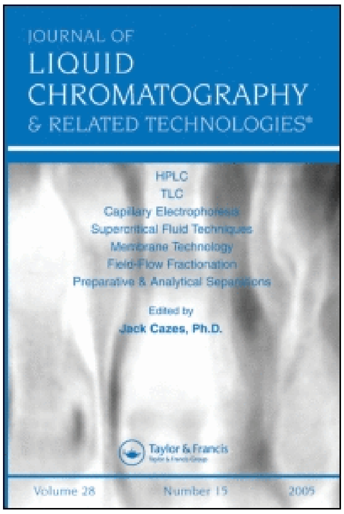

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Open-Tubular Capillary Electrochromatography Using Capillaries Modified with *p-tert*-Butylcalix[4]arene-1,3-bis(allyloxyethy)ether

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To cite this Article Tian, Yun , Zeng, Zhaorui , Chen, Yuanyin , Xie, Chuanhui , Li, Haibing and Yang, Xiangliang(2005) 'Open-Tubular Capillary Electrochromatography Using Capillaries Modified with *p-tert*-Butylcalix[4]arene-1,3-bis(allyloxyethy)ether', Journal of Liquid Chromatography & Related Technologies, 28: 18, 2931 — 2943

To link to this Article: DOI: 10.1080/10826070500274356

URL: http://dx.doi.org/10.1080/10826070500274356

Journal of Liquid Chromatography & Related Technologies®, 28: 2931–2943, 2005

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070500274356

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Abstract: Preparation and characterization of *p-tert*-butylcalix[4]arene-1,3-bis(allyloxyethy)ether (CA[4]-BAE) chemically coated capillaries via free radical reaction with vinyltriethoxysilane (VTES), which was attached onto the inner wall, was reported here previously IR spectra, SEM characterization, and decreased electroosmotic flow (EOF) suggested that the capillary was coated with CA[4]-BAE successfully. A slight slope of EOF *versus* pH ranging from 5 to 9 would help to make the separation reproducible. The CA[4]-BAE-coated capillary had shown improved separations of isomeric toluidines, isomeric naphthols, biphenyl derivatives, and polycyclic aromatic hydrocarbons (PAHs) in comparison with an uncoated capillary. The special selectivity on isomeric aromatic compounds indicated that there was a certain extent of host-guest interactions between solutes and the CA[4]-BAE coating.

Keywords: Capillary electrochromatography, Open-tubular capillary electrochromatography, Calixarene, Positional isomers

INTRODUCTION

Capillary electrochromatography (CEC), which combined the advantages of the high efficiency of capillary electrophoresis (CE) and the high selectivity of liquid chromatography (LC), has developed very quickly in recent years.[1–3] Among

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Address correspondence to Dr. Zhaorui Zeng, Department of Chemistry, Wuhan University, Wuhan 430072, P.R. China. E-mail: zrzeng@whu.edu.cn the various kinds of CEC used, open-tubular CEC (OTCEC) with the attachment of organic moiety to the inner wall of capillary has brought much attention not only due to its valuable designing concept, but also due to its simplicity and high efficiency. Thus, the coating agents played an important role of chromatographic stationary phases completely, or partially, in OTCEC separations and exhibited selective interactions with solutes. Macrocyclic molecules were the most important coating agents in this field. For example, a cyclodextrin-coated capillary was used to resolve the enantiomers of binaphthyl derivatives and 1-phenylethanol.[4,5] A permethyl-β-cyclodextrin-modified silica was used in the micropacked CEC to realize efficient enantiomers separation.[6] Macrocyclic polyamine coated capillaries were utilized for the electrophoretic separation of organic anions, inorganic anions, and aromatic organic acids.[7–9] Macrocyclic dioxopolyamine was attached successfully onto the inner surface of capillaries via chemical bonding or the sol-gel technique for separation of phenolic compounds.[10,11] The sol-gel technique was also used to attach 2,6-dibutyl-β-cyclodextrins onto the capillaries inner wall to separate phenolic compounds and enantiomers of ibuprofen and binaphthol.[12]

Calixarenes, cyclic oligomers of *p*-substituted phenol-formaldehyde condensates, has emerged as attractive macrocyclic molecules after crowns and cyclodexrins in the host-guest chemistry.[13] The outstanding recognition ability of the calixarenes as receptors was mainly based on their variable pliability, which allowed a kind of induced fit to the shape of a suitable guest molecule.[13] Until now, many functionalized calixarenes had already been synthesized. Numerous applications of calixarenes had been reported in various fields, such as modifiers of ion-selective electrode,[14] chromatography stationary phases,[15] and mobile phase additives.[16] However, very few papers had been devoted to using calixarene as modifiers for CEC, in comparison with many reports found in fields of gas and liquid chromatography. More recently, we prepared a calix[6]crown coated capillary to separate aromatic compounds successfully.[17] To the best of our knowledge, 1,3-bridged calix[4]arenes were easily synthesized in higher yields than those of 1,2-bridged calix[4]arenes. Meanwhile, most of 1,3-bridged calix[4]-arenes trended to adopt a stable cone conformation, which played an important role in complexing the guest molecules. Therefore, it was reasonable to believe that 1,3-bridged calix[4]arenes had more essential value in chromatographic separation.

We report, here, a new approach to coat 1,3-bridged calix[4]arene, *p-tert*-butylcalix[4]arene-1,3-bis(allyloxyethy)ether (CA[4]-BAE) for open-tubular electrochromatography. Its chromatographic behavior and separation mechanism were also investigated.

EXPERIMENTAL

Apparatus and Reagents

Analyses were performed on the HPCE system (Beijing New Technology Application Institute, China). Fused silica capillaries of 50 μm ID were purchased from Yongnian Optical Fiber Factory (Hebei Province, China). All capillaries had a total length of 50 cm with effective length of 38 cm. Detection was carried out by on-column spectrophotometric measurement at 254 nm. Samples were introduced hydrostatically by elevation of the sample vials to a height of 5 cm 10 s. Doubly de-ionized water (18.2 mΩ) from a Milli-Q system (Millipore, Bedford, MA) was used for preparation of all standard and buffer solutions.

Melting points were recorded on a Gallenkamp melting point apparatus in open capillaries and were uncorrected. ^{1}H NMR spectra were recorded on a Varian Mercury VX300 at ambient temperature. TMS was used as an internal standard for NMR. FAB-MS spectra was obtained from a Kratos MS80RF mass spectrometer, with *m*-nitrobenzyl alcohol as a matrix. Elemental analyses were performed by the Analytical Laboratory of the Department of Chemistry in Wuhan University (Wuhan, China). All solvents were purified by standard procedures. Petroleum ether referred to the fraction with b.p. 60°C~90°C. All other chemicals were analytically pure grade and used without further purification. Vinyltriethoxysilane was obtained from the Chemical Plant of Wuhan University (Wuhan, China). Isomers (*o-, p-, m-*) of toluidines, (α-, β-) of naphthols, biphenyl derivatives, and PAHs were obtained from Beijing Chemical Corp. (Beijing, China). The stock solutions of toluidines, naphthols, biphenyl derivatives, and PAHs were prepared by dissolving them in methanol, pure water or their mixture, and diluted to the desired concentration using the electrophoretic running buffers and passed through a 0.45 μm membrane filter before use. In all experiments, 20 mM aqueous sodium phosphate buffers were used. Electroosmotic mobility velocities of modified and bare fused-silica columns were both determined by acetone, which served as a neutral marker.

Synthesis of *p-tert*-Butylcalix[4]arene-1,3-bis(allyloxyethoxyethy)ether CA[4]-BAE

A suspension of *p-tert*-butylcalix[4]arene (1.30 g, 2.0 mmol), K_2CO_3 (0.41 g, 3.0 mmol), and 2-(allyloxy)ethyltosylate (1.23 g, 4.8 mmol) in dry CH_3CN (60 mL) was refluxed for 24 h. The reaction mixture was cooled, and the solvent was removed under reduced pressure. The solid residue was then dissolved in CH_2Cl_2 and was filtered. The filtrate was evaporated under reduced pressure, and the residue was recrystallized from CH_3CN to give CA[4]-BAE. Yield: 81.3%. m.p. 164–6°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35 and 1.16 (s each, 18H each, Bu^t), 4.04 (4H, t, J = 4.8 Hz, OCH_2), 4.29 (8H, m, OCH_2), 4.50 and 3.41 (8H, d, AB, J = 13.2 Hz, $ArCH_2Ar$), 5.38 (4H, m, $C{=}CH_2$), 6.02 (2H, m, $CH{=}C$), 7.07 (4H, s, ArH), 7.15 (4H, s, ArH), 7.87 (2H, s, ArOH), Anal. Calcd. for $C_{54}H_{72}O_6$: C, 79.37; H,8.88%. Found: C, 79.32; H, 9.02%. FAB-MS: m/e = 817 (MH^+).

Column Preparation

A fused-silica capillary of 50 cm × 50 μm ID was sequentially rinsed with 1 M NaOH for 30 min, pure water for 15 min, 1 M HCl for 15 min, and pure water for 15 min. After being purged with nitrogen for 20 min, the capillary was placed in the oven at 150°C and purged with nitrogen for 4 h.

To the 250 μL toluene solution of 25 mg VTES, 5 μL 5% trifluoroacetic acid (TFA) was added under irradiation of ultrasonic waves. The solution was introduced into the pretreated capillary under 0.6 Mpa nitrogen pressure, then the capillary was placed in an oven at 120°C for 4 h with both ends sealed.

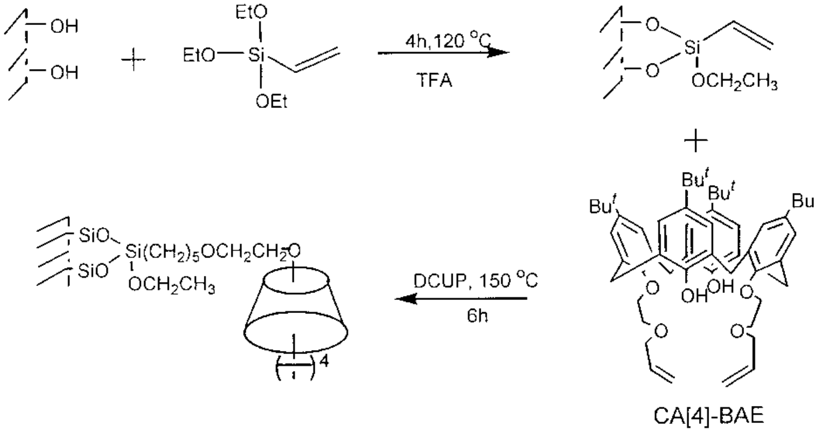
To the 100 μL toluene solution of 5 mg CA[4]-BAE, 100 μg di(*iso*-propylbenzene) peroxide (DCUP) was added under irradiation of ultrasonic waves. The solution was introduced into the above capillary under 0.6 Mpa nitrogen pressure, then the capillary was placed in an oven at 150°C for 6 h with both ends sealed.

Finally, the capillary was flushed with toluene, THF, and methanol, and blown with nitrogen for 30 min. Prior to use, the capillary was washed with methanol and H_2O for 30 min sequentially, and then equilibrated with a running buffer before use. The prepared scheme was shown in Scheme 1.

RESULTS AND DISCUSSION

Characterization

In this fashion of column preparation, the crosslinking reaction time was critical for the successful preparation. Reaction times were studied for 3 h,



Scheme 1. The inner wall modification of the fused-silica capillary.

4.5 h, and 6 h, respectively, and it was found that the stability of the capillary columns increased with the increase of crosslinking reaction time. This could be explained by insufficient crosslinking time leading to incomplete crosslinking reaction and the coated layer with insufficient crosslinking time cracking easily and breaking from the capillary walls when rinsed with the mobile phase.

The coating processes were proven to be successful by IR spectra, SEM characterization, and the result of electroosmotic flow (EOF). For measuring IR spectra, the external coating of polyimide was removed prior to grinding, and a higher ratio of coated capillary to potassium bromide than the conventional one was used for blending.[7–9] The IR data contained features identified on silica materials modified by silanization reaction and bonding process: peaks in the benzene ring stretching region at $1484\sim1428\,\text{cm}^{-1}$, the C-O-C stretching band at 1203, $1042\,\text{cm}^{-1}$, and peak at $868\,\text{cm}^{-1}$ for *tetra*-substituted benzene ring. Neither of three features was observed on an etched capillary without a chemically modified and silanized column. Figure 1 showed the SEM photographs of bare fused-silica capillary and CA[4]-BAE coated capillary, respectively. The smooth surface of the bare capillary (Fig. 1A) became rough after coated by coating with CA[4]-BAE (Fig. 1B). These results confirmed the successful bonding of the CA[4]-BAE moiety to the capillary wall.

EOF Measurement

Here, the dissociation of both residual silanol groups of the inner capillary surface and hydroxyl groups of the stationary phase might contribute to the

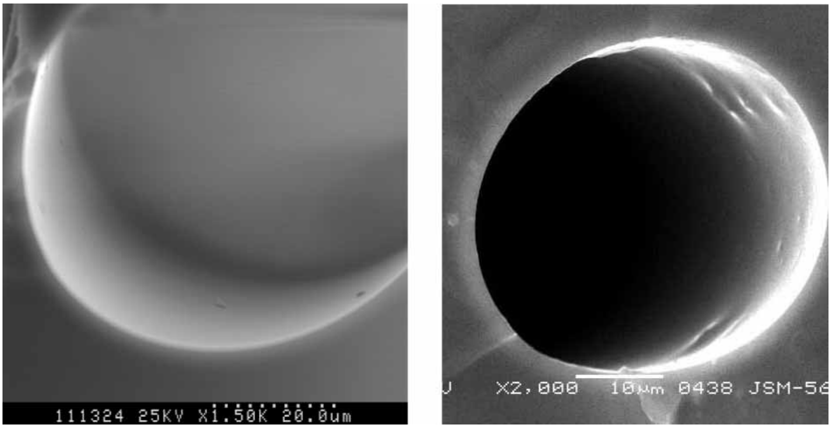


Figure 1. SEMs of the cross-sectional view of (A) uncoated capillary column; (B) a CA[4]-BAE coated capillary column.

electroosmotic flow. The magnitude and direction of EOF reflected the effectiveness of the coating processes and the features of the functionalities. Figure 2 showed a plot of EOF as a function of pH for CA[4]-BAE-coated, as well as bare capillaries using 20 mM phosphate buffer ranging from pH 5 to 9. It was observed that EOF for CA[4]-BAE-coated column had lower EOF than an uncoated one, even lower than a silanized column. Over the entire pH range studied, the magnitude of EOF remained positive, which was an indication of a negatively charged surface and an electroosmotic flow from anode to cathode. The bonded functionalities effectively shielded the silanol groups and greatly reduced EOF. In addition, the slight slope of EOF versus pH for coated columns was favorable for achieving better resolutions and reproducible results.

Separation of Solutes

Separation of Isomeric Toluidines

Figure 3 showed that the toluidine isomers were successfully separated by CA[4]-BAE-coated capillaries. As could be seen from Fig. 3, toluidines were not separated in a bare silica capillary, whereas in a coated one they were well separated; besides, the migration times increased, which made it reasonable to believe that the CA[4]-BAE coating played an important role in separation. Calixarenes had a cavity composed of electron-rich benzene π-systems to easily include organic amines and ammonium cations, especially

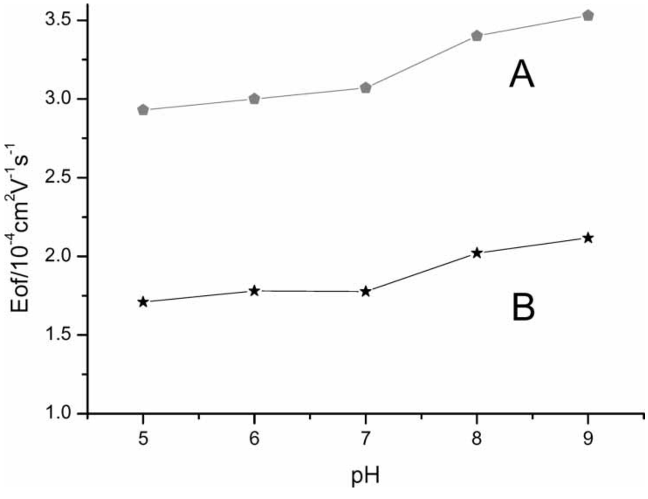


Figure 2. Effect of pH on electroosmotic flow in various capillary materials. Curve A: untreated fused-silica capillary. Curve B: CA[4]-BAE-coated column.

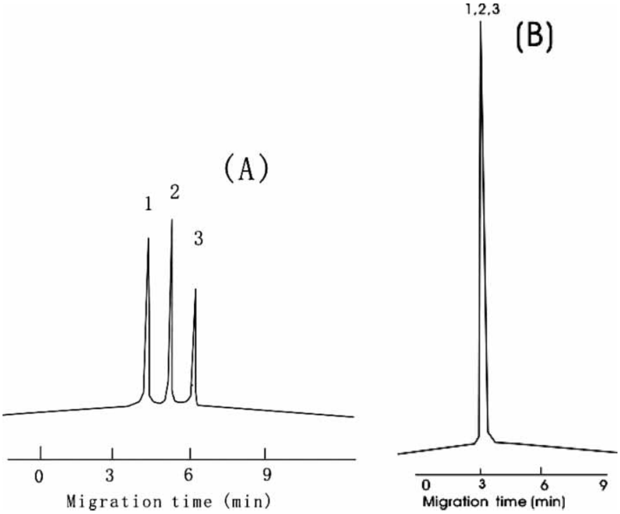


Figure 3. Separation of toluidine isomers in OTCEC and in CZE columns (A) OTCEC, 50 cm (effective length: 38 cm) × 50 μm fused-silica capillary chemically bonded with CA[4]-BAE, (B) CZE, 50 cm (effective length: 38 cm) × 50 μm untreated fused-silica capillary. Mobile phase, 20 mM aqueous sodium phosphate, pH 6.0; Voltage, 20 KV; hydrodynamic injection 10 s, wavelength, 254 nm. Peak identification, 1 = *p*-toluidine, 2 = *m*-toluidine, 3 = *o*-toluidine.

for aromatic amines.[18] Crown ether was also known to include organic amines and primary amine salts, due to a hydrogen-bonding interaction between the analyte and the macrocyclic polyether.[19] Because 1,3-bridged CA[4]-BAE adopted a stable cone conformation, and it combined the advantage of calixarene and open-ring crown ether moieties, it exhibited a superior recognition ability toward selected analytes, which greatly facilitates separation. A similar phenomenon was observed by calix[6]crown-coated capillaries.[17]

Separation of Isomeric Naphthols

Figure 4 shows that the naphthols isomers were successfully separated by CA[4]-BAE-coated capillaries. Naphthol isomers were not separated in a bare silica capillary, although the pKa of α-naphthol and β-naphthol were 9.30 and 9.57, respectively. It was found that the pH of the buffer had little effect on their separation in the CZE mode, even when using the pH 9.0 of the running buffer. It was noted that CA[4]-BAE-coated capillary columns could easily obtain a baseline separation of naphthol isomers. The broadened bands, as well as the effect of decreased efficiency of mass transfer, showed a strong solute-bonded phase interaction. A similar phenomenon was observed for the calixarene-based stationary phase of

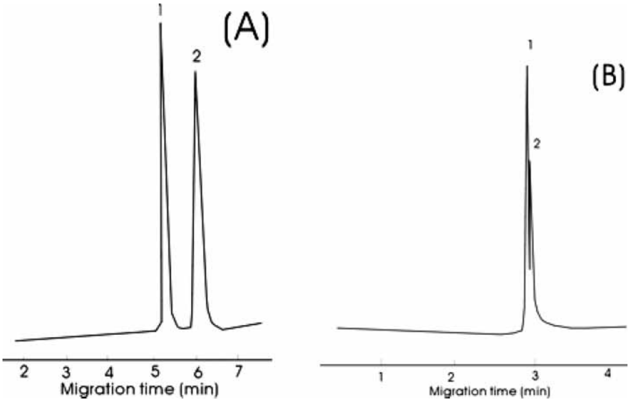


Figure 4. Electrophorograms of the separation of the naphthols in OTCEC coated with CA[4]-BAE and in CZE. (A) OTCEC, 50 cm (effective length: 38 cm) × 50 μm fused-silica capillary coated with CA[4]-BAE (B)CZE, 50 cm (effective length: 38 cm) × 50 μm bare capillary. Mobile phase: 20 mM aqueous sodium phosphate, pH: 6.0; voltage: 20 Kv, hydrodynamic injection: 10 s. wavelength: 254 nm. Peaks: 1: β-naphthol; 2: α-naphthol.

HPLC.[20,21] This can be attributed to a synergistic effect of the π-π interaction and a hydrogen-bonding interaction. Compared with β-napthol, α-naphthol can be easily ionized into more α-napthoxylic anions, which are better electron-donors with a high electron density and hydrogen acceptors. Thus, the joint effect of the stronger π-π interaction with CA[4]-BAE, and the formation of hydrogen bonds with an open ring-crown ether structure and the residual phenolic hydroxyl groups of CA[4]-BAE, determined that α-naphthol was finally eluted.

Separation of Biphenyl Derivatives

The resulting electrochromatograms for the determination of biphenyl derivatives were shown in Figure 5. Coelution of the three biphenyl derivatives was observed (Fig. 5B) due to their similar electrophoretic mobilities. Complete resolution of biphenyl derivatives was obtained on the CA[4]-BAE-coated column. The above results showed that the chromatographic retention mechanism played an important role in the separation, as was evident in the successful bonding of the stationary phases onto the capillary wall.

It was known that biphenyl derivatives were undissolved in water due to their strong hydrophobicity, although the substituted groups introduced such as carboxyl, amino, and chloro had changed their polarity. Thus, methanol was added into the mobile phase to enhance their solubility. The plots of migration times of biphenyl derivatives versus methanol content in the

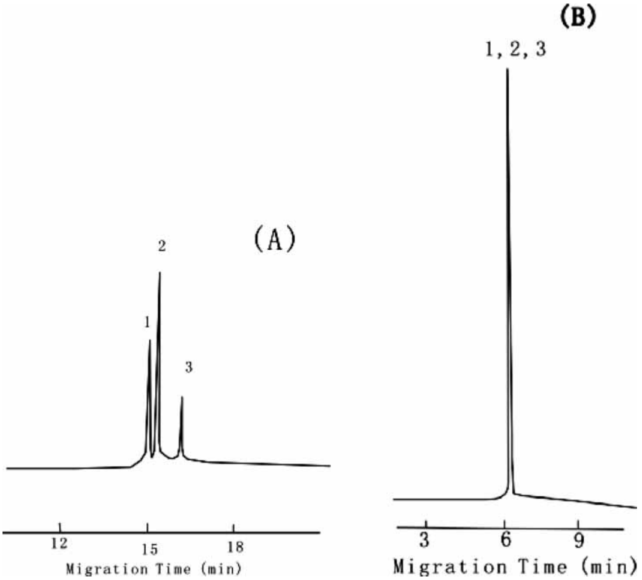


Figure 5. Electrophorograms of the separation of biphenyl derivatives in OTCEC coated with CA[4]-BAE and in CZE. (A) OTCEC, 50 cm (effective length: 38cm) × 50 μm fused-silica capillary coated with CA[4]-BAE (B) CZE, 50 cm (effective length: 38 cm) × 50 μm bare capillary. Mobile phase, 20 mM aqueous sodium phosphate containing 40% methanol, pH8.0. voltage: 20 Kv, hydrodynamic injection: 10 s. wavelength: 254 nm. Peak identification, 1 = 4,4′-diamidobiphenyl; 2 = 4-amido-4’-chlorobiphenyl; 3 = 4-carboxybiphenyl.

mobile phase were shown in Fig. 5. As could be seen from Fig. 5, the addition of an appropriate amount of methanol (40% v/v) into the buffer apparently improved the separation of biphenyl derivatives containing carboxyl and amino groups, which indicated that CA[4]-BAE coating played an important role in this separation. As *p-tert*-butylcalix[4]arene-1,3-bis(allyloxyethy)ether possessed well pre-organized structures and more rigid binding sites in comparison with calixarenes and crown ethers, they exhibited superior recognition ability toward various organic molecules by the cooperation effect of calixarene and the additional polyether moieties, which greatly facilitated the separation.

Separation of PAHs

Figure 6 illustrated the separation of PAHs. The mixture could be clearly resolved into five peaks, whereas when all were coeluted on a bare column, the migration times increased for all of the components. The synthesized new stationary phase showed excellent separation abilities for PAHs.

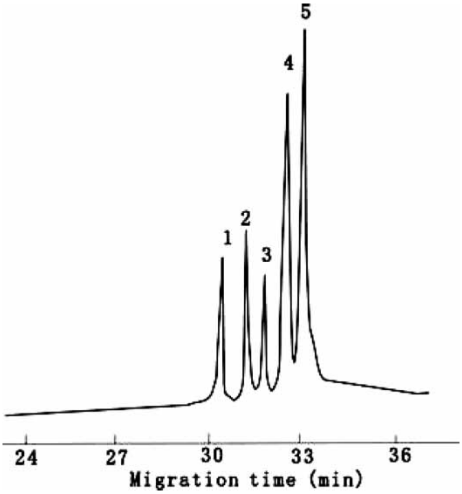


Figure 6. Separation of PAHs in OTCEC coated with CA[4]-BAE. OTCEC, 50 cm (effective length: 38 cm) × 50 μm fused-silica capillary coated with CA[4]-BAE. Mobile phase: 20 mM aqueous sodium phosphate containing 40% methanol, pH 8.0 voltage: 20 Kv, hydrodynamic injection: 10 s. wavelength: 254 nm. Peak identification, 1 = naphthalene; 2 = biphenyl; 3 = fluorene; 4 = phenanthrene; 5 = anthracene.

Studies of the retention behaviors of PAHs showed a predominantly reversed-phase character of this new stationary phase. It was interesting to note that the elution sequence was not only dependent on the hydrophobic interaction; the π-π interaction and the inclusion interaction between the analytes and the stationary phase might also play an important role in separation. The existence of an inclusion interaction between PAHs with calix[4]arene was also reported by Leyton et al.[22] Nevertheless, the inclusion property of CA[4]-BAE had to be further verified.

Column Evaluation

In the following experiments, the column performance of CA[4]-BAE-modified columns using 20 mM phosphate buffer was evaluated with different analytes. As could be seen from Table 1, the column efficiencies (N) were in the range from 7.21×10^4 to 1.362×10^5 plates/m. The relative standard deviation (RSD %) of retention times were less than 4.07% for within-column in five consecutive runs. Limit of detection (LOD), precision (RSD), linear range, and correlation coefficients (R) were included in Table 2. For all the analytes, the LODs were from 1.3 mg·mL^{-1} to 5.0 mg·mL^{-1} and correlation coefficients were more than 0.9990. With

Table 1. Repeatability of migration time and separation efficiency of solutes for OTCEC modified by CA[4]-BAE[a]

Solute ($\times 10^4$plates/m)	Sepn effic, N (min)	Av t_R ($n = 5$)	RSD_t(%)
4-Carboxybiphenyl	7.21	16.20	3.72
Naphthalene	13.62	30.85	3.14
Biphenyl	11.98	31.82	2.55
Fluorene	7.88	32.47	4.07

[a] Experimental conditions are the same as shown in Fig. 3.

o-toluidine as the analyte, LOD calculated at signal to noise ratio of 3 was $1.0\,mg{\cdot}L^{-1}$ for the uncoated capillary and $2.0\,mg \cdot L^{-1}$ for the CA[4]-BAE coated capillary, respectively.

CONCLUSION

Calixarenes have received increasing attention in supramolecular chemistry. Due to introducing additional polyether moieties, *p-tert*-butylcalix[4]arene-1,3-bis(allyloxyethy)ether possessed well preorganized structures and more rigid binding sites in comparison with calixarenes and polyethers, which make them have great potential in analytical chemistry. Coated capillaries with CA[4]-BAE could be prepared *via* chemical bonding. Successful CA[4]-BAE coating was proven by IR spectra, SEM characterization, and low electroosmotic flow. Compared with a bare silica capillary, such a CA[4]-BAE-coated capillary could improve the separation of

Table 2. Limit of detection(LOD), Precision(RSD), Linear ranges, and correlation coefficients for the analysis of different solutes for OTCEC modified by CA[4]-BAE[a]

Solute	LOD ($mg{\cdot}L^{-1}$)	RSD (%) ($n = 5$)	Linear Range ($mg{\cdot}mL^{-1}$)	R
p-Toluidine	4.7	7.9	0.001–1.0	0.9997
o-Toluidine	2.0	3.4	0.001–1.0	0.9995
m-Toluidine	3.2	5.9	0.0005–1.0	0.9998
β-Naphthnol	2.9	3.1	0.05–2.0	0.9998
Naphthalene	2.3	2.9	0.01–2.0	0.9997
Biphenyl	1.9	4.2	0.03–2.0	0.9991
Fluorine	1.5	4.1	0.05–1.0	0.9994

[a] Experimental conditions are the same as shown in Fig. 3.

[b] LODs were estimated on the basis of 3 : 1 signal to noise ratios.

isomeric toluidines, isomeric naphthols, biphenyl derivatives, and PAHs, which showed that CA[4]-BAE coating played an important role in introducing additional selectivity towards solutes. The preparation and application of more calixarenes derivatives coated capillaries are underway in our group.

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Received March 31, 2005
Accepted June 21, 2005
Manuscript 6622