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Synthesis of *p-tert*-Butylcalix[4]arene Dinitrile Bonded Aminopropyl Silica and Investigating Its Usability as a Stationary Phase in HPLC

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The chromatic separation of adenine, adenosine, cytosine, phenol, benzene, and toluene were investigated by using 5,11,17,23-tert-butyl-25,27-bis(cyanomethoxy)-26-(chloroformyl)-28-hydroxy-calix[4]arene bonded aminopropyl silica (CDBAPS) as a stationary phase. The separation ability of the stationary phase was observed to be good for target species. The effect of non-polar calix[n]arene network was observed in chromatographic processes, and it was thought that, thanks to the relatively polar nitrile groups, further selectivity would be obtained in various chromatographic separations.

Keywords calixarene, immobilization, aminopropyl silica, HPLC, nucleo bases, nucleosides, aromatic compounds

Introduction

Separation and qualitative/quantitative determination of nucleo bases and nucleosides, and aromatic compounds are of great importance in many research areas. Among the analytical and instrumental methods, which can be used for this aim, HPLC is one of the simple, useful, and effective ones. The efficiency of HPLC depends on the efficiency of the stationary phase, on a large scale. There are many studies focused on the investigation of novel stationary phases for reverse phase, normal phase, and ion-exchange liquid chromatography, as obtaining more effective, and less time-consuming chromatographic operations. These endeavors are generally hindered by inappropriate chemical and physical properties of solids, which will be used as a stationary phase. Therefore, the solids possessing good mechanic strength, controllable particle size, excellent chromatographic characteristics, and are favorable in cost, are always desirable (1–12).

The calixarenes, third generation host molecules after crown ethers and cyclodextrins, possess cavity-shaped cyclic molecules prepared by the ring-closing condensation of *p-tert*-butyl phenol and formaldehyde under alkaline conditions, and exist in a "cup" like shape with a defined upper and lower rim. By functionally modifying either the upper and/or lower rims, it is possible to prepare various derivatives with differing

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selectivity for various guest ions and small molecules. Considering their unique cavity architecture and high thermo stability, it was expected that the calixarenes and their derivatives might play an important role in analytical chemistry (13–27).

Through the careful immobilization of calixarene derivatives onto an appropriate solid support, such as silica derivatives, effective stationary phases, which exhibit low back-pressure and good mechanic strength in the column arrangement, can be obtained (1). Column packing material is always a key factor in the development of HPLC methods. In 1993, Glennon et al. (1, 2) prepared silica-bonded calix[4]arene tetraester and silica-bonded calix[4]arene tetradiethylamide stationary phases to separate metal ions and amino esters for the first time. Lee et al. (3) reported the separation of some substituted phenol regio-isomers and some other aromatic positional isomers on a calix[6]arene-*p*-sulfonato bonded silica stationary phase. The substituted aromatics, nucleosides, urasil derivatives, estradiol epimers, and cis/trans isomers of proline-containing peptides on calix[n]arene-bonded silica gel were successfully separated by Gebauer et al. (4–6). Menyes et al. (7) reported that a hexapropylether of *p-tert*-butylcalix[6]arene covalently linked to silica, was used for the separation of polycyclic aromatic hydrocarbons and fullerenes, and showed higher selectivity and lower consumption of solvent than conventional RP-C_{18}. Healy et al. (8) prepared an L(−)-ephedrinyl-calix[4]arene-bonded phase and used it for the separation of R(−) and S(+)-phenyl-2,2,2-trifluorethanol. In the past few years, Li et al., prepared *p-tert*-butyl-calix[6]arene-bonded silica gel stationary phase and *p-tert*-butyl-calix[n]arene-bonded (n = 4, 6, 8) silica gel stationary phases with different coupling reagents in one-pot method (10, 11) and investigated the chromatographic separation of some positional isomers, polycyclic aromatic hydrocarbons, nucleosides, and sulfonamide drugs (12). The results show that calixarene-bonded stationary phases are excellent reverse-phase packing with inclusion capability.

The present paper includes chromatographic separation of adenine, adenosine, cytosine, phenol, toluene, and benzene by using *p-tert*-butylcalix[4]arene dinitrile bonded aminopropyl silica (CDBAPS) as a stationary phase.

Experimental

Materials

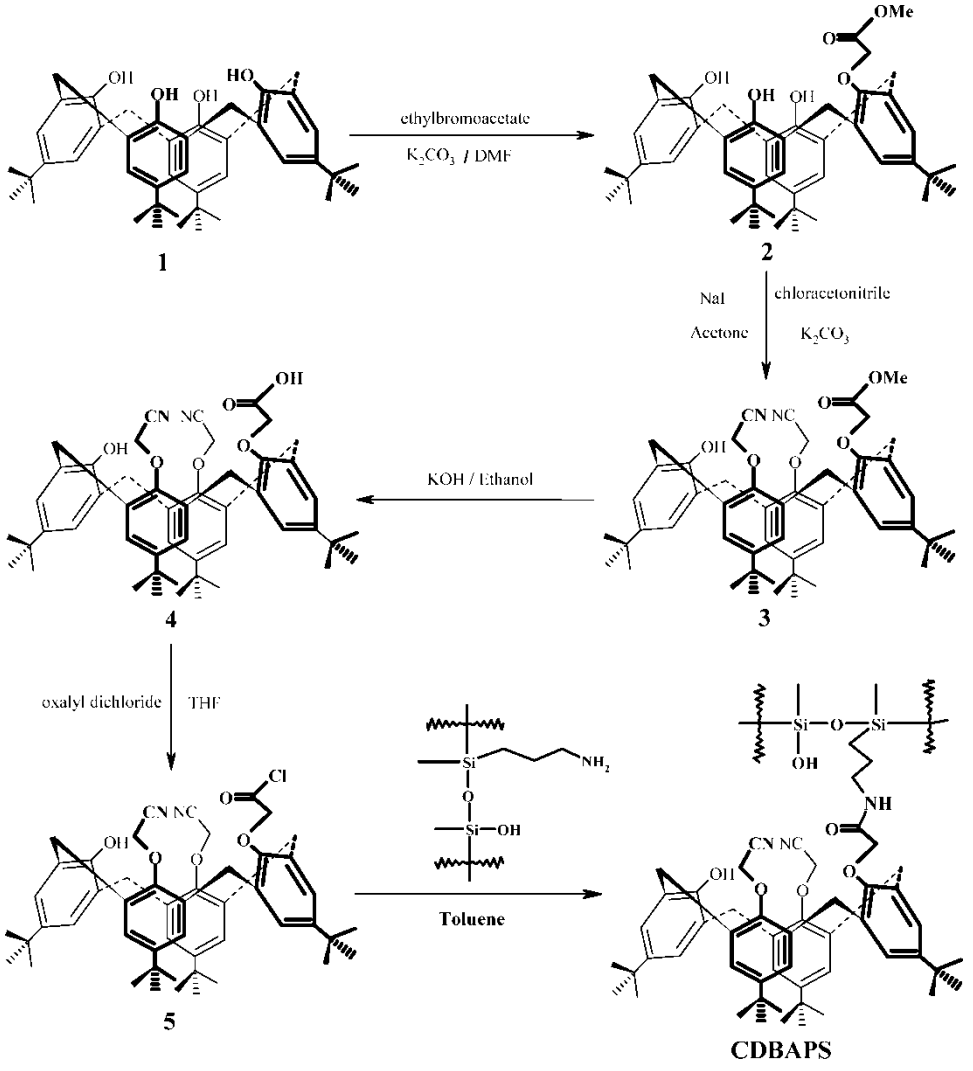
High-purity aminopropyl silica (APS) was purchased from Aldrich (No: 364258) and was used as support material for immobilization of CDBAPS. Analytical TLC was performed on pre-coated silica gel plates (SiO_2, Merck PF_{254}), while silica gel 60 (Merck, particle size 0.040–0.063 mm, 230–240 mesh) was used for preparative column chromatography. Generally, solvents were dried by storing them over molecular sieves (Aldrich; 4 Å, 8–12 Mesh). Acetone, CH_2Cl_2, and MeOH was distilled from $CaSO_4$, $CaCl_2$, and over Mg, respectively. Tetrahydrofuran (BDH) and toluene was dried by refluxing over sodium/benzophenone and CaH_2, respectively, fractionally distilled, and then stored over molecular sieves. All the chemicals were purchased from Merck or Aldrich. All aqueous solutions were prepared with deionized water that had been passed through a Millipore Milli-Q Plus water purification system.

Adenine, adenosine, and cytosine were purchased from Sigma or Merck and used as received. Phenol, toluene, and benzene were obtained from Merck. Solutions of adenine, adenosine, and cytosine, phenol, toluene, and benzene were prepared with HPLC grade methanol (Lab-Scan), and filtered through 0.45 μm Nylon filter. A phosphate buffer (0.05 mol L^{-1}, pH 5.00) was prepared from KH_2PO_4 (Merck) with ultra high quality

pure water, and filtered through a 0.45 μm Nylon filter before use. The acetonitrile was HPLC grade and obtained from Riedel.

Instrumentation

IR spectra were recorded on a Perkin-Elmer 1605 FTIR spectrometer as KBr pellets. Agilent 1100 series HPLC system was used in order to conduct chromatographic operations. HPLC system consisted of a G1311A model quaternary pump, a G1314A model Variable Wavelength UV-VIS detector, a 7725i model Rheodyne manual injector system with 20 μL loop, a G1316A model thermostatted column compartment, a G1379A model degasser, and a Chemstation 2001 data processor.



Scheme 1. The schematic representation of synthesis and immobilization of 5,11,17,23-*tert*-butyl-25,27-bis(cyanomethoxy)-26-(chloroformyl)-28-hydroxy-calix[4]arene.

Synthesis

The *p-tert*-butylcalix[4]arene dinitrile bonded aminopropyl silica (CDBAPS) was synthesized according to Scheme 1 the first time. The other compounds abbreviated as **1–5** were prepared according to the reported procedures (28–30).

Immobilization of 5,11,17,23-tert-butyl-25,27-bis(cyanomethoxy)-26-(chloroformyl)-28-hydroxy-calix[4]arene onto APS (CDBAPS)

Compound **5** (0.51 g; 0.63 mmol), APS (1.5 g), and dry toluene (25 mL) were added to a 50 mL round-bottomed flask equipped with a magnetic stirrer, and stirred at room temperature for 5 h under nitrogen atmosphere and then refluxed for 5 h. The cooled mixture was filtered and washed in sequence three times with warm toluene, acetone, methanol, and distilled water. The product was dried at 120°C to give 1.5 g of CDBAPS, under vacuum, for 3 h and kept in a desiccator before use.

Chromatographic Procedure

CDBAPS was packed into a stainless-steel column (180 × 4.6 mm I.D.) by using a slurry technique, and the column was left with 100% methanol in order to avoid the stationary phase drying. Methanol, acetonitrile, phosphate buffer (0.05 mol L^{-1}, pH 5.00), and ultra high quality pure water were used as mobile phases and separation of compounds were realized by gradient elution. All samples were prepared with HPLC grade methanol and filtered through a 0.45 μm Nylon filter before injection. The detection wavelength was 254 nm. The concentration of samples was $1 \cdot 10^{-3}$ mol L^{-1}, and generally 10 μL of sample solutions were injected. All chromatographic operations were carried out at a definite temperature (30 ± 0.5°C), and repeated at least twice. Between the subsequent chromatographic operations, before all injections, the stationary phase was conditioned by loading 100% methanol or acetonitrile for 20 min.

Results and Discussion

Characterization of CDBAPS

CDBAPS was characterized by elemental analysis and FTIR. According to the carbon, hydrogen, and nitrogen content shown in Table 1, it was concluded that the bonded calixarene amount onto APS was found to be approximately 0.748 mmol g^{-1}. Also, from the FTIR results shown in Fig. 1, it was observed that compound **5** immobilized onto APS,

Table 1
Elemental analysis results of CDBAPS[a]

C (%)	H (%)	N (%)	Bonded-amount (mmol g^{-1})
47.0	5.2	3.1	0.748

[a]Calculated according to the carbon, hydrogen, and nitrogen content.

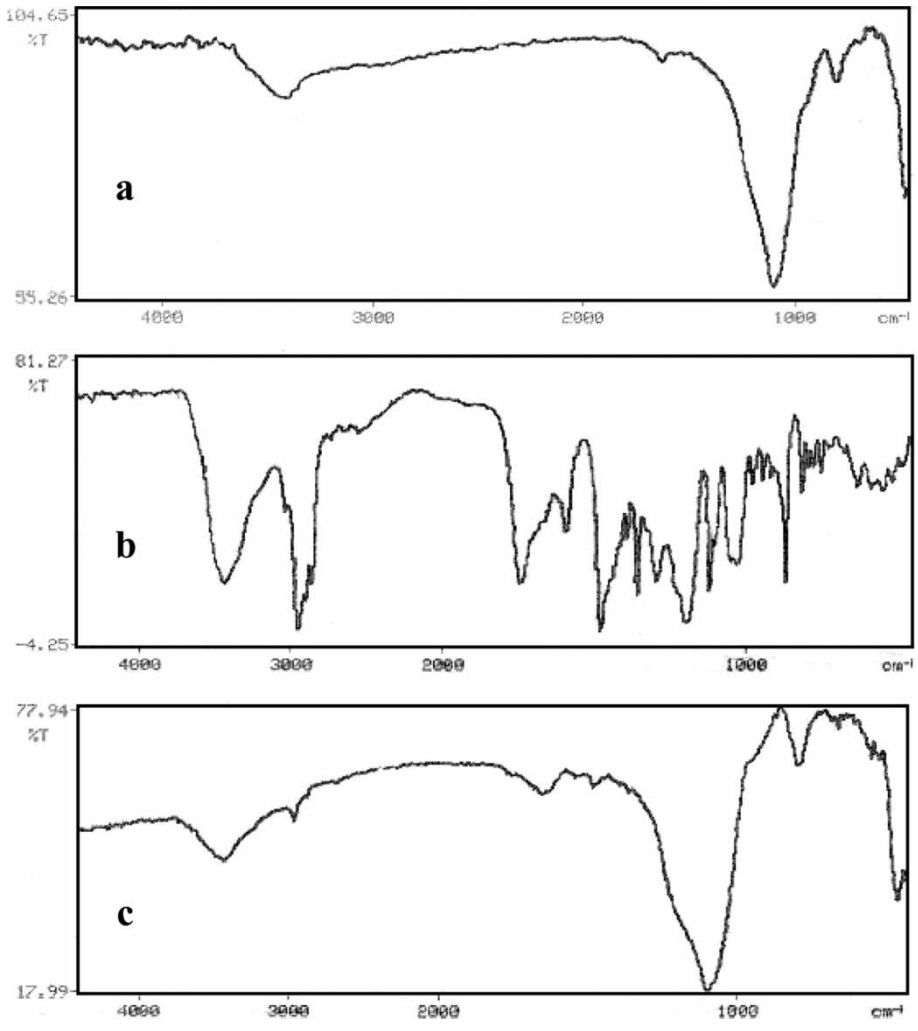


Figure 1. FTIR spectrums of APS (a), *p-tert*-butyl-calix[4]arene dinitrile derivative **4** (b), and CDBAPS (c).

due to 3426 cm^{-1}, 1647 cm^{-1}, and 799 cm^{-1} bands in IR spectra corresponded to O–H, NH–C═O and Si–O, respectively.

The Column Efficiency

The column efficiency of the novel stationary phase (CDBAPS) was determined as follows: The cytosine was selected as a probe solute and some chromatographic operations were performed, while the mobile phase consisted of 99% phosphate buffer (0.05 mol L^{-1}, pH 5.00) and 1% methanol and flow rate was 0.5 mL min^{-1}. The temperature of the column compartment was fixed at $30 \pm 0.5^{\circ}C$, and 5 μL of cytosine solution was injected in all repeated chromatographic processes ($n > 10$). The retention time of cytosine on the stationary phase was 1.54 ± 0.1 min ($n > 10$). At these chromatographic

conditions, the mean theoretical plate number was calculated as 1035 per meter. CDBAPS was found to be effective in separation and repeatable in chromatographic operations.

Because of its hydrophobic character, the calix[4]arene network can collapse when it interacts with water molecules, and this can cause the following:

Poor resolution
Increment in back pressure
Unrepeatable flow rates
Unrepeatable chromatographic analysis.

Therefore, it is recommended that the methanol (or acetonitrile) content of the mobile phase should be at least 1%.

The Separation of Aromatic Compounds

The separation of phenol, toluene, and benzene was reached by gradient elution as follows: Until the third minute, the mobile phase consisted of 1% methanol and 99% water, and then the methanol content of the mobile phase was gradually increased to 50% until the 20th min. The chromatogram is given in Fig. 2.

It was found that there was a relationship between the elution order of the three aromatic compounds and their polarity. This result indicates that the hydrophobic interaction was a significant factor in the separation of these compounds. Besides the hydrophobic interactions, π-π interactions between the aromatic moieties of calix[4]arene and aromatic compounds, and interactions between the nitrile groups of calix[4]arene and relatively polar compounds can be considered, and these interactions can support additional selectivity. However, the hydrophobic interactions between the calix[4]arene network and aromatic compounds were thought to be the main effective interactions.

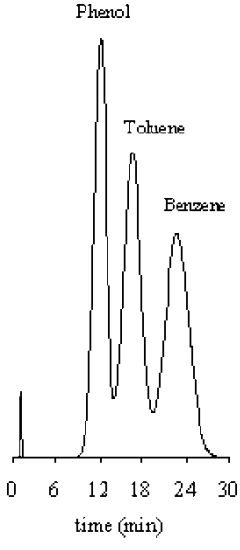


Figure 2. Chromatogram of aromatic compounds on CDBAPS.

Table 2
Retention factors of phenol, toluene, and benzene

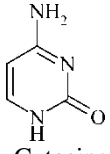
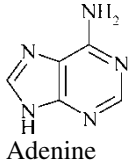
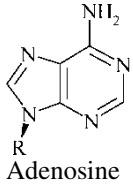
Compounds	Retention capacities (k)	Resolution (Rs)
Phenol	8.51	0.84 (Phenol-Toluene)
Toluene	12.05	0.87 (Toluene-Benzene)
Benzene	16.86	

The retention factors of phenol, toluene, and benzene are given in Table 2. It was found that the elution times of the compounds decreased with increasing the methanol content in the mobile phase.

Separation of Adenine, Adenosine, and Cytosine

The chemical structures and dissociation constants of cytosine, adenine, and adenosine are given in Table 3. Separation of these compounds was attained by gradient elution as follows: Until the third minute, the mobile phase consisted of 1% acetonitrile and 99% phosphate buffer, and then until the 5th minute, the percentage of acetonitrile in the mobile phase was gradually increased to 10%. The chromatogram is given in Fig. 3.

Table 3
The chemical structures and dissociation constants of cytosine, adenine, and adenosine

Compounds	pKa1	pKa2
 Cytosine	4.15 (+1)[a]	12.2
 Adenine	4.15 (+1)[a]	9.8
 Adenosine	3.5 (+1)[a]	12.5

[a](+1): i.e., $-NH^+$.

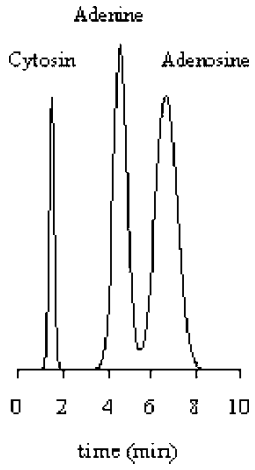


Figure 3. Chromatogram of cytosine, adenine, and adenosine on CDBAPS.

From the results of various experiments, it was concluded that with increasing the acetonitrile content of the mobile phase, the retention capacities of three compounds were decreased. However, when the acetonitrile content of the mobile phase was relatively high, the resolution was lost.

The retention factors of adenine, adenosine, and cytosine are given in Table 4. As can be seen in the table, the retention factor of cytosine is the lowest, and this situation can be attributed to weak hydrophobic interactions between this compound and CDBAPS.

In the chromatographic separation of purines, pyrimidines, and nucleosides, the pH of the mobile phase is another important parameter, because of protonation and deprotonation of these compounds. According to the reported results, nucleo bases and nucleosides can be successfully separated on ODS at pH 3.5 and pH 4.8–5.2, respectively (31). In the present study, the separation of adenine, adenosine, and cytosine was achieved, while the mobile phase consisted of a phosphate buffer (0.05 mol L^{-1}, pH 5.00) and acetonitrile.

Separation of Cytosine, Adenine, and Phenol

Separation of cytosine, adenine, and phenol was achieved by gradient elution as follows: Elution was started while the mobile phase consisted of 1% methanol and 99% phosphate buffer, and methanol content of the mobile phase was gradually increased to 10% until the

Table 4
The retention factors of cytosine, adenine, and adenosine

Compounds	Retention capacities (k)	Resolution (Rs)
Cytosine	0.20	2.20 (Cytosine-Adenine)
Adenine	2.63	0.88 (Adenine-Adenosine)
Adenosine	4.25	

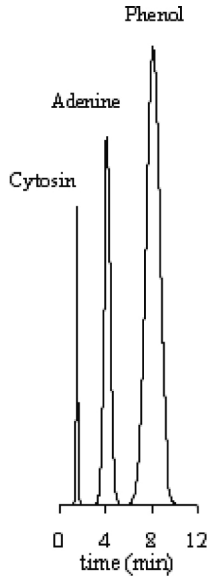


Figure 4. The chromatogram of cytosine, adenine, and phenol on CDBAPS.

Table 5
The retention factors of cytosine, adenine, and phenol

Compounds	Retention capacities (k)	Resolution (Rs)
Cytosine	0.19	1.89 (Cytosine-Adenine)
Adenine	2.30	1.32 (Adenine-Phenol)
Phenol	5.36	

third miute, and then the percentage of the methanol in the mobile phase was gradually increased to 50% until the 10th minute. The chromatogram is given in Fig. 4.

The separation of cytosine, adenine, and phenol was achieved in a short analysis time ($\sim$10 min). Therefore, CDBAPS was thought to be effective for routine analyses of such compounds. The retention factors for compounds are given in Table 5.

Conclusions

Chromatographic separation of cytosine, adenine, adenosine, phenol, toluene, and benzene was investigated by using CDBAPS as a stationary phase. The novel stationary phase was thought to be effective for routine analysis of targeted compounds. It was observed that the hydrophobic interactions were the main effective interactions, which caused the separation. Besides the hydrophobic interactions, the dipole-dipole, hydrogen bonds and π-π interactions were thought to be effective in the separation. These additional interactions can give further selectivity and efficiency to the novel stationary phase in comparison to ODS.

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References

1. Glennon, J.D., O'Connor, K., Srijaranal, S., Manley, K., Harris, S.J., and McKervey, M.A. (1993) *Anal. Lett.*, 26: 153–162.
2. Glennon, J.D., Horne, E., O'Connor, K., Kearney, G., Harris, S.J., and McKervey, M.A. (1994) *Anal. Proc.*, 31: 33–35.
3. Lee, Y.K., Ryu, Y.K., Ryu, J.W., Kim, B.E., and Park, J.H. (1997) *Chromatogr.*, 46 (9–10): 507–510.
4. Friebe, S., Gebauer, S., Krauss, G.J., Goermar, G., and Krueger, J. (1995) *J. Chromatogr. Sci.*, 33 (6): 281–284.
5. Gebauer, S., Friebe, S., Gubitz, G., and Krauss, G.J. (1998) *J. Chromatogr. Sci.*, 36 (8): 383–387.
6. Gebauer, S., Friebe, S., Scherer, C., Gubitz, G., and Krauss, G.J. (1998) *J. Chromatogr. Sci.*, 36 (8): 388–394.
7. Menyes, U., Roth, U., and Troeltzsch, C. (1997) *Eur. Pat. Appl.*, 786: 661A2.
8. Healy, L.O., Mcenery, M.M., McCarthy, D.G., Harris, S.J., and Glennon, J.D. (1998) *Anal. Lett.*, 31 (9): 1543–1551.
9. Li, L.S., Da, S.L., Feng, Y.Q., and Liu, M. (2004) *Talanta*, 64: 373–379.
10. Xiao, X.Z., Feng, Y.Q., Da, S.L., and Zhang, Y. (1999) *Chromatogr.*, 49 (11–12): 643–648.
11. Xu, W., Li, J.S., Feng, Y.Q., Da, S.L., Chen, Y.Y., and Xiao, X.Z. (1998) *Chromatogr.*, 48 (3–4): 245–250.
12. Xiao, Y.X., Xiao, X.Z., Feng, Y.Q., Wang, Z.H., and Da, S.L. (2001) *J. Liq. Chromatogr. Rel. Technol.*, 24 (19): 2925–2942.
13. Gutsche, C.D. (1998) Calixarenes revisited. Stoddart, J.F., ed.; The Royal Society of Chemistry: Cambridge.
14. Asfari, Z., Bohmer, V., Harrowfield, J., and Vicens, J. (2001) Calixarenes 2001. Kluwer Academic Press: Dordrecht.
15. Ikeda, A. and Shinkai, S. (1997) *Chem. Rev.*, 97: 1713–1734.
16. McMahon, G., O'Malley, S., Nolan, K., and Diamond, D. (2003) Important calixarene derivatives—their synthesis and applications, ARKIVOC (VII) : 23–31.
17. Mandolini, L. and Ungaro, R. (2000) Calixarenes in Action. Imperial College Press: London.
18. Memon, S., Tabakci, M., Roundhill, D.M., and Yilmaz, M. (2005) *Polymer*, 46 (5): 1553–1560.
19. Tabakci, M., Memon, S., Sap, B., Roundhill, D.M., and Yilmaz, M. (2004) *J. Macromol. Sci.-Pure Appl. Chem.*, 41 (7): 811–825.
20. Ediz, O., Tabakci, M., Memon, S., Yilmaz, M., and Roundhill, D.M. (2004) *Supramol. Chem.*, 16 (3): 199–204.
21. Tabakci, M., Memon, S., Yilmaz, M., and Roundhill, D.M. (2004) *React. Funct. Polym.*, 58 (1): 27–34.
22. Tabakci, M., Memon, S., Yilmaz, M., and Roundhill, D.M. (2004) *J. Polym. Sci.; Part A-Polym. Chem.*, 42 (1): 186–193.
23. Tabakci, M., Memon, S., Yilmaz, M., and Roundhill, D.M. (2003) *J. Incl. Phenom. Macrocycl. Chem.*, 45 (3–4): 265–270.
24. Yilmaz, A., Memon, S., and Yilmaz, M. (2002) *Tetrahedron*, 58: 7735–7740.
25. Demirel, A., Dogan, A., Canel, E., Memon, S., Yilmaz, M., and Kilic, E. (2004) *Talanta*, 62: 123–129.
26. Alpoguz, H.K., Memon, S., Ersoz, M., and Yilmaz, M. (2004) *Sep. Sci. Technol.*, 39 (4): 799–810.

27. Memon, S., Akceylan, E., Sap, B., Tabakci, M., Roundhill, D.M., and Yilmaz, M. (2003) *J. Polym. Environ.*, 11: 67–74.
28. Gutsche, C.D., Iqbal, M., and Stewart, D. (1986) *J. Org. Chem.*, 51 (5): 742–745.
29. Cheriaa, N., Abidi, R., and Vicens, J. (2004) *Tetrahedron Lett.*, 45 (41): 7795–7799.
30. Tabakci, M., Ersoz, M., and Yilmaz, M. (2006) *J. Macromol. Sci.-Pure Appl. Chem.*, 43: 1 (accepted).
31. Galushko, S.V. and Shishkina, I.P. (1988) *J. Chromatogr. A*, 445: 59–70.