



# Investigation on the preparation and chromatographic behavior of a new *para*-*tert*-butylcalix[4]arene-1,2-crown-4 stationary phase for high performance liquid chromatography

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## ABSTRACT

In the present work, a new *para*-*tert*-butylcalix[4]arene-1,2-crown-4 bonded silica stationary phase (CBS4-4) was synthesized, structurally characterized, and employed to separate polycyclic aromatic hydrocarbons (PAHs), phenols, aromatic amines, benzoic acid and its derivatives. The chromatographic behaviors of the prepared stationary phase were investigated and compared with ODS. The effects of methanol concentrations on the retention index show that CBS4-4 exhibits high selectivity for the above analytes. The separation mechanisms based on the different interactions between calixarene and the analytes were discussed. With the assistance of quantum chemistry calculation, the interaction Gibbs free energy change  $\Delta G_{\text{solv}}$  (in the mobile phase) of *p*, *m* and *o*-phenylenediamine positional isomers and *para*-*tert*-butylcalix[4]arene-1,2-crown-4 were obtained. The  $\Delta G_{\text{solv}}$  values were consistent with the retention behavior of *p*, *m* and *o*-phenylenediamine on the CBS4-4. According to the chromatographic data, it can be concluded that the selectivity of CBS4-4 for analytes is mainly ascribed to hydrophobic interaction, accompanied by other effects such as hydrogen bonding interaction,  $\pi$ - $\pi$  and inclusion interaction. The CBS4-4 column has been successfully employed for the analysis of benzoic acid in Sprite drink.

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## 1. Introduction

With the development of high performance liquid chromatography (HPLC), various new bonded stationary phases were continuously explored and applied. However, until now there exists no universally accepted chromatographic test to choose an appropriate packing material for a particular separation problem [1]. The search for more efficient stationary phases with the widest possible spectrum of applications has become a significant direction in the study of compound separation by HPLC. Recently, macrocyclic compounds (cyclodextrins, crown ethers and calixarenes), which are capable of forming inclusion complexes with guest molecules, are commonly used in modern chromatography. Many chromatographic scientists have paid the exceptional attention to calixarenes because their unique structures. Calixarenes, cavity-shaped cyclic molecules consisting of phenol units linked via methylene bridges, are known as a typical representative of the

third-generation host after crown ethers and cyclodextrins [2]. The peculiar configurations lead to the formation of typical host-guest interaction between calixarenes and numerous compounds, and result in widely varied applications in ion-selective membranes and electrodes [3–9], electrophoresis [10–15] and chromatography [16–27].

In the field of chromatography, their ability to provide  $\pi$ - $\pi$  interactions or  $\pi$ -electron transfer [28–30] and form inclusion complexes [30–33] enable them to be a valuable tool for HPLC stationary phases. To date, more and more applications of different calixarene bonded stationary phases have been reported for the analyses, such as water-soluble vitamins [34], sulphonamides [35,36], estradiol epimers [37], metal ions [38], amino acid esters [39,40], aromatic positional isomers [41–44], polycyclic aromatic hydrocarbons (PAHs) [44–47], nucleosides [48], and so on.

Very recently, we developed six LC calixarene-bonded stationary phases [44] for HPLC and *p*-*tert*-butylcalix[4]arene modified sol-gel column for open-tubular capillary electrochromatography [49], and investigated their separation performance and separation mechanism assisted with quantum chemistry calculation [44,50,51]. These previous reports have shown that calixarene bonded stationary phases are excellent in reversed-phase chro-

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matography and exhibiting promising application in HPLC. In the present work, we describe the preparation of a new *para*-tert-butylcalix[4]arene-1,2-crown-4 bonded silica stationary phase (CBS4-4) and with its chromatographic behavior investigated. The new developed stationary phase has been characterized by using elemental analysis, FT-IR and thermal gravimetric analysis. The chromatographic performance was investigated by using PAHs, phenols, aromatic amines, benzoic acid and its derivatives as probes in comparison with ODS. The influence of methanol concentrations and pH on the chromatographic behavior of the solutes was also investigated.

## 2. Experimental

### 2.1. Apparatus and materials

Chromatographic analyses were carried out by 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. The home-made calixarene column was filled using a packing machine (Kerui Tech. Co. Ltd., Dalian, China) under the pressure of 50 MPa. An Eclipse XDB-C18 column (Agilent, 150 mm × 4.6 mm i.d., 5 μm) was used as a comparison with the home-made calixarene column. Elemental analysis was performed with a Flash EA 1112 elemental analyzer. <sup>1</sup>H NMR spectrum was recorded with a Bruker 400 MHz spectrometer in CDCl<sub>3</sub>. Mass spectrum was acquired by using a Agilent XCT Trap mass spectrometer equipped with a gas nebulizer probe, nitrogen was used as drying gas at a flow rate of 5.0 L/min, the nebulizer pressure was 15.0 psi, the capillary was typically held at 3500 V and the source temperature was maintained at 325 °C. IR spectra were recorded with a Bruker Vector 22 instrument. Thermal gravimetric analysis (TGA) was carried out with 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.

All analytes and solvents used in this study were of analytical grade and obtained from Beijing Chemical Plant (Beijing, China) unless specially mentioned. Silica gel (with particle size of 5 μm, pore size of 100 Å and specific surface area of 300 m<sup>2</sup>/g) was provided by Lanzhou Institute of Chemical and Physics of Chinese Academy of Sciences (Lanzhou, China). γ-Glycidoxypropyltrimethoxysilane (KH-560) was purchased from Wuhan University Chemical Plant (Wuhan, China). A phosphate buffer (0.5%, w/w) (pH 3.5–7.5) was prepared by mixing KH<sub>2</sub>PO<sub>4</sub> with ultra-high quality pure water, and filtered through a 0.45 μm filter before use. HPLC-grade methanol (MeOH) was purchased from the Luzhong Reagent Plant of Shanghai (Shanghai, China). Water was purified by using Milli-Q purification equipment.

### 2.2. Synthesis of *para*-tert-butylcalix[4]arene-1,2-crown-4

Scheme 1 shows the synthesis scheme of *para*-tert-butylcalix[4]arene-1,2-crown. *Para*-tert-butylcalix[n]arene was prepared in good yield according to the previous literature [52]. *para*-tert-butylcalix[4]arene-1,2-crown-4 was obtained by reacting of *para*-tert-butylcalix[4]arene and triethylene glycol ditosylate [53]. NaH (0.43 g, 18 mmol) was added to a suspension of compound 2 (1.00 g, 1.54 mmol) in anhydrous DMF (100 mL) under the protection of argon, the mixture was stirred at room temperature for 1 h to ensure the material completely transformed into phenol sodium salt. Then triethylene glycol ditosylate (0.35 g, 0.77 mmol) dissolved in DMF (10 mL) was added dropwise, after that the mixture continued to stir at 50–70 °C. Progress of the

reaction was detected by TLC. When the starting triethylene glycol ditosylate had disappeared, methanol (10 mL) was added dropwise to remove the excess NaH. The resulting solution was evaporated to dryness and the residue taken up with aqueous HCl (10%, 100 mL), extracted with methylene chloride (100 mL) for three times. The organic layer was dried by anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to give a yellow residue. The product was purified by column chromatography with EtOAc–Petroleum ether (1:3, v/v) as eluent to give 0.40 g (68%, yield) white solid. Mp: 111–114 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 8.89 (s, 2H, OH), 7.25 and 6.93 (2d, 4H, J = 1.9 Hz, ArH), 7.06 and 6.98 (2d, 4H, J = 1.7 Hz, ArH), 4.68 (d, 1H, J = 12.3 Hz, ArCH<sub>2</sub>Ar axial), 4.30 (d, 3H, J = 12.6 Hz, ArCH<sub>2</sub>Ar axial), 4.26–3.89 (m, 12H, CH<sub>2</sub>–CH<sub>2</sub>), 3.38–3.33 (m, 4H, ArCH<sub>2</sub>Ar equatorial), 1.20 and 1.13 (2s, 36H, C(CH<sub>3</sub>)<sub>3</sub>). From the <sup>1</sup>H NMR data, we can see that the chemical shift of phenolic hydroxyls is in low field, which is due to the existence of intra-molecular hydrogen bonds, indicating that the crown ether ring is connected on the two adjoining hydroxyls. The product can easily form molecular ion ([M–H]<sup>−</sup>) in negative detection mode with the MS result of m/z = 761 (M<sup>−</sup>).

### 2.3. Preparation of *para*-tert-butylcalix[4]arene-1,2-crown-4 bonded silica stationary phase (CBS4-4)

Scheme 1 shows the synthesis process of a new calix[4]arene-bonded silica gel stationary phase. Details of the bonding procedure are as follows. Active silica gel (5.0 g) was suspended in 50 mL dry toluene (freshly distilled), and then 6.0 mL KH-560 and 1.0 mL triethylamine (used as a catalyst) was added to this suspension. The mixture was stirred and heated to 80 °C under the protection of nitrogen atmosphere for 8 h. The solid was filtered by 1.5 μm filter, and washed in sequence with toluene and acetone, then dried at 80 °C under vacuum for 8 h. Finally, γ-glycidoxypropyl bonded silica gel (GBS, Scheme 1) was obtained and used as a precursor in the following reaction.

A mixture of 2.0 g *para*-tert-butylcalix[4]arene-1,2-crown-4, 0.6 g NaH, and 60 mL toluene (freshly distilled) were stirred at 80 °C for 30 min, then the supernatant liquid was transferred to a 100 mL three-neck flask, 3.0 g GBS was added, after that the mixture was refluxed with the catalyst for 48 h. The whole process was carried out under nitrogen atmosphere. After the reaction finished, the product was filtered and washed in sequence with toluene, acetone, methanol and distilled water. Subsequently, CBS4-4 was obtained, and was dried at 100 °C under vacuum for 8 h, then cooled to room temperature in a desiccator.

### 2.4. Characterization of *para*-tert-butylcalix[4]arene-1,2-crown-4 bonded silica stationary phase (CBS4-4)

As can be seen from Scheme 1, *para*-tert-butylcalix[4]arene-1,2-crown-4 (compound 3) was prepared by the reaction of *para*-tert-butylcalix[4]arene (compound 2) and triethylene glycol ditosylate in the presence of NaH, and with DMF as the solvent. Then the compound 3 was bonded onto γ-glycidoxypropyl bonded silica gel (GBS) by the breaking ring reaction method [44]. The characterization of the developed stationary phase was carried out by elemental analysis, IR and thermal gravimetric analysis.

The elemental analysis results of GBS and CBS4-4 are given in Table 1. The carbon content of CBS4-4 was obviously higher than that of GBS, which confirmed that the calixarene was successfully immobilized onto the silica gel. The bonded amount of *para*-tert-butylcalix[4]arene-1,2-crown-4 onto the silica gel was calculated by subtracting that of GBS. According to the carbon content of the bonded silica gel stationary phases, the resulting stationary phase contains 13.46% carbon corresponding 0.105 mmol *para*-

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