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# Calix[4]pyrroles: Highly selective stationary phases for gas chromatographic separations

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ABSTRACT

Calix[4]pyrroles offer a great potential as stationary phases for gas chromatography (GC) due to their unique structures and physicochemical properties. Herein we present the first report of using two calix[4]pyrroles, namely *meso*-tetra-cyclohexylcalix[4]pyrrole (THCP) and *meso*-octamethylcalix[4]pyrrole (OMCP). These stationary phases were statically coated onto capillary columns and investigated in terms of column efficiency, polarity, separation performance, thermal stability and repeatability. The columns achieved column efficiencies of 2200–3000 plates/m and exhibited nonpolar nature with an average polarity of 67 for THCP and 64 for OMCP, respectively. THCP stationary phase shows high selectivity for analytes of different polarity and exhibits nice peak shapes, especially for aldehydes, alcohols and anilines that are prone to severe peak tailing in GC analysis. Interestingly, THCP stationary phase possesses superior resolving ability for aniline and benzenediol positional isomers while OMCP shows preferential selectivity for nonpolar analytes such as hexane isomers. Moreover, calix[4]pyrrole columns also have good thermal stability up to 260 °C and repeatability with a relative standard deviation (RSD%) of less than 0.10% for run-to-run and less than 5.2% for column-to-column. This work demonstrates the unique separation performance of calix[4]pyrroles and their promising future as a new class of GC stationary phases.



## 1. Introduction

Calix[4]pyrroles are a class of macrocycles composed of four pyrrole rings linked in the *meso*-position, which are characteristic of heterocyclic ring systems and cup-shaped cavity structures [1–7]. *Meso*-octamethylcalix[4]pyrrole (OMCP), the first calix[4]pyrrole, was synthesized over a century ago by Baeyer using the condensation of pyrrole and acetone. *Meso*-tetracyclohexyl calix[4]pyrrole (THCP) was reported by Brown and coworkers [2]. These two calix[4]pyrroles have high stability, good solubility in organic solvents and easy availability by synthesis, and their chemical structures are shown in Fig. 1. Over the past decades, researches have proven the selective interactions of calix[4]pyrroles with halide anions [8–12] and neutral molecules such as alcohols, amides, phenols and other oxygen-containing neutral species [13,14] *via* H-bonding or π–π stacking interactions. In addition, calix[4]pyrroles possess structural flexibility by adjusting their conformation to accommodate different substrates [3,8,13,15–18]. For example, the preferential cone conformation favors H-bonding interactions of calix[4]pyrroles with polar molecules and may also exhibit specific interactions with aromatics *via* the shallow aromatic cavity defined by the pyrrole rings.

As shown in Fig. 1, calix[4]pyrroles have a quite similar structure to their counterpart calix[4]arenes that have benzene rings instead of pyrrole rings. Phenol-derivatized calix[4]arenes were reported as gas chromatography (GC) stationary phases [19–23]. For this purpose, derivatization of the parent calix[4]arene *via* phenol groups is mandatory in order to improve the solubility of the parent calix[4]arene in organic solvents and thermal stability. Even so, the derivatized calix[4]arenes are often needed to be used together with polysiloxanes either in physical mixtures [19,22] or in chemically grafted polymers [20,21] in order to improve their column efficiency and GC separation performance. In contrast, calix[4]pyrroles show advantages over calix[4]arenes as ideal candidates for GC stationary phases on the basis of their physicochemical properties and selective interactions with different

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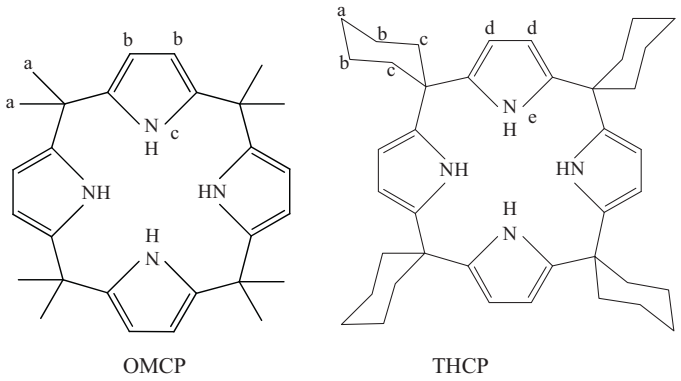


**Fig. 1.** Structures of *meso*-octamethylcalix[4]pyrroles (OMCP) and *meso*-tetracyclohexylcalix[4]pyrroles (THCP).

analytes mentioned above. However, to the best of our knowledge, no reports on calix[4]pyrroles for this purpose are available. In fact, calix[4]pyrroles are barely reported in chromatography. To date, only two reports for high-performance liquid chromatography (HPLC) [24,25] are available, in which calix[4]pyrrole-bonded silica gels were used for HPLC separation of anionic and neutral molecules.

Herein, we report the first example of using calix[4]pyrroles as GC stationary phases, in which two calix[4]pyrroles, namely THCP and OMCP, were investigated. Separation performance of calix[4]pyrroles as GC stationary phases was evaluated by separation of various mixtures including *n*-alkanes, aldehydes, alcohols, ketones, the Grob mixture, halogenobenzenes, polycyclic aromatic hydrocarbons (PAHs), hexane isomers, aniline isomers and benzenediol isomers. Meanwhile, a well-recognized commercial polysiloxane column with comparable polarity was also used for comparison. Moreover, thermal stability and separation repeatability of calix[4]pyrrole columns were also examined.

## 2. Experimental

### 2.1. Materials and equipment

Pyrrole was purchased from Alfa Aesar (Heysham, Lancashire, UK). Cyclohexanone, acetone, dichloromethane and the analytes used in different mixtures were purchased from Beijing Chemical Reagent Company (Beijing, China). All the chemicals were at least of analytical grade and the mixture solutions were prepared in dichloromethane. Untreated fused-silica capillary tubing (0.25 mm, i. d.) was purchased from Yongnian Ruifeng Chromatogram Apparatus Co., Ltd. (Hebei, China). A HP-5MS capillary column (12 m × 0.25 mm, i. d.) was purchased from Agilent Technologies.

An Agilent 7890A gas chromatograph (Agilent Technologies, USA) equipped with a split/splitless injector, a flame ionization detector (FID) and a ChemStation software was used. All the GC separations were performed under the following conditions: nitrogen of high purity (99.999%) as carrier gas at a flow rate of 1 mL/min; injection port at 300 °C; split ratio of 30:1; FID at 300 °C. A Bruker Avance-400 NMR Spectrometer (Bruker, Switzerland) was used for the characterization of the synthesized calix[4]pyrrole stationary phases.

### 2.2. Methods

#### 2.2.1. Synthesis of the calix[4]pyrrole stationary phases

The OMCP stationary phase was synthesized following the reported method [1–3] with minor modification. Briefly, pyrrole (230 mmol) was dissolved in methanol (250 mL) in a three-pecked flask, and then hydrochloric acid (2 mL) was added dropwise while stirring at room temperature. To this solution, acetone (230 mmol) was slowly dropped over a time period of 30 min and the mixture solution was refluxed for 12 h at room temperature. Afterwards, a crude product of OMCP was obtained and then washed with methanol. After recrystallization, the final product of OMCP was obtained as white crystalline powder. Using the aforementioned method except using cyclohexanone instead of acetone, the product of THCP was also obtained. The obtained $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm) data for OMCP are: a:1.511 (s, 24H, $CH_3$), b:5.895–5.901 (d, 8H, β-H), c:7.053 (s, 4H, NH), and for THCP are: a:1.402–1.413 (m, 8H, $CH_2$), b:1.484–1.540 (m, 16H, $CH_2$), c:1.926 (m, 16H, $CH_2$), d:5.902 (d, 8H, β-H), e:7.056 (s, 4H, NH). See Fig. 1 about the hydrogen atoms labeled as a–e.

#### 2.2.2. Preparation of capillary columns

Prior to static coating, a fused-silica capillary column (10 m × 0.25 mm, i. d.) was purged with nitrogen at 200 °C for 3 h and pretreated with a saturated solution of sodium chloride in methanol for the surface roughing to facilitate uniform coating of a stationary phase on the capillary inner wall. Then, the pretreated capillary column was statically coated with the solution of OMCP or THCP stationary phase in dichloromethane (0.25% or 0.35%, *w*/*v*) at 40 °C. During the coating process, one end of the capillary column was sealed and the other end was connected to a vacuum system to gradually remove the solvent under vacuum. The coated capillary column was then conditioned from 40 °C to 180 °C at the rate of 1 °C/min and held at the high-end temperature for 8 h under a constant flow of nitrogen at 1 mL/min.

## 3. Results and discussion

### 3.1. Characterization of the calix[4]pyrrole columns

Table 1 lists the column dimensions and efficiencies of the prepared columns as well as the commercial column. The column efficiencies expressed as the number of theoretical plates per meter were determined by isothermal determination of *n*-dodecane at 100 °C at a flow rate of 1 mL/min (34 cm/s). As shown, the THCP and OMCP columns achieved the column efficiencies of 2200–3000 plates/m. It should be mentioned that column 3 was only used for evaluation of the impact of coating thickness on separation performance and all the rest determinations and

**Table 1**
Characteristics of calix[4]pyrroles and commercial columns.

| Column No. | Stationary phase | Column dimension (m × μm i.d.) | Coating thickness (μm) | Column efficiency[a] (plates/m) |
|---|---|---|---|---|
| 1 | THCP | 10 × 250 | 0.16 | 3000 |
| 2 | OMCP | 10 × 250 | 0.16 | 2600 |
| 3 | OMCP | 10 × 250 | 0.22 | 2200 |
| 4 | HP-5MS | 12 × 250 | 0.25 | 2700 |

[a] Determined by *n*-dodecane at 100 °C at a linear velocity of 34 cm/s.