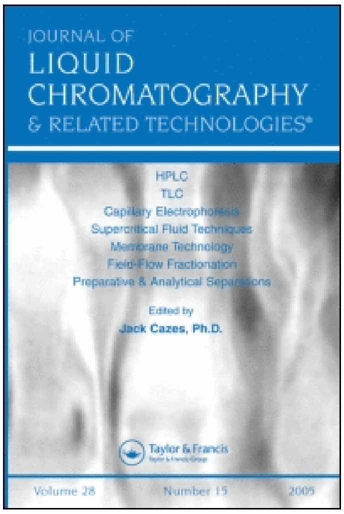

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Shape Selectivity of Polycyclic Aromatic Hydrocarbons and Fullerenes with Tri-*Tert*-Butylphenoxy Bonded Silica Phase in Microcolumn Liquid Chromatography

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SHAPE SELECTIVITY OF POLYCYCLIC AROMATIC HYDROCARBONS AND FULLERENES WITH TRI-TERT-BUTYLPHENOXY BONDED SILICA PHASE IN MICROCOLUMN LIQUID CHROMATOGRAPHY

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ABSTRACT

Retention behaviors of various planar and non-planar polycyclic aromatic hydrocarbons (PAHs), and all carbon compounds so-called fullerenes with 2,4,6-(tri-tert-butylphenoxy)dimethyl (TBP) silica phase and benzyldimethyl (Benzyl) silica phase have been investigated using microcolumn liquid chromatography system. The results clearly indicate that these phases possess a "non-planarity" recognition capability of PAHs. Non-planar solutes were retained longer than planar ones with these phases under certain separation conditions, although the opposite trend where the planar solutes are retained longer than the non-planar ones was observed with typical octadecylsilica (ODS) phases. This unique behavior can be explained by the specific molecular-molecular interaction between non-planar solutes and the stationary phase moiety having a cavity like structure made by the three tert-butyls and phenyl ring of the moiety. The separation of C_{60} and C_{70} were also examined and the difference of the structural uniqueness between TBP phase and Benzyl phase showed interesting results for such bulky fullerenes separation.

INTRODUCTION

The analysis of PAHs based on molecular shape and planarity is very important because the molecular shape and planarity are directly related to its physicochemical properties and biological activities.[1,2] Therefore, it is important to separate PAHs based on the small difference of their molecular shapes and to accomplish this target the separation mechanism should be studied in detail.

As the most powerful method of separating PAHs reverse phase liquid chromatography (RPLC) has been known. The chemically bonded octadecyl silica (ODS) phases have been used as the stationary phase for the separation of PAHs. Generally, ODS phases can be devided into two types; i.e. polymeric and monomeric phases. Polymeric ODS phases have a higher molecular planarity recognition capability than monomeric ones. Non-planar molecules are eluted faster than planar ones with polymeric phases because these phases have a "slot-like" structure on the silica surface as proposed by Wise et al.[3-9] The development of new stationary phases which can accomplish more selective and unique separation has been necessary, because of difficulty to completely separate PAHs by the small difference of their molecular shapes and planarities using polymeric and monomeric ODS phases.

In our previous studies,[10,11] multidentate phenyl bonded phases were synthesized and evaluated for their molecular shape recognition power for various PAHs molecules. The planar solutes were eluted faster than non-planar ones with one of those, 1,3,5-tris(dimethyl phenyl) (TP) phase. The TP phase has a very unique structure of which moiety covers the silica surface horizontally, whereas common phenyl and ODS phases are attached vertically to silica surface by siloxane bonding. These results clearly have indicated that the retention behaviors of PAHs with these newly synthesized chemically bonded phases are very different from each other based on the steric structures of the bonded phase ligand on silica surfaces.[10,11]

Fullerenes are unique compounds made by all carbons in their structures and the purification of such compounds from carbon soot is the important task for separation chemistry because only LC can realize this goal. A number of works have been reported to use different types of stationary phases in LC to get better selectivity for fullerenes especially the most popular C_{60} and C_{70}.[12-18] The authors have also been investigating to find the most suitable stationary phase for such target and the latest conclusion we got from our basic investigations are that the phenyl group in the stationary phase structure is the first key and the shape of the bonded phase moiety is the second key to enhance the separation selectivity for C_{60} and C_{70}.

As an extension of these studies, we synthesized two new chemical bonded stationary phases which are a 2,4,6-(tri-tert-butylphenoxy)dimethyl (TBP) phase and benzyldimethyl (Benzyl) phase (Figure 1). The TBP phase has a specific structure formed by the phenyl ring and three bulky tert-butyl groups. The benzyl phase has a framework similar to the TBP phase, but no tert-butyl groups.

In this study, we examined the basic retention mechanism for PAHs and fullerenes with TBP and Benzyl phases and the influence of three bulky tert-butyl groups to the selectivity for PAHs and fullerenes.

EXPERIMENTAL

Reagent

Hexamethyldisilazane (HMDS), 2,4,6-(tri-tert-butylphenoxy)chlorosilane and benzyldimethylchlorosilane were purchased from Petrarch Systems (Bristol, PA, USA). Base silicas were obtained from Nomura Chemicals (Seto, Japan). The particle diameter is ca. 5 μm, the pore diameter is 150 Å and the specific surface area is ca. 280 m^2/g. Develosil ODS-5 packing (Nomura Chemicals) as a monomeric ODS phase was used as the reference. Other reagents and organic solvents were of analytical-reagent grade.

Bonded Phase Synthesis

As described previously,[19-21] 7 g of dried silica were added to 70 mL of 3.4 % solution of 2,4,6-(tri-tert-butylphenoxy)chlorosilane or benzyldimethylchlorosilane in dry toluene containing 3 mL triethylamine.

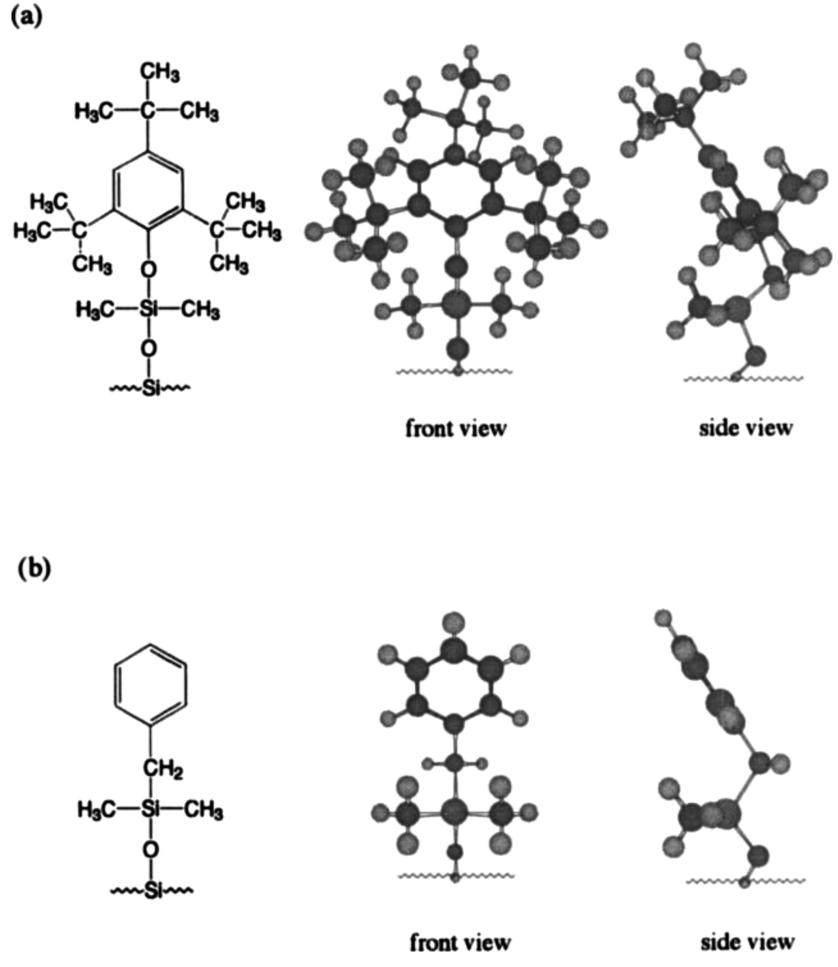


Figure 1. Two and three dimensional chemical structures of bonded phases investigated. (a) TBP phase and (b) Benzyl phase. The characteristics of these bonded phases are summarized in Table 1.

The silica suspension was refluxed for 5 h, filtered with a glass filter (1μm), washed several times with toluene, chloroform, methanol, and acetone and then dried in vacuo at 70 °C for 2 days. Furthermore, in accordance with the Buszewski method,[22] a TBP phase or Benzyl phase was added to 70 mL of toluene and 4 mL of HMDS for end capping. The basic characteristics of these

Table 1

Basic Characteristics of New Chemically Bonded Phases and Commercially Available ODS Phase as the Reference

Stationary Phase	Pore Size (Å)	Carbon Content (%)	Surface Coverage (μmol/m^2)
TBP	150	3.33	0.49
Benzyl	150	7.04	2.58
TP	120	7.46	2.27
Develosil ODS-5	100	20	3.31

phases are given in Table 1, and one of the commercially available ODS phase, Develosil ODS-5 is also listed in Table 1. The carbon contents of the treated silicas were determined by elemental analysis using an MT-3 CHN elemental analyzer (Yanagimoto, Kyoto, Japan). The specific surface areas and pore diameters of the silicas were determined with an MOD-220 porosimeter (Carlo Erba, Milan, Italy), SA-1000 surface area pore-volume analyzer (Shibata, Tokyo, Japan), and FT-IR 1640 (Perkin-Elmer, Yokohama, Japan) and then the surface coverages N (μmol/m^2) were calculated.

Chromatographic Measurements

The bonded phases were packed using a slurry method into a fused-silica capillary (Tokyo Kasei, Tokyo, Japan) of 0.53 mm i.d. x 150 mm length.

The microcolumn LC system consisted of a microfeeder MF-2 pump (Azuma Electric, Tokyo, Japan), a Rheodyne 7520 injector (Cotati, CA, USA) with a 0.2 μL injection volume and a Shodex M-315 UV detector (Showa Denko, Tokyo, Japan) set at 254, 275 or 300 nm.

The mobile phases were prepared from guaranteed reagent grade methanol (Kishida Chemical, Osaka, Japan) and deionized water purified by using Milli-Q water purification system (Millipore, Bedford, MA, USA). The typical flow-rate was 2 μL/min. The chromatographic measurements were done at least three times. For the column dead-volume measurements, Uracil was used.

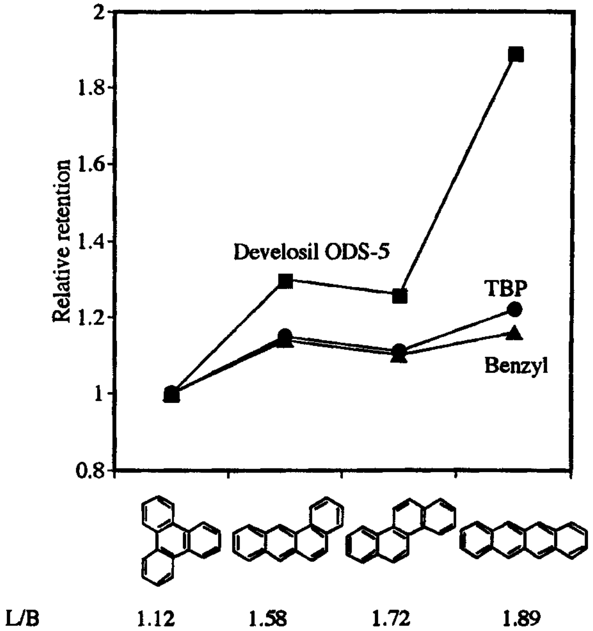


Figure 2. Relative retention plots of three PAHs to triphenylene with various phases.

Sample Solutes

PAHs used for evaluation in this study were commercially available (Tokyo Kasei, Tokyo, Japan) except phenanthro[3,4-c]phenanthrene (PhPh) which were obtained from Dr. W. Schmidt (Greifenberg am Ammersee, Germany) via. Dr. J.C.Fetzer (Chevron Research and Technology, Richmond, CA, USA) and Aldrich Chemical Co., Milwaukee, WI. Fullerenes of C_{60} and C_{70} were isolated from the carbon soot produced by using Ar-discharge in Toyohashi Science Core, Toyohashi, Japan and the mixture of C_{60} and C_{70} were obtained by toluene extraction from the soot.

Molecular Modeling

Molecular modeling was carried out using Chem 3D Plus software (Cambridge Scientific Computing Inc., MA, USA).

Table 2

Retention Factors of Various PAHs with Three Phases

F	Sample	Retention Factor(k')		
		TBP	Benzyl	Develosil ODS-5
3	benzene	0.12	0.20	0.77
5	naphthalene	0.17	0.32	1.64
6.5	fluorene	0.22	0.53	3.34
7	diphenylmethane	0.24	0.50	2.43
7	anthracene	0.23	0.58	4.25
7	phenanthrene	0.21	0.55	3.74
7	cis-stilbene	0.26	0.62	3.52
7	trans-stilbene	0.26	0.64	3.35
8	pyrene	0.25	0.68	5.48
8.5	benzo[ghi]fluoranthene	0.30	0.85	10.2
9	benzo[c]phenanthrene	0.31	0.92	9.49
9	triphenylene	0.27	0.80	6.99
9	o-terphenyl	0.33	0.88	4.50
9	m-terphenyl	0.33	0.99	6.82
9	p-terphenyl	0.36	1.02	8.10
9	benzo[a]anthracene	0.31	0.87	9.10
9	chrysene	0.30	0.88	9.78
9	naphthacene	0.33	0.93	13.2
10	bnezo[a]pyrene	0.35	1.11	17.2
10	benzo[e]pyrene	0.33	1.04	12.9
12	coronene	0.53	1.58	46.2
13	PhPh	0.40	1.53	12.6

Mobile phase; methanol/water = 80/20.

RESULTS AND DISCUSSION

Molecular Linearity Recognition for PAHs

To evaluate the chromatographic characteristics of the synthesized materials, the retention time of various PAHs samples were measured. The retention factors (k') were summarized in Table 2. The mobile phase was methanol/water = (80/20) for all stationary phases evaluation. In general, the

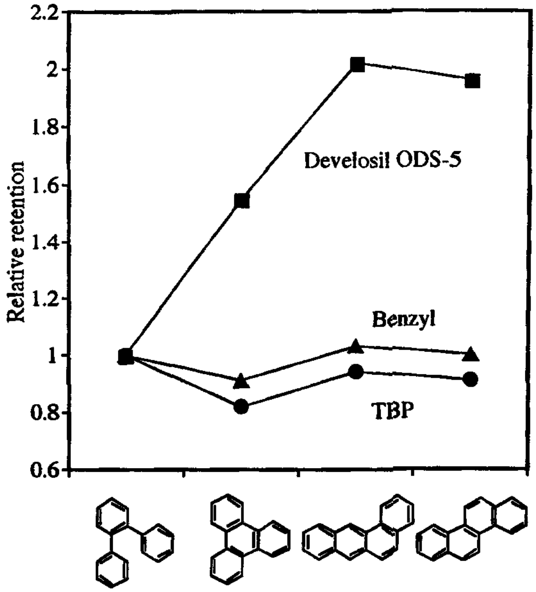


Figure 3. Relative retention plots of three PAHs to o-terphenyl with various phases.

retention values of PAHs in RPLC have a high correlation with the F number proposed by Schabron et al.,[23] where F is defined as the number of double bonds plus the number of primary or secondary carbons minus 0.5 times the number of non-aromatic rings.

As shown in Table 2, the elution orders of some planar and non-planar PAHs pairs with these synthesized bonded phases were different from that of ordinary ODS phases.[5-9] Figure 2 shows the plots of relative retention of benz[a]anthracene, chrysene and naphthacene compared to triphenylene. These PAHs have the same F number (F=9), but they have different L/B ratio.[24,25] L/B ratio is one of the molecular shape parameters which indicates molecular linearity of PAHs molecules. Therefore, more linear shape molecule has a larger L/B ratio.

Generally, it is known that in the case of isomeric PAHs which have the same F value the LC retention increases with increasing L/B ratio. The retention of linear molecule on these synthesized phases increases slightly

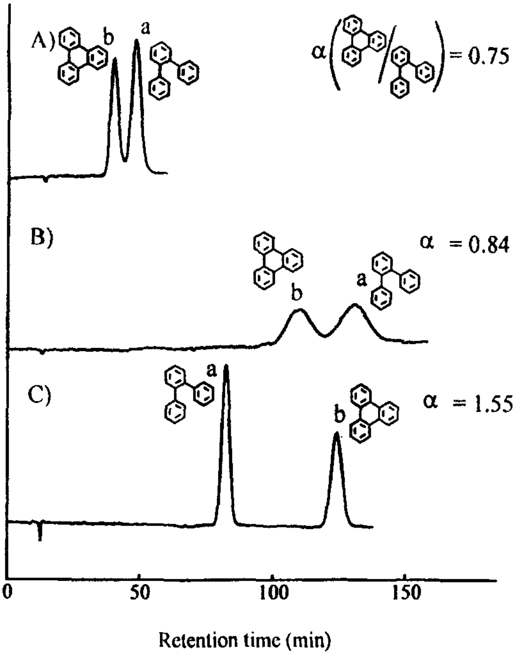


Figure 4. Typical chromatograms for the separation of o-terphenyl (a) and triphenylene (b). Mobile phase composition are as follows: (A) TBP phase, methanol/water=60/40; (B) Benzyl phase, methanol/water=60/40; and (C) ODS phase, methanol/water=80/20.

compared to that on the ODS phase. Therefore, it is found that these synthesized phases have a smaller linear molecular recognition power than the monomeric ODS.

Molecular Planarity Recognition for PAHs

Figure 3 shows the plots of relative retention of triphenylene, benz[a]anthracene and chrysene compared to o-terphenyl, where o-terphenyl is a non-planar molecule and others are planar molecules. The non-planar molecule was retained longer than planar ones with these synthesized phases although the opposite trend was observed with the ODS phase.

From these results, it was found that TBP and Benzyl phases have a non-planarity recognition capability. The chromatograms for the separation of triphenylene and o-terphenyl are shown in Figure 4. The mobile phases are

Table 3

Retention Factors (k') forTriphenylene and o-Terphenyl and the Separation Factors (α) with Three Stationary Phases Using Different Mobile Phase Compositions

Stationary Phase	k'						$\alpha = k'_{tri}/k'_{o\text{-}ter}$		
	Triphenylene			o-Terphenyl					
	80/20	70/30	60/40	80/20	70/30	60/40	80/20	70/30	60/40
TP	0.92	3.36	14.8	1.08	4.55	22.9	0.85	0.74	0.65
TBP	0.27	0.60	1.78	0.33	0.80	2.38	0.82	0.78	0.75
Benzyl	0.80	2.23	7.11	0.88	2.56	8.42	0.91	0.87	0.84

Mobile phase; methanol/water.

methanol/water=(60/40) for TBP (A) and Benzyl (B), and methanol/water=(80/20) for Develosil ODS phase (C). These separation factors are 0.75, 0.84 and 1.55 with TBP, Benzyl and Develosil ODS phase, respectively.

The experimental data obtained with TBP, Benzyl and TP phase are summarized in Table 3, where the retention factors of triphenylene and o-terphenyl and the separation factors with three different compositions of methanol/water mobile phase are shown. The elution order of triphenylene and o-terphenyl with TBP and Benzyl phase is the same as with TP phase. The reason for this trend with these phases can be explained by the similarity of these chemical structures on silica support.

Figure 5 shows three dimensional structures of PAHs pairs which have similar molecular size and different planarity, e.g., a pair of o-terphenyl and triphenylene. These separation factors are summarized in Table 4. As shown in Table 4, for these planar and non-planar PAHs pairs except the pair of coronene and PhPh, the two phases provided different elution order from the ODS phase, that means, that they provided different selectivity from the ODS phase, i.e., non-planar ones retained longer than planar ones. Due to the large molecular size of coronene and PhPh toward the phenyl-ring of TBP and Benzyl phase and to the relatively smaller distortion of PhPh molecule than other non-planar PAHs such as o-terphenyl, the pair of coronene and PhPh showed different retention behavior from other pairs with two new phases.

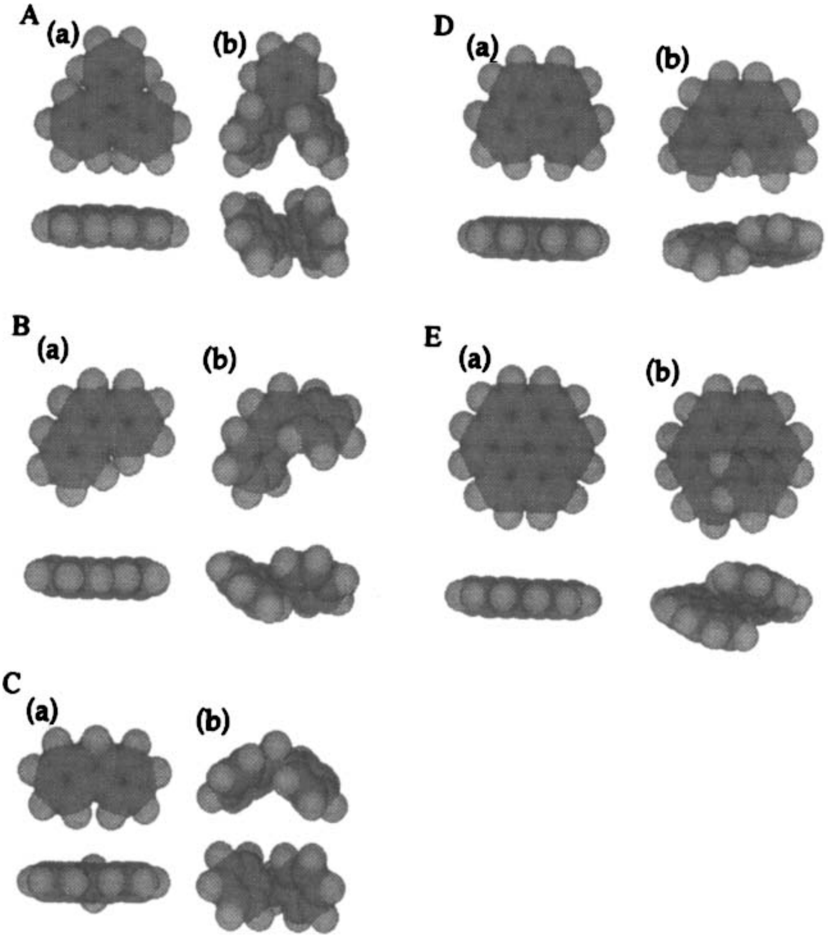
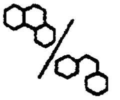
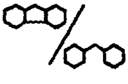
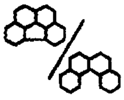
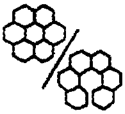


Figure 5. Three dimensional structures of planar and non-planar PAHs.
A: (a) triphenylene, (b) o-terphenyl; B: (a) phenanthrene, (b) cis-stilbene; C: (a) fluorene, (b) diphenylmethane; D: (a) benzo[c]phenanthrene, (b) benzo[ghi]fluoranthene; and E: (a) coronene, (b) PhPh.

From the data obtained above and these PAHs structures, it can be concluded that TBP and Benzyl phases have the non-planarity recognition capability for only small size PAHs.

Table 4

Separation Factors (α) for Planar and Non-planar Solute Pairs

	Separation Factor α (k′ Planar / k′ Non-planar)			
Stationary Phase				
TBP	0.71	0.89	0.87	1.33*
Benzyl	0.73	0.91	0.85	1.03*
Develosil ODS-5	1.06	1.37*	1.08*	3.67*

mobile phase: methanol/water = 60/40.
*mobile phase: methanol/water = 80/20.

Figure 6 shows the molecular-molecular interaction model between two solutes and TBP phase. Figure 6 (a) shows these three bulky tert-butyl groups disturb the interaction between TBP phase and planer solute such as triphenylene, because these tert-butyl groups covered the phenyl ring in the cavity-like structure and then the π-π interaction between planar PAHs and the phenyl ring in TBP moiety is prevented. However, as shown in Figure 6 (b), TBP phase having three bulky tert-butyl groups can produce more effective interaction between non-planar molecules such as o-terphenyl and the phenyl group than planar ones such as triphenylene. As the result, TBP phase retained non-planar solutes longer than planar ones.

Benzyl phase which has no tert-groups, can retain the solutes by π-π interaction without the interference caused from tert-butyl groups in TBP phase. For small size PAHs Benzyl phase has also a non-planarity recognition capability.

The phase retains the solutes by π-π interaction between the solute molecule and the stationary phase without the solute molecular planarity recognition. However if PAHs size is becoming larger, the moiety size is not enough to catch the solute molecules and therefore the non-planarity recognition power is decreased as in the case of coronene and PhPh.

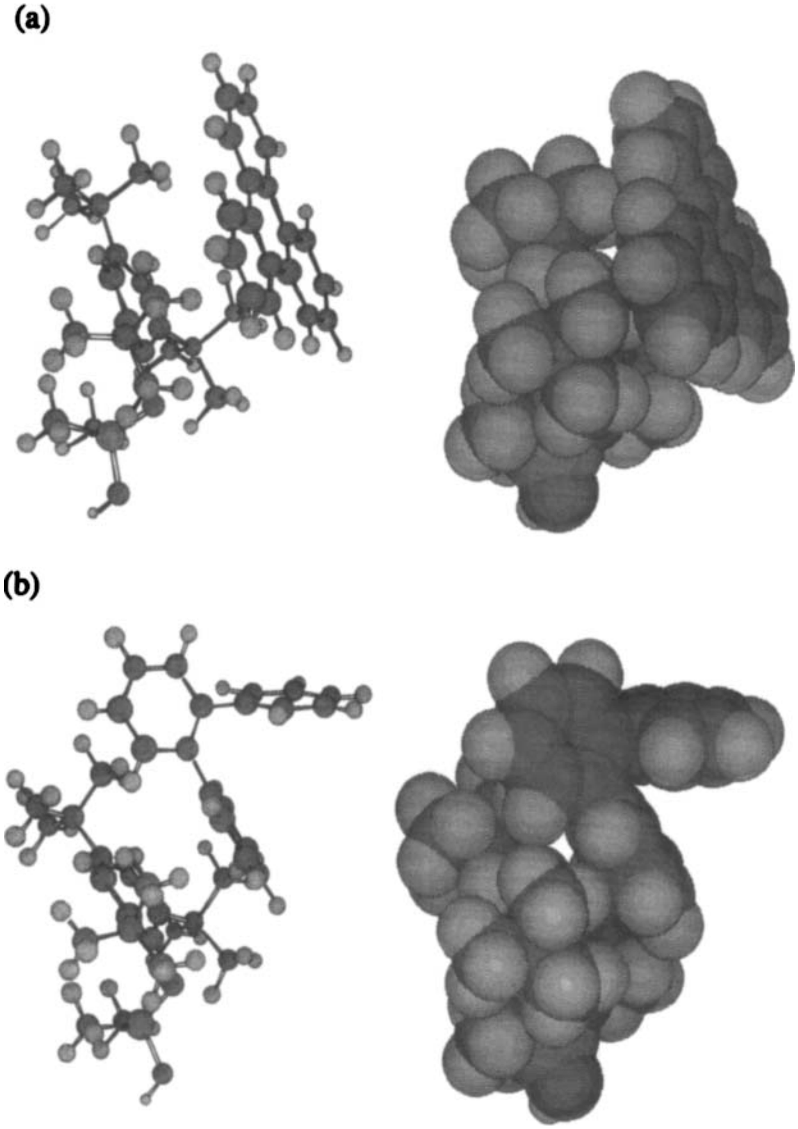


Figure 6. Three dimensional models of the interaction between solutes and TBP phase.
(a) triphenylene with TBP phase and (b) o-terphenyl with TBP phase.

Table 5

Retention Factors (k′) for C_{60} and C_{70} and the Separation Factors (α) with three Stationary Phases

	Retention Factor (k′)		Separation Factor (α)
	C_{60}	C_{70}	$k'C_{70}/k'C_{60}$
TBP	0.37	0.69	1.86
Benzyl	0.90	1.37	1.50
Develosil ODS-5	47.8	170	3.55

Mobile phase: acetonitrile/toluene = 70/30.

Selectivity for C_{60} and C_{70}

In addition, we also examined the separation of fullerenes with these stationary phases. The retention factors (k') for C_{60} and C_{70} and these separation factors (a) with TBP and Benzyl phases are shown in Table 5.

As shown in Table 5, it seems that these stationary phases are less useful than ODS phase for the separation of fullerenes using acetonitrile/toluene mobile phase. The fullerenes provided small retention and separation factor on two new stationary phases, because of low surface coverage and selectivity comparing to ODS phase. The reason of low selectivity is that molecular sizes of fullerenes are too large for these stationary phases to be recognized the shape difference between C_{60} and C_{70}.

CONCLUSION

In this study, it is found that TBP phase has a non-planarity recognition for relatively small size PAHs. The planar-nonplanar selectivity on Benzyl phase is not so pronounced as obtained with TBP phase.

It is also apparent that tert-butyl groups of TBP phase contribute to the non-planarity recognition capability toward small PAH molecules. In view of the separation science, the results obtained are very useful to reveal the sight of the interactions between solutes and surface structures of the stationary phase in LC.

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REFERENCES

1. I. A. Smith, G. D. Berger, P. G.Seybold, M. P.Serve, Cancer Res., **38**, 2968-2977 (1978).

2. G. H.Loew, J. Phillips, J. Wong, L. Hieldmeland, G. Pack, Cancer Biochem. Biophys., **2**, 113-122 (1978).

3. L. C. Sander, S. A. Wise, Anal. Chem., **56**, 504-510 (1984).

4. L. C.Sander, S. A. Wise, Anal. Chem., **59**, 2309-2313 (1987).

5. K. Jinno, T. Ibuki, N. Tanaka, M. Okamoto, J. C. Fetzer, W. R. Biggs, P. R. Griffiths, J. M. Olinger, J. Chromatogr., **461**, 209-(1989).

6. K. Jinno, T. Nagoshi, N. Tanaka, M. Okamoto, J. C. Fetzer, W. R. Biggs, J. Chromatogr., **392**, 75-82 (1987).

7. K. Jinno, S. Shimura, N. Tanaka, K. Kimata. J. C. Fetzer, W. R. Biggs, Chromatographia, **27**, 285-291 (1989).

8. L. C.Sander, S. A. Wise, LC.GC, **8**, 378-390 (1990).

9. L. C.Sander. S. A. Wise, HRC & CC, **8**, 248-255 (1985).

10. K. Jinno, K. Yamamoto, H. Nagashima, T. Ueda, K.Itoh, J. Chromatogr., **517**, 193-207 (1990).

11. K. Jinno, K. Yamamoto, T. Kuwamoto, H. Nagashima, T. Ueda, S. Tajima, E. Kimura, K.Itoh, J. C. Fetzer, W. R. Biggs, J. Chromatogr., **34**, 381-385 (1992).

12. K. Jinno, K. Yamamoto, T. Ueda, H. Nagashima and K. Itoh, J. Chromatogr., **594**, 105-109 (1992).

13. K. Jinno, K. Nakagawa, Y. Saito, H. Ohta, H. Nagashima, K. Itoh, J. Archer, Y. Chen, J. Chromatogr., **691**, 91-99 (1995).

14. Y. Saito, H. Ohta, H. Nagashima, K. Itoh, K. Jinno, M. Okamoto, Y. Chen, G. Luehr, J. Archer, J. Liq. Chromatogr., **18 (10)**, 1897-1908 (1995).

15. K. Jinno, Y. Saito, H. Nagashima, K. Itoh, Y. Chen, G. Luehr, J. Archer, J. C.Fetzer, W. R. Biggs, J. Microcol. Sep., **5**, 135-140 (1993).

16. Y. Saito, H. Ohta, H. Nagashima, K. Itoh, K. Jinno, J. J. Pesek, J. Microcol. Sep., **7**, 41-49 (1995).

17. K. Jinno, T. Uemura, H. Nagashima, K. Itoh, J. High Resolt. Chromatogr., **15**, 627-628 (1992).

18. H. Ohta, Y. Saito, K. Jinno, H. Nagashima, K. Itoh, Chromatographia, **39**, 453-459 (1994).

19. M. Okamoto, K. Nobuhara, K. Jinno, J. Chromatogr., **556**, 407-414 (1991).

20. M. Okamoto, F. Yamada, J. Chromatogr., **283**, 61-76 (1984).

21. M. Okamoto, K. Nobuhara, M. Masatani, K. Jinno, Chromatographia., **33**,203-207 (1992) .

22. B. Buszewski, Chromatographia, **28**, 574-578 (1989).

23. J. F. Schabron, R. J.Hurtubise, Anal.Chem., **49**, 2253-2260 (1977).

24. L. C.Sander, S. A. Wise, "Investigations of Selectivity in RPLC of Polycyclic Aromatic Hydrocarbons," in **Advances in Chromatography vol. 25**, J. C Giddings, E. Grushka, J. Cazes, P. R. Brown, eds., Marcel Dekker, Inc., New York, 1986, pp.139-218.

25. R. A. Hites, W. J. Simonsick Jr., **Physical Science Data 29, Calculated Molecular Properties of Polycyclic Aromatic Hydrocarbons**, Elsevier Science Publishers B. V., Amsterdam, 1987.

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