0.30	1.69	0.14	0.43
Nitrotoluene	*o*-	–	2.30	9.36	–	10.3
	m-	–	2.45	10.0	–	12.3
	p-	–	2.37	11.2	–	10.9
Xylene	*o*-	–	3.16	4.82	–	6.07
	m-	–	3.20	4.82	–	6.33
	p-	–	3.15	4.82	–	5.73

Mobile phase: methanol–water (40/60, v/v) flow rates: 1.0 mL min^{-1}; detection wavelength, 254 nm.
pK_a and log $K_{o/w}$ data taken from SRC PhysProp database.

The extra-rentention of linear *p*-nitrophenol may partly ascribe to inclusion complexation with calix [2] arene [2] triazine. The results of the optimized supramolecule and quantum chemistry calculation (Table S1, Fig. S2e and f) also indicate that *p*-nitrophenol could form the more stable supramolecular complex (more negative ΔG value) with tetrocalix[2]arene[2]triazine and retain stronger than *m*-nitrophenol. So it can be concluded that hydrophobic, hydrogen bonding, π—π, π-electron transfer interactions as well as inclusion complexation were responsible for the chromatographic behavior of isomers of nitrophenol and nitroaniline.

From Table 2, it can be seen that most of the positional isomers were well separated except xylene isomers. Beacuse nonpolar xylene position isomers contained nonpolar substituted group —CH_3, and had the similar hydrophobicity and π—π interaction with OCATS, three xylene position isomers could not be separated from each other. The result was similar to those obtained on the conventional calixarene stationary phases [11,13–15], which may be the general character of calixarene stationary phase. Therefore, it is noteworthy that the presence of polar groups such as —OH, —NO_2 and —NH_2 in the aromatic probes might tune the separation selectivity on OCATS stationary phase.

3.3. Determination of melamine in infant fromula on OCATS column

Melamine with high nitrogen content (67%) has been added illegally to milk and dairy products to suggest deceptively misleading "high" protein content when measured using Kjeldahl nitrogen analysis. In 2008, there were incidents in China in which a large number of cases of renal failure in infants caused by powdered milk that had been mixed with melamine. Due to the serious health concerns associated with melamine consumption and the extensive scope of affected products, rapid and sensitive methods to detect melamine are essential. The RP-HPLC method performed on a Kromasil C18 column is presently the popular choice for quantitative determination of melamine [26,27] involving expensive acetonitrile or ion pair reagents (sodium *n*-heptanesulfate, trifluoroacetic acid) in mobile phase. Thus, in this paper, we try to develop the rapid analysis of melamine in powder infant formula samples by simple HPLC method using a green and economical mobile phase on OCATS column.

The infant milk powder was treated with trichloroacetic acid solution to precipitate proteins and to dissociate the target analyte from the sample matrix. The mixed solution was centrifuged and the supernatant was applied to a Clearnert PCX-SPE column clean up. The optimized chromatogram of melamine on an OCATS column was shown in Fig. 7, from which we can see that melamine obtained better separation from the matrix with symmetric shape of peaks.

The linearity was satisfactory in the range of 0.3–50 μg/mL with a correlation coefficient of 0.9998. Under the optimal conditions, the limit of detection (LOD) was 0.1 μg/mL. The recovery of

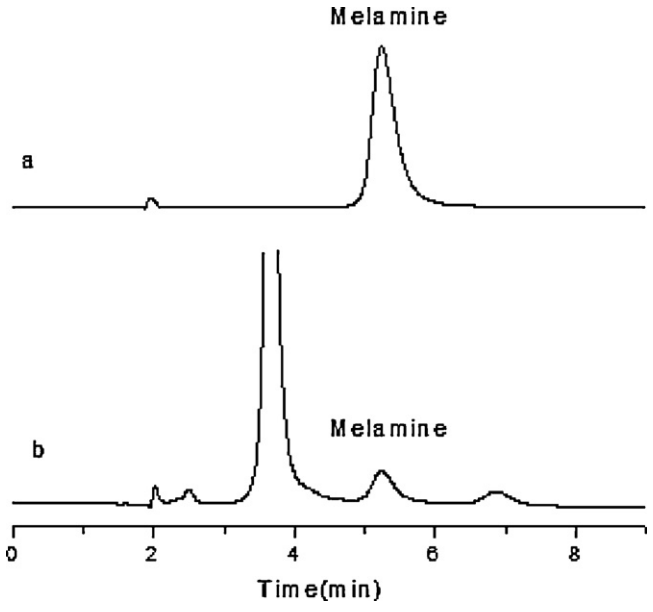

Fig. 7. Chromatograms of standard melamine (a) and infant fromula (b) on OCATS column mobile phase, methanol–water (3/97, v/v); flow rates, 1.0 mL min^{-1}; detection wavelength, 240 nm.

melamine for infant formula samples spiked with 0.4 μg/g was in the range of 94–103% with the RSDs less than 2.5% ($n = 3$).

4. Conclusion

A new oxo-bridged calix[2]arene[2]triazine bonded stationary phase was prepared and characterized by elemental analysis, FT-IR, thermal analysis. The chromatographic characteristics of this new stationary phase were investigated by using PAHs, mono-substituted benzenes, and isomers of disubstituted benzene, and the results were compared with those on ODS. The experimental data showed that the new material was a novel stationary phase with various action sites for different solutes. As a combination of calixarene and crown ether, the chromatographic performance of OCATS was dependent on both the moiety of calixarene and ether-bridge. The hydrophobic, hydrogen bonding, π—π, inclusion interactions altogether contribute to the chromatographic character of OCATS. The conjugated OCATS system with aromatic rings and bridging-oxygen atoms could improve the selectivity. The quantum chemistry calculation results were employed to provide supplementary support on the retention behaviors of solutes on OCATS. Melamine and infant formula matrix could be baseline separated within 8 min on OCATS column with the rich water mobile phase (3% methanol) avoiding expensive acetonitrile and ion pair reagents.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.12.031.

References

[1] Z.R. Zeng, C.Y. Wu, X.H. Fang, Z.F. Huang, Y.T. Wang, J. Chromatogr. 589 (1992) 368.
[2] R.N. Fu, C. Huang, C. Huang, W. Xu, J. Chromatogr. A 653 (1993) 173.
[3] H.S. Cho, H.J. Choi, M.H. Hyun, J. Chromatogr. A 1216 (2009) 7446.
[4] Y.H. Gong, Y.Q. Xiang, B.F. Yue, G.P. Xue, J.S. Bradshaw, H.K. Lee, M.L. Lee, J. Chromatogr. A 1002 (2003) 63.
[5] Y.Q. Feng, M.J. Xie, S.L. Da, Anal. Chim. Acta 403 (2000) 187.
[6] K. Si-Ahmed, F. Tazerouti, A.Y. Badjah-Hadj-Ahmed, Z. Aturki, G. D'Orazio, A. Rocco, S. Fanali, J. Chromatogr. A 1217 (2010) 1175.
[7] C.H. Ding, K. Qu, Y.B. Li, K. Hu, H.X. Liu, B.X. Ye, Y.J. Wu, S.S. Zhang, J. Chromatogr. A 1170 (2007) 73.
[8] M. Śliwka-Kaszyńska, K. Jaszcołt, I. Anusiewicz, J. Sep. Sci. 32 (2009) 3107.
[9] T. Sokoliess, U. Menyes, U. Roth, T. Jira, J. Chromatogr. A 898 (2000) 35.
[10] S. Erdemir, M. Yilmaz, Talanta 82 (2010) 1240.
[11] M. Śliwka-Kaszyńska, S. Karaszewski, J. Sep. Sci. 31 (2008) 926.
[12] S.K. Thamarai Chelvi, E.L. Yong, Y.H. Gong, J. Chromatogr. A 1203 (2008) 54.
[13] M. Śliwka-Kaszyńska, K. Jaszczołt, A. Kołodziejczyk, J. Rachoń, Talanta 68 (2006) 1560.
[14] K. Jaszczołt, M. Śliwka-Kaszyńska, Chromatographia 66 (2007) 837.
[15] M. Śliwka-Kaszyńska, K. Jaszczołt, D. Witt, J. Rachon, J. Chromatogr. A 1055 (2004) 21.
[16] L.S. Li, S.L. Da, Y.Q. Feng, M. Liu, Talanta 64 (2004) 373.
[17] M. Barc, Śliwka-KaszyńskaF M., J. Chromatogr. A 1216 (2009) 3954.
[18] C. Schneider, T. Jira, J. Chromatogr. A 1216 (2009) 6285.
[19] L.S. Li, M. Liu, S.L. Da, Y.Q. Feng, Talanta 63 (2004) 433.
[20] M.X. Wang, H.B. Yang, J. Am. Chem. Soc. 126 (2004) 15412.
[21] D.X. Wang, Q.Y. Zheng, Q.Q. Wang, M.X. Wang, Angew. Chem. Int. Ed. 47 (2008) 7485.
[22] Q.Q. Wang, D.X. Wang, H.B. Yang, Z.T. Huang, M.X. Wang, Chem. Eur. J. 16 (2010) 7265.
[23] H.B. Yang, D.X. Wang, Q.Q. Wang, M.X. Wang, J. Org. Chem. 72 (2007) 3757.
[24] L.S. Li, S.L. Da, Y.Q. Feng, M. Liu, Anal. Sci. 20 (2004) 561.
[25] W. Xu, J.S. Li, Y.Q. Feng, S.L. Da, Y.Y. Chen, X.Z. Xiao, Chromatographia 48 (1998) 245.
[26] S. Ehling, S. Tefera, I.P. Ho, Food Addit. Contam. A 24 (2007) 1319.
[27] H.W. Sun, L.X. Wang, L.F. Wang, L.F. Ai, S.X. Liang, H. Wu, Food Control 21 (2010) 686.