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

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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.

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.

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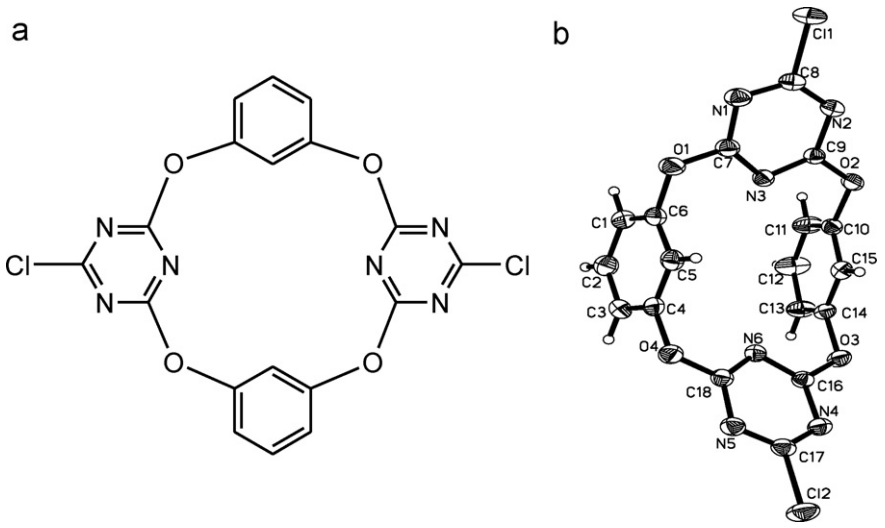

Fig. 1. Chemical and single-crystal structure of oxo-bridged calix[2]arene[2]triazine.

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,