

# Linear Polystyrene-Grafted Silica Gels for High-Performance Liquid Chromatography

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Linear polystyrene, having a trimethoxysilyl group at one side of the terminal group, was newly synthesized and readily grafted onto porous silica gels using the terminal reactive group. Its packed column showed good selectivity based on the  $\pi$ - $\pi$  interaction for polyaromatics as well as the usual reversed-phase mode separation for alkyl compounds. The elution behavior (including the elution order) agreed with those that were observed in non-silica-supported poly(styrene-divinylbenzene) spherical particles. However, the asymmetric factor ( $A_s$ ) of the elution peaks for polyaromatics was much better in silica-supported polystyrene than in non-supported polystyrene; for example, the values of  $A_s$  for triphenylene were 1.4 and 4.7, respectively. This difference is attributable to the flexibility of the polystyrene chain.

**Keywords** Polystyrene, linear polymer, phenyl-bonded silica gel,  $\pi$ - $\pi$  interaction, polyaromatics, reversed phase chromatography

Poly(styrene-divinylbenzene) (PS-DVB) spherical particles are one of the most useful packing materials for various column liquid chromatographies. Especially, macroporous PS-DVB is most popularly used in the field of size-exclusion chromatography in organic eluents. In addition, PS-DVB provides hydrophobic and aromatic properties in polar eluents, like aqueous solutions. These properties provide a good separation factor for aromatic compounds compared with alkyl-bonded silica gels, like octadecyl-bonded silica gels (ODS). The  $\pi$ - $\pi$  interaction is perhaps effective in this molecular-recognition process. However, there is a serious problem with PS-DVB in this chromatography: we have often observed excessive retention with remarkable peak-tailing for polyaromatics, such as pyrene and triphenylene. We have estimated that this undesirable retention is not derived from styrene units, because phenyl-bonded silica gels have no such peak-tailing. Therefore, we believe that the elimination of the cross-linking structure due to divinylbenzene would solve this problem.

In this communication we report on silica-supported linear polystyrene (Sil-PS<sub>n</sub>, where  $n$  stands for average degree of polymerization; Fig. 1) with non-cross-linking structures. This new packing material was synthesized by modifying our previous technique.<sup>1-4</sup> This paper also describes good separation for polyaromatics with no peak-tailing.

## Experimental

### Materials

Silica-supported polystyrene (Sil-PS<sub>n</sub>) was prepared

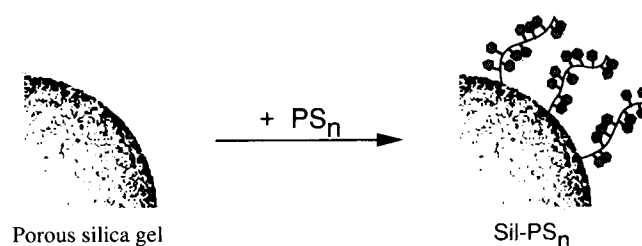


Fig. 1 Schematic illustration of linear polystyrene-grafted silica gels. PS<sub>n</sub> is polystyrene with a reactive group at the one side of the terminal group, where  $n$  is the degree of polymerization.

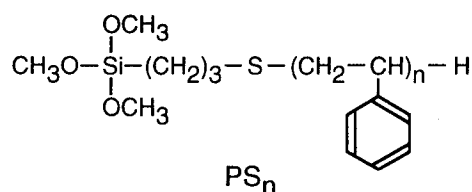


Fig. 2 Chemical structures of PS<sub>n</sub>.

by the telomerization of styrene with 3-mercaptoptrimethoxysilane and subsequent immobilization onto porous silica gels. The typical synthetic procedure was as follows: distilled styrene and 3-mercaptoptrimethoxysilane (in the molar ratio of 30 : 1) were dissolved in ethanol. After bubbling N<sub>2</sub> for 20 min, azobisisobutyronitrile (0.2 wt% for a monomer) as an initiator was added to the mixture at 80°C and stirred for 15 h at 80°C under an N<sub>2</sub>

atmosphere. After cooling to room temperature, white powders were obtained upon adding methanol and performing filtration. The structure of the obtained polystyrene was confirmed by  $^1\text{H-NMR}$  spectroscopy and elemental analysis.

Immobilization was carried out by mixing polystyrene with porous silica gels (YMC-120 S5, diameter 5  $\mu\text{m}$ ; pore size 120  $\text{\AA}$ ; specific surface area 300  $\text{cm}^2 \text{g}^{-1}$ ) in tetrachlorocarbon at reflux temperature. The immobilization was confirmed by IR spectroscopy and elemental analysis.

#### Measurement

Silica-supported polystyrene, Sil-PS<sub>21</sub> (the average degree of polymerization was estimated to be 21 by  $^1\text{H-NMR}$  spectroscopy) was packed into a stainless-steel column (250 mm  $\times$  4.6 mm i.d.), and the liquid-chromatographic property was examined using methanol- and acetonitrile-water solutions as eluents. The chromatograph included a JASCO 880 PU pump and a Shimadzu UV-visible photodiode array SPD-M6A. Five microliters of the sample dissolved in methanol was injected through a Reodyne Model 7125 injector. Chromatography was carried out at a flow-rate of 0.5  $\text{ml min}^{-1}$  at 25°C.

## Results and Discussion

#### Preparation and characterization of silica-supported polystyrene

Polystyrene (PS<sub>21</sub>) with a trimethoxysilyl group at one side of the terminal group was prepared by one-step telomerization of styrene and 3-mercaptopropyltrimethoxysilane at a molar ratio of 30:1.  $^1\text{H-NMR}$  spectroscopy of the obtained polymer showed  $n=21$  in the degree of polymerization ( $n$ ): the chemical shifts in  $\text{CDCl}_3$  were as follows:  $\delta=0.75$  ( $\text{SiCH}_2$ ),  $\delta=3.55$  ( $\text{SiOCH}_3$ ) and  $\delta=6.3-7.2$  ( $\text{C}_6\text{H}_5$ ) ppm. A molecular weight of 2380 (as  $n=21$ ) was determined by using the proton ratio between the trimethoxy and phenyl groups. This value was close to the value ( $M_n=2740$ ) that was determined by size-exclusion chromatography (SEC) using a Shimadzu HSG-15 column (500 mm  $\times$  7.9 mm i.d.) in chloroform. The SEC analysis also showed  $M_w/M_n$  to be 1.47. This indicates that the molecular-weight distribution of PS<sub>21</sub> is very narrow compared to conventional polystyrene prepared by the usual-type radical polymerization: for example, polystyrene with  $M_w/M_n=3.4$  has been reported by Kosfeld.<sup>5</sup> This significant difference between telomerization and polymerization can be explained by the fact that the mercapto group of 3-mercaptopropyltrimethoxysilane is a good chain-transfer agent.

PS<sub>21</sub> was readily immobilized onto porous silica gels by mixing in tetrachlorocarbon at reflux temperature. The elemental analysis of PS<sub>21</sub>-immobilized silica gels (Sil-PS<sub>21</sub>) showed that 21.5 wt% of PS<sub>21</sub> was introduced into silica gels, and that successive washing with chloroform produced no significant change. This value shows that

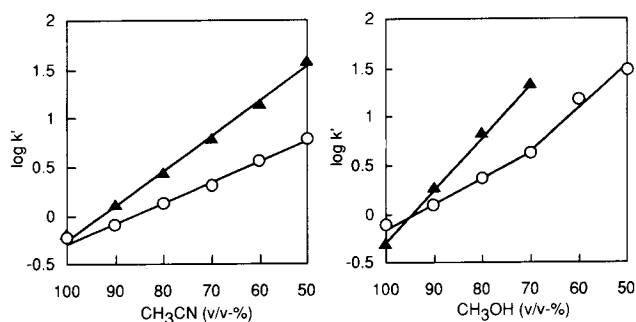


Fig. 3 Relationships between  $\log k'$  and the composition of aqueous eluents. Sample: naphthalene ( $\circ$ ), octylbenzene ( $\blacktriangle$ ).

the surface concentration of the phenyl groups of Sil-PS<sub>21</sub> is 6.8  $\mu\text{mol m}^{-2}$ . In addition, the  $M_w/M_n$  value of the PS<sub>21</sub> which remained in the solution was determined to be 1.52, even after the immobilization process. This indicates that PS<sub>21</sub> is not self-condensed during the immobilization process.

The conformation of polystyrene (degree of polymerization, 20) was estimated using CAChe-mechanics. The optimization with MM2 parameters continued until the energy change was less than the 0.001  $\text{kcal mol}^{-1}$ . According to this calculation, the center-to-center distances between the 4-phenyl carbon atoms were estimated to be 3.6 and 5.1  $\text{\AA}$  in the *meso* and *racemi* diad conformations, respectively.

#### Retention mode for alkylbenzenes

The Sil-PS<sub>21</sub> column showed complete separation for a mixture of ethyl-, butyl-, hexyl-, octyl-, decyl- and dodecylbenzenes in acetonitrile-water and methanol-water as eluents. The elution order agreed with those that were observed in octadecyl-bonded silica gels (Inertsil ODS-2, 250 mm  $\times$  4.6 mm i.d., GL Science Inc.). In addition, the retention capacity ( $k'$ ) increased along with an increase in the polarity of the eluents, as shown in Fig. 3. These results indicate that Sil-PS<sub>21</sub> includes a retention mode similar to that of reversed-phase liquid chromatography.

#### Separation for polyaromatics

As shown in Fig. 4, the Sil-PS<sub>21</sub> column showed complete separation for a mixture of benzene, naphthalene, anthracene, pyrene and triphenylene. A similar elution order was observed with the PS-DVB column (PRP-1, 5  $\mu\text{m}$ , 150 mm  $\times$  4.1 mm i.d., Hamilton Company). However, in the two chromatograms, the asymmetric factor ( $A_s$ , measured for triphenylene at 50% of the peak height), the height equivalent to a theoretical plate (HETP) and the resolution factor ( $R_s$ ), are much different:  $A_s=1.4$  and 4.7, HETP=0.08 and 1.4 mm for triphenylene and  $R_s=5.9$  and 1.7 between peaks 4 and 5 with Sil-PS<sub>21</sub> and PS-DVB columns, respectively. This remarkable peak-tailing in PS-DVB can be generally explained by rigid structures or micropores due to cross-

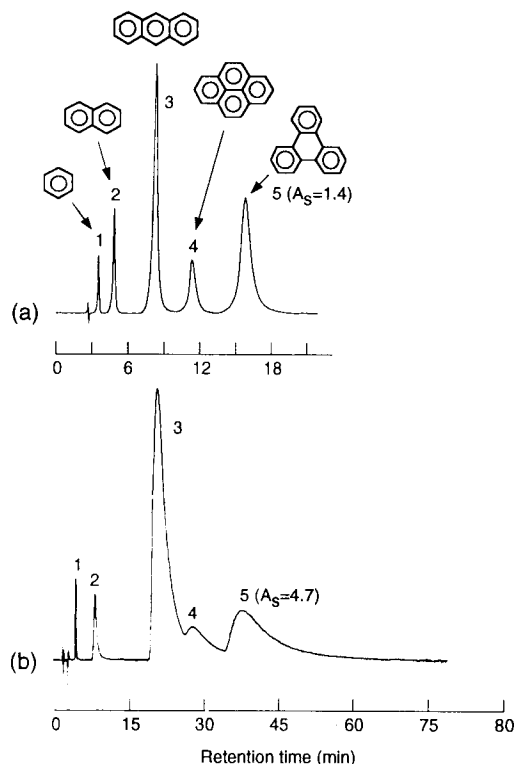


Fig. 4 Chromatograms of aromatic hydrocarbons with Sil-PS<sub>21</sub> (a) and PS-DVB (b) columns. Mobile phase: (a) methanol-water (9:1); (b) acetonitrile-water (9:1). 1, benzene; 2, naphthalene; 3, anthracene; 4, pyrene; 5, triphenylene.

linking structures.<sup>6-8</sup> Supporting this, when non-aromatic or non-rigid compounds were selected as samples, PS-DVB showed almost no peak-tailing for these compounds. On the other hand, PS<sub>21</sub> includes no cross-linking structures because of its linear polymer, and, thus, can maintain flexibility after immobilization. Perhaps, this flexibility inhibits an undesirable excessive retention for polyaromatics causing peak-tailing.

The selectivity for aromatic compounds should be compared with that of conventional hydrophobized silica gels. Therefore, triphenylene and *o*-terphenyl were selected as solutes. For these samples, ODS-2 showed a separation factor ( $\alpha$ ) of 1.6. On the other hand, the Sil-PS<sub>21</sub> column showed a better selectivity ( $\alpha=2.6$  for triphenylene and *o*-terphenyl) than did the ODS-2 column. Since the PS-DVB column also showed a good separation factor ( $\alpha=2.2$  for triphenylene and *o*-terphenyl), but remarkable peak-tailing, we estimated that the phenyl group for polystyrene provides specific affinity for polyaromatics as well as hydrophobicity. In this assumption, the  $\pi$ - $\pi$  interaction between the stationary phase and solutes probably has the most effect on the selectivity.

#### Comparison of separation factor with phenyl-bonded silica gels

Phenyl-bonded silica gel is well-known as a hydrophobic stationary phase for liquid chromatography.

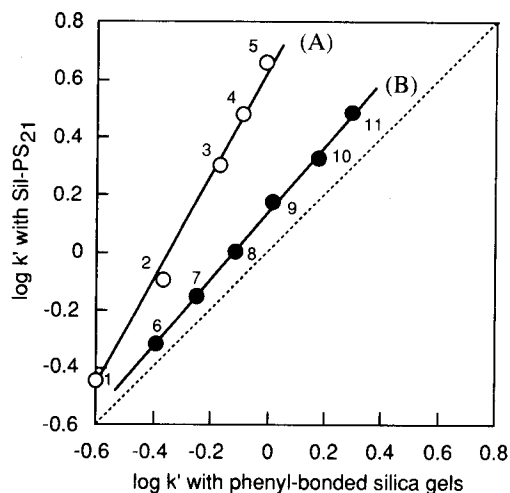


Fig. 5 Relationships between  $\log k'$  with Sil-PS<sub>21</sub> and phenyl-bonded silica gel columns. Mobile phase: methanol-water (9:1) at 25°C. Samples: 1, benzene; 2, naphthalene; 3, anthracene; 4, pyrene; 5, triphenylene; 6, ethyl-; 7, butyl-; 8, hexyl-; 9, octyl-; 10, decyl-; 11, dodecylbenzenes.

The property of this packing has been investigated by Hanai and Hubert.<sup>9</sup> The separation mode for aromatic compounds can include the  $\pi$ - $\pi$  interaction, since phenyl groups are introduced as organic phases. Therefore, the chromatographic performance of Sil-PS<sub>21</sub> should be compared with that of phenyl-bonded silica gels.

Phenyl-bonded silica gels (Inertsil PH, 5  $\mu$ m, 250 mm  $\times$  4.6 mm i.d., GL Sciences Inc.) showed good separation for a mixture of ethyl-, butyl-, hexyl-, octyl-, decyl- and dodecylbenzenes. Plots of the number of their carbon atoms vs.  $\log k'$  showed good linearity. This indicates that the separation for alkylbenzenes can be explained by the usual reversed-phase mode. A similar linearity was observed in a Sil-PS<sub>21</sub> column, and the slope of the plots was very close to that in phenyl-bonded silica gels; this is represented by the fact that the plots of  $\log k'$  with phenyl-bonded silica gels vs.  $\log k'$  with Sil-PS<sub>21</sub> provide a slope of *ca.* 1, as shown in the plots (A) of Fig. 5. These results indicate that the alkylbenzene separation mode is similar in both phenyl-bonded silica gels and Sil-PS<sub>21</sub> to each other.

Phenyl-bonded silica gels showed good separation for polyaromatics such as benzene, naphthalene, anthracene, pyrene and triphenylene. In addition, their elution peaks showed good asymmetries (*e.g.*  $A_s=1.4$  in triphenylene) like Sil-PS<sub>21</sub> ( $A_s=1.4$ ). These good peak-shapes can be explained by the non-cross-linking structure of the phenyl-bonded phase, similar to that of Sil-PS<sub>21</sub>. This demonstrates that one of the disadvantages of phenyl-bonded silica gels is their smaller capacity factor due to the smaller carbon content in the bonded phase, compared with that of conventional hydrophobic packings. However, this disadvantage can be resolved by immobilizing the polymeric phenyl groups.

Figure 5 also shows the ratio of  $\log k'$  for polyaromatics between phenyl-bonded silica gels and Sil-PS<sub>21</sub>.

Figure 5(B) shows that Sil-PS<sub>21</sub> showed longer retentions for all polyaromatic compounds used in this study than did phenyl-bonded silica gels; for example,  $k'$  for triphenylene was 4.3 and 0.98 with Sil-PS<sub>21</sub> and phenyl-bonded silica gels, respectively. This remarkable difference can be partially explained by that fact that elemental analyses (19.8wt%) of Sil-PS<sub>21</sub> showed a 1.5-times higher value of carbon content than that (13.2wt%) of the phenyl-bonded silica gels.

Figure 5 shows that Sil-PS<sub>21</sub> provides a better selectivity for polyaromatics than do phenyl-bonded silica gels; for example, the separation factor for triphenylene and pyrene was 1.43 and 1.2 with Sil-PS<sub>21</sub> and phenyl-bonded silica gels, respectively. This difference in selectivity was further emphasized by comparing the separation factors for triphenylene and *o*-terphenyl: 2.3 and 1.04 with Sil-PS<sub>21</sub> and phenyl-bonded silica gels, respectively. This remarkable difference can not be explained based only on that of the immobilized carbon content. We estimate that the conformational effect derived from the polymeric structure of PS<sub>21</sub> provides a more effective  $\pi$ - $\pi$  interaction between the phenyl groups and solutes than the isolated phenyl groups of phenyl-bonded silica gels.

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