Chemically bonded phenylsilicone stationary phases for the liquid chromatographic separation of polycyclic aromatic hydrocarbons and cyclosiloxanes

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Abstract

Chemically bonded phenylsilicone stationary phases, prepared from polymethylphenylsiloxane (PMPS) bonded silica gel, have been developed for use in high-performance liquid chromatography (HPLC) to separate compounds containing aryl groups through $\pi-\pi$ interaction. Silanol-terminated PMPS was synthesized through living anionic polymerization of methylphenylcyclotrisiloxane. The PMPS bonded phases, with different degrees of polymerization (DP), were prepared by bonding silanol-terminated PMPS directly onto silica gel. The stationary phases thus prepared were characterized by elemental analysis, gel permeation chromatography, IR, ^{1}H NMR and ^{29}Si NMR. Evaluation of their chromatographic behavior in relation to small PAHs (polycyclic aromatic hydrocarbons) and various cyclosiloxanes showed them to have reasonable separation ability in both normal-phase and reversed-phase. Copolymerization reactions of trifluoropropylmethylcyclotrisiloxane (F_3) and hexaphenylcyclotrisiloxane (P_3) were followed on these stationary phases, and their performance in the retention of phenyl-containing siloxane copolymers with different compositions was also evaluated. Comparison of these PMPS phases with a commercial ODS (C_{18}) phase in analyses of PAHs and cyclosiloxanes indicates clearly the significant contribution made by the phenyl moiety on the siloxane backbone to the $\pi-\pi$ interaction in the HPLC separation process.

1. Introduction

Phenyl-substituted polysiloxanes have been used extensively as stationary phases for gas chromatography [1]. OV-17 is the best-known example. Stark et al. [2] have suggested that the primary mode of interaction between the phenyl silicones and analytes is due to polarizability rather than the interaction of any permanent dipoles. In the work described in our previous reports [3,4], it was found that, in the HPLC analysis, the presence of an aromatic ring in the bonded phase system played an important role,

due to a $\pi-\pi$ or $n-\pi$ interaction with the analytes. A liquid-chromatography review has suggested that the molecular association between the analytes and the stationary phase is due at least partially to donor–acceptor interactions [5]. However, the immobilization of the polymethylphenylsiloxanes (PMPS) on the silica surface has not been reported previously, perhaps owing to synthesis problems. Taking advantage of our experience in the synthesis of silicones, we are interested in learning whether phenyl-silicones provide donor–acceptor interactions with analytes and, if so, whether they show sufficient selectivity for the separation of the analytes concerned by high-performance liquid

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chromatography (HPLC). In this work, five chemically bonded phenylsilicone (PMPS) stationary phases were prepared by bonding silanol-terminated PMPS of different degrees of polymerization (DP) directly onto silica gel. The aromatic hydrocarbons, and also various cyclosiloxanes used as monomers for ring-opening polymerization to give the silicone polymers, served as analytes for the evaluation of PMPS selectivity. The contribution of the phenyl moiety on the siloxane backbone to π–π interaction in the HPLC separation process was examined by comparison of these phases prepared in-house with a commercial ODS phase. Tracing the path of a copolymerization reaction is a useful way to find out suitable conditions for the synthesis of a desired copolymer, as well as to establish whether side reactions, such as back-biting and chain transfer are involved. Gel permeation chromatography (GPC) is often used for such a purpose. However, for effective resolution of the comonomers, cyclic oligomers (products of back-biting), and the intermediate products, GPC analysis is tedious and not recommended for quantitative measurement. In the copolymerization to produce a phenyl-containing polysiloxane, this tracing analysis may be achieved by HPLC using a π–π interaction-type stationary phase. HPLC on these PMPS phases has been examined, therefore, as a potential alternative to GPC for tracing the copolymerization reactions that take place during phenyl-containing copolymer formation.

2. Experimental

2.1. Chemicals

Silica gel (Nucleosil; pore size 100 Å, particle size 7 μm, surface area 350 m^2/g) was obtained from Macherey-Nagel. Oligocyclosiloxanes (Dow Corning) and polycyclic aromatic hydrocarbons (PAHs; Merck) were used as received. The concentration of n-butyl lithium (n-BuLi) (1.5 M in hexane; Merck) was pretested by the Gilman method [6]. Tetrahydrofuran (THF), which was used as a solvent in the polymerization, was

freshly distilled just before use. The solvents used for HPLC were LC-grade n-hexane, acetonitrile and deionized water.

2.2. Preparation of silanol-terminated PMPS

1,3,5-Trimethyl-1,3,5-triphenylcyclotrisiloxane (D_3^{ph}), obtained via pyrolysis of oligomethylphenylcyclosiloxane, was used as monomer. PMPS was synthesized from this monomer in dry THF under reflux for 30 min through anionic polymerization with the appropriate amount of n-BuLi as an initiator and acetic acid as a terminator. The number averaged molecular mass (M_n) and the mass averaged molecular mass (M_w) of the polymer were determined by GPC. The average molecular mass of the polymer was also confirmed by ^{1}H NMR.

2.3. Preparation of PMPS stationary phases

Silica gel (5 g) was dried at 140°C for 10 h in a reaction flask. A solution of an excess amount of silanol-terminated PMPS in 20 ml THF was added to, and mixed well with, the silica gel under an N$_2$ stream. The mixture was then heated to 120°C (oil bath) under vacuum for 12 h, with stirring. The siloxane-modified silica gel thus obtained was washed thoroughly with THF followed by dichloromethane, and then dried under vacuum. Five PMPS stationary phases, with different degrees of polymerization (DP = molecular mass of polymer/molecular mass of MephSiO unit), were thus prepared and named as XXMP, XX indicating the DP of the corresponding bonded PMPS (MP = MephSiO). The characteristics of these PMPS phases are given in Table 1.

2.4. Chromatographic studies

The chromatographic studies were carried out with a Kratos liquid chromatographic system, which consisted of a Spectroflow 400 solvent-delivery system, a Spectroflow 480 injector and an Applied Biosystem 757 variable-wavelength UV detector or a Waters 410 Differential Refractometer. Data collection was done with a Chem/

Table 1
Characteristics of the prepared PMPS stationary phases

Phase[a]	Polymer		Carbon loading (C wt%)[c]	Polymer loading (mmol/g)	Phenyl loading (mmol/g)[d]	IR (cm^{-1})	^{29}Si-NMR (ppm)
	Mn[b]	Mw/Mn[b]					
17MP	2400	1.17	17.30	0.117	1.989	3072, 2965, 1580, 1425, 733, 698	−33.775
26MP	3600	1.11	19.32	0.087	2.262	3071, 2963, 1588, 1425, 732, 698	−33.721
45MP	6200	1.11	24.07	0.063	2.835	3057, 2967, 1587, 1425, 733, 698	−33.738
58MP	7900	1.09	24.38	0.050	2.900	3071, 2963, 1588, 1426, 732, 698	−33.482
76MP	10400	1.15	24.91	0.039	2.980	3070, 2961, 1587, 1425, 731, 698	−33.304

[a] Silica gel (7 μm–100 Å), surface area: 350m^2/g, monomer: D$_3^{ph}$. Numbers denote the degree of polymerization of PMPS.
[b] By GPC.
[c] By elemental analysis.
[d] Based on C%.

Win data station developed by SISC (Taiwan). Stainless-steel columns (300 mm × 4 mm I.D.) were packed by the balanced-density slurry method. Reversed-phase ODS (Nucleosil 7 C$_{18}$) was obtained commercially. The mobile-phase flow-rate was 1.0 ml min^{-1} and the UV detector was operated at 254 nm.

2.5. Measurements

^{1}H NMR spectra of the PMPSs were obtained with a Bruker (300 MHz) spectrometer. Solid-state ^{29}Si NMR spectra of the bonded phases were obtained with a Bruker MSL 200 MHz solid-state NMR spectrometer. IR spectra of the PMPS and the corresponding bonded phases were obtained with a Perkin-Elmer 938 G IR spectrometer. M_n, M_w and molecular mass distribution (M_w/M_n) were measured with a GPC

instrument using polystyrenes as the standards. The GPC system consisted of a Kratos Model Spectroflow 400 Gel Permeation Chromatograph with four Shodex columns in series, KF-804, KF-803, KF-802, KF-801, in THF, operated at a flow-rate of 1.0 ml min^{-1} with an RI detector (Shodex Model RI-71).

3. Results

The degree of polymerization of the polymers synthesized in this way is considered to be well-controlled, judging by the molar ratio of D$_3^{ph}$ and n-BuLi; the molecular weight distribution (M_n/M_w) is also quite narrow (<1.2). The bonded phases were characterized by ^{29}Si NMR (δ: ca. −34 ppm for MephSi-O), IR (>3000 and 698–

733 cm^{-1} for the phenyl group; ca. 2960 and 1425–1588 cm^{-1} for the methyl group) and elemental analysis (Table 1). The carbon loading, which is directly related to the phenyl loading, increased smoothly with increasing polymer chain length (i.e. DP) but levelled out at around 24 wt% when the DP of the polymer was larger than 45 (Fig. 1). The decrease in polymer loading with increasing polymer DP may be ascribed to steric factors.

Chromatographic results of testing for the separation of PAHs and cyclosiloxanes $(R_1R_2SiO)_{3-4}$ (F_3, F_4: $R_1 = CH_3$, $R_2 = CH_2CH_2CF_3$; D_3, D_4: $R_1 = R_2 = CH_3$; D_3^{ph}, D_4^{ph}: $R_1 = CH_3$, $R_2 = ph$; P_3, P_4: $R_1 = R_2 = ph$) are shown in Figs. 2–4 and Table 2. Chromatograms for 17MP and 26MP phases in all separations showed broad peaks with poor resolution, too poor for distinction between the analytes, perhaps due to their lower carbon and phenyl loadings. The representative chromatographic behavior for the other three bonded phases in different mobile phases (Figs. 3 and 4) showed that PAH separation is achieved effectively either in the normal-phase (NP, n-hexane as the mobile phase on 45MP) or the reversed-phase (RP; different ratios of CH_3CN-H_2O as the mobile phases on 76MP). While no effective separation of a mixture of various cyclotrisilox-

Fig. 2. Plots of k' values of PAHs against C% for PMPS stationary phases. Mobile phases: (a) $n\text{-}C_6H_{14}$, (b) CH_3CN, (c) CH_3CN-H_2O (95:5); analytes: (●) benzene, (■) naphthalene, (▲) fluorene, (▼) anthrathene, (○) phenanthrene, (◆) pyrene.
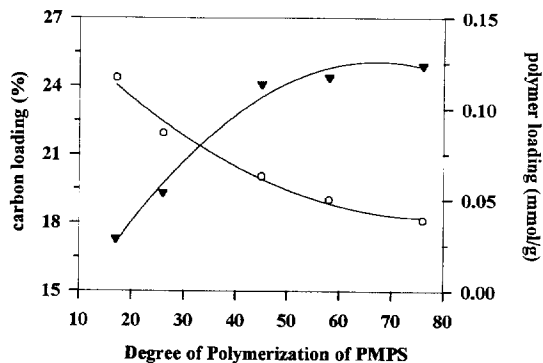

Fig. 1. Correlation of carbon loading (▼) and polymer loading (○) with degree of polymerization (DP) of the PMPS stationary phases prepared in-house.
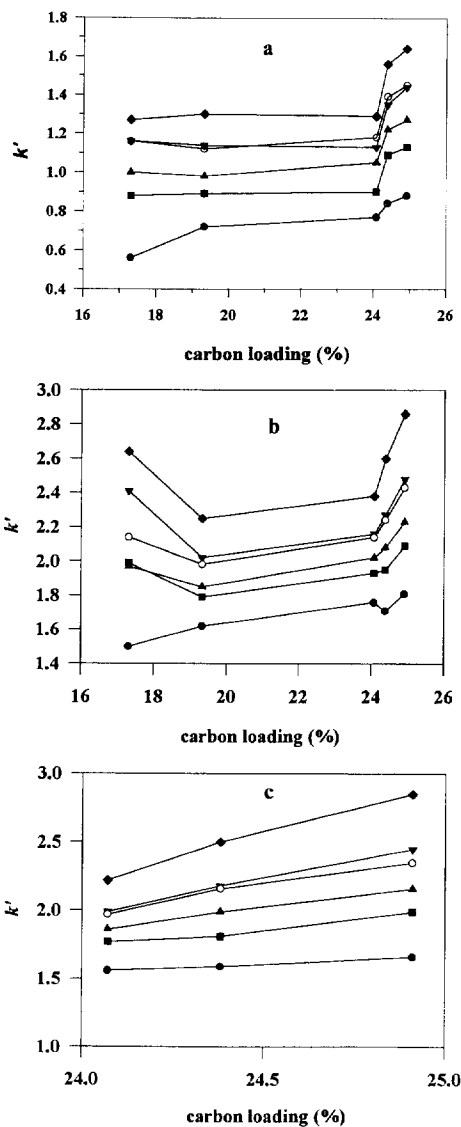
anes and cyclotetrasiloxanes, $(R_1R_2SiO)_{3-4}$, was observed in NP, except diphenylsiloxyoligomers on 45MP, satisfactory selectivity on 76MP was observed in RP.
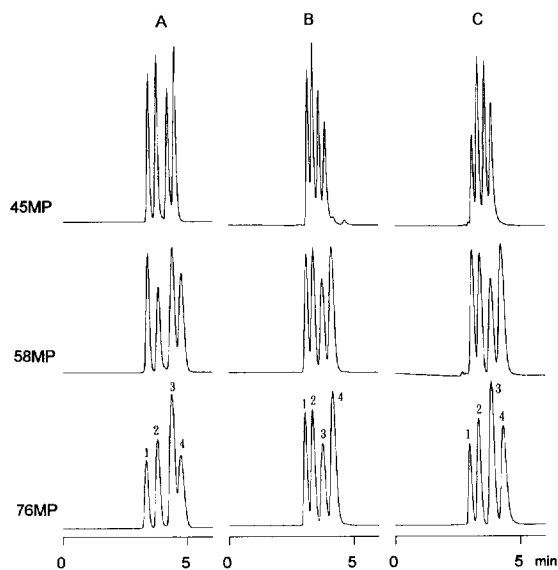
Fig. 3. Effect of the mobile phase on the chromatographic behavior of PAHs on the prepared stationary phases. Mobile phases: (A) n-C_6H_{14}, (B) CH_3CN, (C) CH_3CN–H_2O (95:5); flow-rate: 1 ml min^{-1}; analytes: (1) benzene, (2) naphthalene, (3) anthracene, (4) pyrene.
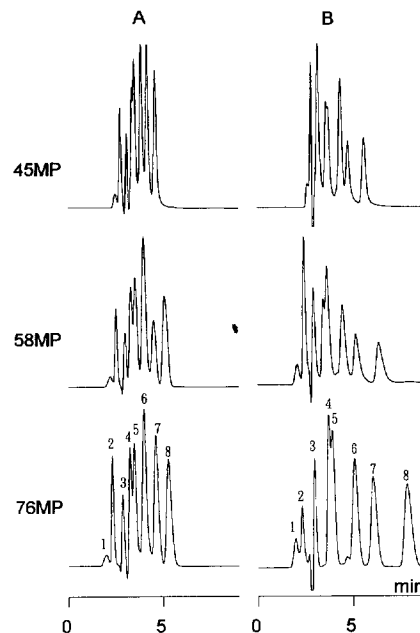

Fig. 4. Comparison of the chromatographic results for the separation of cyclic siloxane oligomers on the prepared stationary phases. Mobile phases: (A) CH_3CN; (B) CH_3CN–H_2O (95:5); flow-rate: 1 ml min^{-1}; analytes: (1) F_4, (2) F_3, (3) D_3, (4) D_4, (5) D_3^{ph}, (6) D_4^{ph}, (7) P_3, (8) P_4.

4. Discussion

4.1. Chromatographic behavior of PAHs on the PMPS phases prepared in-house

Since, in polarity the prepared PMPS phases lie between a pure silica and a C_{18} modified phase, they may be expected to have an amphoteric chromatographic characteristic (NP/RP).

A mobile-phase survey through n-hexane, a nonpolar organic solvent, to polar solvents was performed for relatively small PAHs (Fig. 2). It was found that a contribution of the π–π interaction other than hydrophobic (HB) interaction may be involved in the retention mechanism, and that its significance is determined by the phenyl content of the PMPS phase, the number of rings of the PAH and the polarity of the mobile phase.

A consistent change in the retention factor (k') of all the PAHs with phenyl loading (same tendency as C%) of the PMPS phases was found for the bonded phases with DP ≥ 45 (C% > 24%) in both NP and RP mobile-phase systems. In general, k' increases with increasing number of PAH rings on a given bonded phase. Since the k' of phenanthrene is very similar to that of its isomer with the same number of rings, anthracene, on their PMPS phases (Fig. 2), the π–π interaction would be dominant in the retention mechanism. This hypothesis is also supported by the fact that selectivity towards these two isomers is less effective on 76MP than on the commercial ODS C_{18} phase, as shown in Fig. 5.

In the n-hexane system, the low value of k' was considered to be due to an HB-interaction-dominated retention mechanism. However, in a polar system such as acetonitrile or acetonitrile–water, the larger value of k' can be ascribed to the significant contribution of π–π interaction.

Table 2
Normal-phased chromatographic separation of cyclosiloxane oligomers (n-hexane, flow-rate: 1 ml min^{-1})

Phases	Retention factor (k')				Separation factor (α)	
	D_3^{Ph}	D_4^{Ph}	P_3	P_4	$D_3^{Ph}-D_4^{Ph}$	P_4-P_3
17MP	0.78	0.63	2.47	2.81	1	1.12
26MP	0.92	0.84	2.53	3.03	1	1.12
45MP	1.01	0.93	2.61	3.16	1	1.20
58MP	1.09	0.96	3.01	3.43	1.13	1.12
76MP	1.14	0.96	3.26	3.64	1.18	1.12

The combination of both $\pi-\pi$ and HB interactions also reduces the consistency of the correlation of k' values and C% (or phenyl loading).

A comparison of the separations of a mixture of four PAHs, with different numbers of rings, in

Fig. 5. Comparison of the chromatographic results for the separation of PAHs on (a) the prepared 76MP phase, (b) a commercial ODS C_{18} phase. Mobile phase: CH_3CN-H_2O (95:5); flow-rate: 1 ml min^{-1}; analytes: (1) benzene, (2) naphthalene, (3) fluorene, (4) phenanthrene, (5) anthracene, (6) pyrene, (7) 1,2-benzanthracene.

different mobile-phase systems is shown in Fig. 3. The best selectivity towards relatively small molecules, benzene and naphthalene, is found in the NP system, and HB interaction may serve as the major contributory factor, while better selectivity towards relatively large molecules, anthracene and pyrene, can be achieved in a polar system, with $\pi-\pi$ interaction predominant. By virtue of this additional contribution, the prepared 76MP phase, which provided the greatest $\pi-\pi$ interaction ability among the prepared phases, shows the best selectivity in RP systems for these PAHs with different numbers of rings.

4.2. Chromatographic behavior of cyclosiloxanes on the PMPS phases prepared in-house

A preliminary examination was made of a mixture of only four components with different phenyl contents (D_3^{ph}, D_4^{ph}, P_3 and P_4) in an NP system. Their k' and α values are shown in Table 2. The PMPS phase was found not to be selective towards D_3^{ph} and D_4^{ph} at DP $\leqslant$ 45. Peaks in all chromatograms, except for those for P_3 and P_4 on 45MP phase, are broad and poorly resolved. The solubility of P_3 and P_4 in n-hexane is quite different from that of D_3^{ph} and D_4^{ph}, leading to more complications in the resolution mechanism.

The selectivity of these PMPS phases is much better in a polar mobile phase than in n-hexane. Therefore, a mixture of eight components was investigated in acetonitrile. The elution order is: $P_4 > P_3 > D_4^{ph} > D_3^{ph} > D_4 > D_3 > F_3 > F_4$ on all PMPS bonded phases. Chromatograms of this separation on 17MP and 26MP also show no effective resolution in this polar system. As
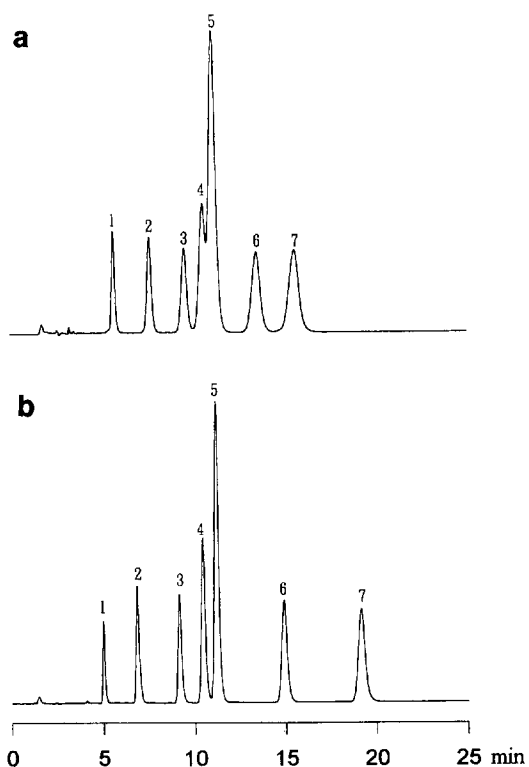
shown in Fig. 4, it is clear that the selectivity increases with an increase in DP (i.e. phenyl loading) of the PMPS bonded phase as well as in the phenyl contents of the cyclosiloxanes. These facts, combined with the elution order shown above, strongly suggest that a $\pi-\pi$ interaction is predominant in this separation system on PMPS with a sufficiently high phenyl content.

On comparison of the separations of this mixture in mobile phases with different compositions (Fig. 4) it is found that, except for D_4/D_3^{ph}, the selectivities among all other components increase when water is added to the mobile phases.

The retentions of larger phenyl-containing molecules also increase significantly with the addition of water. These phenomena, which may be caused by both the solubility of the analytes in the mobile phase and the $\pi-\pi$ interaction between the analytes and PMPS, result in excellent resolution among the phenyl-containing analytes.

4.3. Comparison of 76MP with a commercial ODS stationary phase

In order to confirm the effective contribution of $\pi-\pi$ interaction by the PMPS phase, comparisons of 76MP with a commercial ODS phase were examined for the HPLC separation of PAHs (Fig. 5) and cyclosiloxanes (Fig. 6). In Fig. 5, all the PAH analytes, with the exception of anthracene/phenanthrene, the tested PAHs are separated on 76MP as effectively as on the ODS phase within a shorter time. This means that, instead of HB contributions, $\pi-\pi$ interactions resolve the analytes more effectively. This contribution can be seen more clearly in Fig. 6.

The differences in the elution order of the cyclosiloxanes on these two phases are obviously due to the different contributions of the stationary phases to the recognition mechanism. On the ODS phase, the elution order is parallel to the molecular size of the analytes. However, on 76MP, it is clearly dominated by the phenyl content. This confirmed that a PMPS stationary phase with sufficient phenyl content is accessible
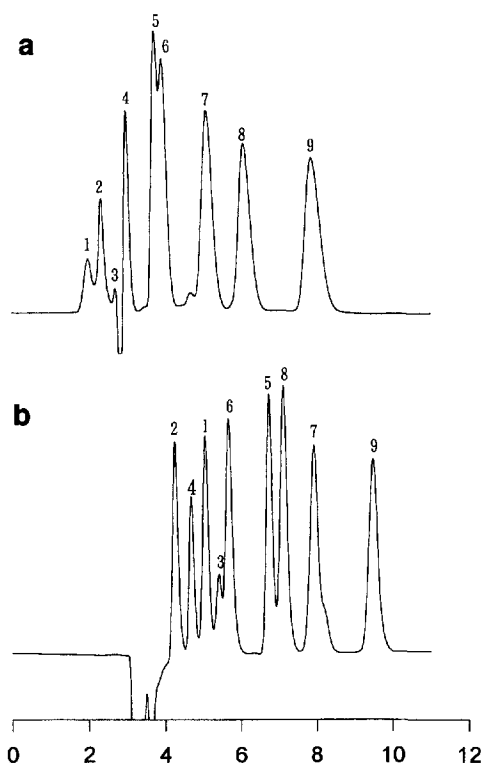

Fig. 6. Comparison of the chromatographic results for the separation of cyclosiloxanes on (a) the prepared 76MP phase, (b) a commercial ODS C_{18} phase. Mobile phase: CH_3CN-H_2O (95:5); flow-rate: 1 ml min^{-1}; analytes: (1) F_4, (2) F_3, (3) F_5, (4) D_3, (5) D_4, (6) D_3^{ph}, (7) D_4^{ph}, (8) P_3, (9) P_4.

for $\pi-\pi$ interactions and results in the formation of a charge-transfer complex with phenyl-containing species, achieving effective resolutions in a short time.

4.4. Test application of PMPS

As a test application of these charge-transfer phases, tracing of the copolymerization reactions of F_3 and P_3, which was initiated by n-butyl lithium in a toluene solution, was investigated on 76MP. The intermediate copolymerization products, with different phenyl contents and/or chain lengths represented as $F_n P_m$ ($n,m \neq 0$), together with unreacted F_3, P_3 and other cyclic oligomers (such as F_4, F_5, P_4, etc.) were found on the chromatogram in an elution order increasing

with phenyl content. The F_nP_m species are not well-resolved; for the purpose of this application, modification of the length of the PMPS and the pore size of the silica gel should be studied further. Nevertheless, when pure copolymers of F_3–P_3 with 0, 20, 40, 50 mol% of P_3, respectively, were tested, it was found that they were differently retained on 76MP in a CH_3CN mobile phase (Fig. 7), and could be distinguished through π–π interactions with this PMPS stationary phase.
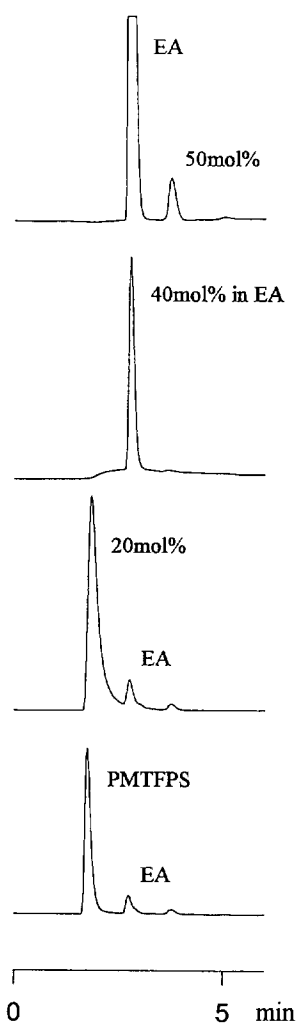

Fig. 7. Chromatographic behaviors of PMTFPS–PDPS co-polymers with different PDPS contents on the 76MP phase. Mobile phase: CH_3CN; flow-rate: 1 ml min^{-1}. (EA: ethyl acetate).

5. Conclusion

The results of the present work can be summarized as follows:

(1) Chemically bonded PMPS stationary phases with a sufficiently high phenyl loading (DP = 45) provide satisfactory selectivity for mixtures of polycyclic aromatic hydrocarbons or cyclosiloxanes whether these contain phenyl or not. A relatively lower phenyl loading (DP < 45) was found to be more effective in the normal-phase system, while a higher phenyl loading (DP = 76) was found to be the most suitable in reversed-phase systems. Effective separation of aromatic hydrocarbons was found in both phases. Satisfactory selectivity in relation to cyclosiloxanes was found in the reversed-phase, and excellent separation of phenyl-containing cyclosiloxanes could be achieved on 76MP.

(2) The contribution of π–π interactions renders 76MP better than a commercial ODS stationary phase for siloxane-monomer recognition.

(3) Different phenyl contents of the poly-methyltrifluoropropylsiloxy (PMTFPS)–poly-diphenylsiloxy (PDPS) copolymers were distinguishable on 76MP, and tracing of the copolymerization of F_3 and P_3 may prove to be an application of PMPS-bonded stationary phase in HPLC.

Acknowledgments

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