A New Stationary Phase Prepared by Polymerization of Styrene on Initiator Bonded Porous Silica

Sung Soon Kim and Won Jo Cheong*

*Department of Chemistry, Nano Fine Center, and Institute of Basic Research, Inha University, Incheon 402-751, Korea
E-mail: wjcheong@inha.ac.kr

There have been a number of studies to attach a polymerization initiator on inorganic surface (porous or nonporous) to make a layer of organic polymer or copolymer (linear or branched) upon the surface. Such organic-inorganic hybrid materials can serve as useful stationary phases in separation sciences.[1-13] Various silica materials were used including porous silica particles,[1,4-6,7,11-13] silica beads,[2,5,10] silica monolith,[9] and silica capillary.[3,8] In the majority[1-10] of those studies, atom transfer radical polymerization (ATRP) was used to form an organic polymer layer on the silica surface. On the other hand, reversible addition-fragmentation chain transfer (RAFT) polymerization was used only in a few cases.[11-12] Attachment of a diazo type initiator to silica surface was also reported.[13] In a particular study, an initiator was bonded to porous spherical organic monolith particles, and ATRP was used to add a polymer layer with ion exchange capability.[14] In some of the above studies, molecule imprinted polymer (MIP) was formed on the silica surface.[12-13]

In ATRP, a halogen-containing initiator is attached to silica surface and a catalytic mixture composed of cuprous/cupric halides and an amine base is used to activate polymerization. The principles and applications of ATRP were well introduced in some review articles.[15-16] It is known that brushes of well determined and high molecular weight polymer with low dispersity are attached to silica surface in ATRP. However, separation efficiencies of the resulting stationary phases were found to be considerably lower than those of commercial C18 phases.

Addition of a diazo type initiator onto the silica surface is not a favorable method since off-surface polymerization also takes place when the initiator is activated.

In RAFT polymerization, a halogen containing ligand is attached to silica surface, and it is reacted with sodium diethyldithiocarbamate to yield the initiator silica. The details of principles and applications of RAFT polymerization can be found in some review articles.[17-18] No additional catalyst is required for polymerization. However, no good separation efficiency was obtained with the resultant stationary phases so far[11-12] in comparison with commercial C18 phases.

In this study, a new initiator was attached to porous silica particles to make a new stationary phase by RAFT polymerization of styrene on the initiator silica. The commercial porous silica particles were reacted with 4-(chloromethyl) phenylisocyanate followed by sodium diethyl dithiocarbarmate. The porous initiator silica particles were then allowed to

undergo radical polymerization with styrene to give a new stationary phase. This stationary phase was packed in a glass-lined stainless steel microcolumn to examine the chromatographic performance. We were able to attain the chromatographic performance comparable to that of the commercially available C18 stationary phase.

Experimental

Materials. Styrene, 4-(chloromethyl) phenylisocyanate, sodium diethyl dithiocarbamate, and anhydrous toluene were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade methanol, acetone, acetonitrile, tetrahydrofuran (THF) were obtained from SK Chemicals (Ulsan, Korea). All the reagents were used as received. Screen frits (1.6 mm diameter, 0.08 mm thickness) were purchased from Valco (Houston, TX, USA). The Alltima C18 stationary phase (5 μm, 80 Å), Adsorbosphere silica (5 μm, 80 Å), and glass lined stainless steel tubing (30 cm, 0.5 mm I.D. 1.6 mm O.D.) were purchased from Alltech (Deerfield, IL, USA).

Synthesis of the initiator silica and the new stationary phase. The initiator attached silica was prepared in two steps. In the first step, the 400 mg silica particles were dried at 120 °C for 3 h or more, and reacted with 100 mg 4-(chloromethyl) phenylisocyanate in 10 mL anhydrous toluene at 80 °C for 48 h. After completion of reaction, the product was thoroughly washed with toluene followed by acetone, filtered, and air dried. In the second step, 100 mg sodium diethyl dithiocar-
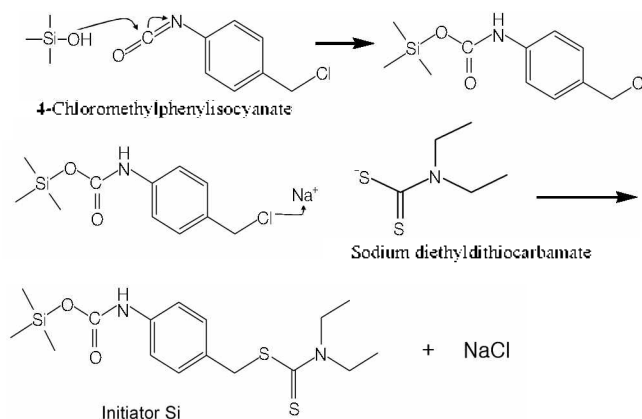
Scheme 1. The two step procedure of synthesis of the initiator attached silica

bamate was dissolved in 5 mL THF, and the solution was purged by nitrogen to remove oxygen, then the silica particles with the halogen containing ligand were suspended in the solution and reacted at 40 °C for 4 h. The product was thoroughly washed with THF, methanol, and acetone in sequence, filtered, dried in air at room temperature and stored in a desiccator before polymerization. The preparation scheme of the initiator attached silica is shown in Scheme 1.

Styrene 2.4 mL was dissolved in 10 mL toluene, and the solution was purged by nitrogen, then the 200 mg inititor silica particles were suspended by stirring, and radical polymerization was carried out under reflux at 110 °C for 24 h. According to the RAFT polymerization mechanism,[17,18] the polymer chain is introduced and grown in the C-S bond located between the methylphenyl carbamate moiety and the diethyldithio carbamate moiety of the initiator silica shown in Scheme 1. The necessary initial radicals are generated by thermal self initiation of styrene.[18] The polystyrene combined silica particles were thoroughly washed with toluene, methanol, and acetone in sequence and dried at 60 °C for 24 h.

μLC. A Shimadzu (Tokyo, Japan) 10AD pump, a Shimadzu DGU-14A membrane degasser, a Valco (Houston, TX, USA) CI4W.05 injector with a 50 nL injection loop, a Jasco (Tokyo, Japan) UV-2075 UV/Vis capillary window detector, and the home-made 0.5 mm I.D. glass-lined microcolumn were combined to compose the μLC system. The chromatographic data were obtained by a PC system, and a software Multichro 2000 from Youlin-Gisul (Sungnam, Korea) was used to acquire and process the data.

The microcolumns were packed basically according to the procedure reported before.[19-20] Thus, a frit (commercial screen frit) was placed in the 1/16 inch outlet of a 1/16-1/32 reducing union, a piece of 30 cm glass lined stainless steel tubing was fitted to the outlet, and the tubing was connected to the packer. The 100 mg particles were suspended in 10 mL methanol, and sonicated for 20 min. After filling the slurry reservoir with the suspension (slurry), the pressure of the slurry packer was instantly raised to 14,000 psi for 2 min, adjusted to 10,000 psi for the next 10 min followed by setting at 8,000 psi for 30 min. The column was then connected to the injector through a union with a piece of 5 cm stainless steel tubing (100 μm I.D., 1.6 mm O.D.) and a frit. The 1/32 inch outlet of the other column end was connected to the capillary window detector by installing a 1/32 inch graphite ferrule and a connecting

capillary (50 μm I.D., 365 μm O.D.). A piece of short Teflon tubing was used to connect the two capillary ends.

A test mix composed of phenol, 2-nitroaniline, acetophenone, benzene, and toluene was prepared in methanol and stored in a refrigerator at 4 °C before use. Various samples of different solute concentrations were prepared including 0.5-5.0 μL phenol, 0.3-3.0 mg 2-nitroaniline, 0.1-1.0 μL acetophenone, 2.0-20 μL benzene, and 1.0-10 μL toluene in 1 mL solvent. All the samples were found to be dilute enough to avoid sample overloading, and the column efficiency data obtained with the samples were all used to compute statistical parameters (Table 2).

Results and Discussion

Architecture of stationary phase. The SEM photos of the polystyrene bound initiator silica in different view scales are shown in Figure 1, and the elemental analysis results of the initiator silica, the polystyrene bound silica, and the commercial C18 phase were summarized in Table 1. As shown in Figure 1, the thickness of polystyrene layer was not uniform over the silica surface, and some lumps of aggregated poly-

Table 1. Comparison of the EA results among the initiator silica, the polystyrene bound initiator silica, and the commercial C18 phase.

Element	Initiator silica	Polystyrene bound initiator silica	Commercial C18 phase
Carbon	4.8%	16.7%	15.4%
Hydrogen	0.6%	1.4%	3.1%

Table 2. Comparison of the values of number of theoretical plates (averages and standard deviations) based on 5 batches of microcolumns (0.5 × 300 mm) packed with the stationary phase between the commercial C18 and the polystyrene bound silica.

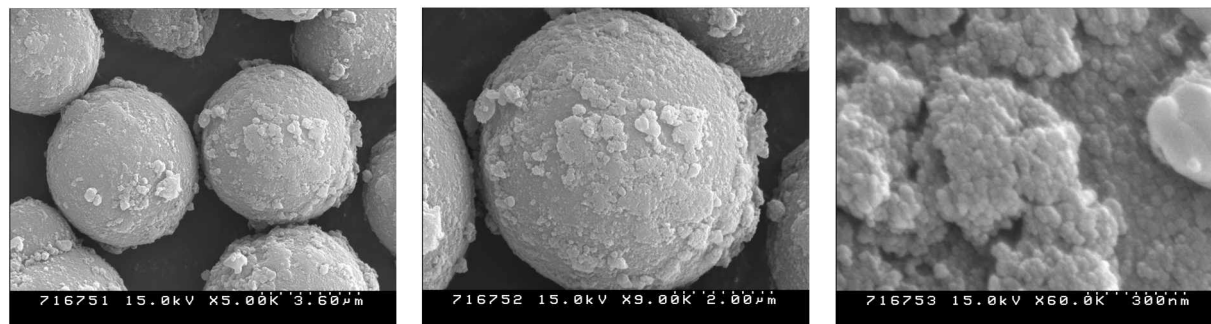| solute	Commercial C18	Polystyrene bound silica
phenol	12500 ± 900	17700 ± 500
2-nitroaniline	14600 ± 1000	18700 ± 800
acetophenone	14800 ± 1200	16800 ± 700
benzene	15100 ± 1300	19100 ± 600
toluene	15000 ± 1000	21600 ± 500

Figure 1. The SEM images of the polystyrene bound silica. From the left, the view of multiple particles, the view of single particle, and the expanded view of the surface of single particle are shown.

styrene were formed, which means that formation of poly-styrene brushes was rather heterogeneous and that polystyrene brushes of quite high molecular weights were formed so that such large lumps could be made. The reason for heterogeneous propagation of polystyrene polymer on the silica surface is not clear at present.

Comparison of separation performance between the poly-styrene combined silica and the commercial C18 phase. Two identical microcolums (300 × 0.5 mm) were packed with the polystyrene combined initiator silica particles and a commercial C18 phase (Alltima C18), respectively, to examine the chro-matographic performance of the polystyrene bound initiator silica in comparison with that of the commercial C18 phase. The same chromatographic conditions were used for both columns. The chromatographic results were compared in Figure 2. The data of number of theoretical plates (N) were compared between the two columns in Table 2.

It should be noted that the column efficiency (N) of the polystyrene (PS) combined silica column shown in Table 2 was found to be higher than that of the commercial C18 phase while retention times of the standard materials at the PS
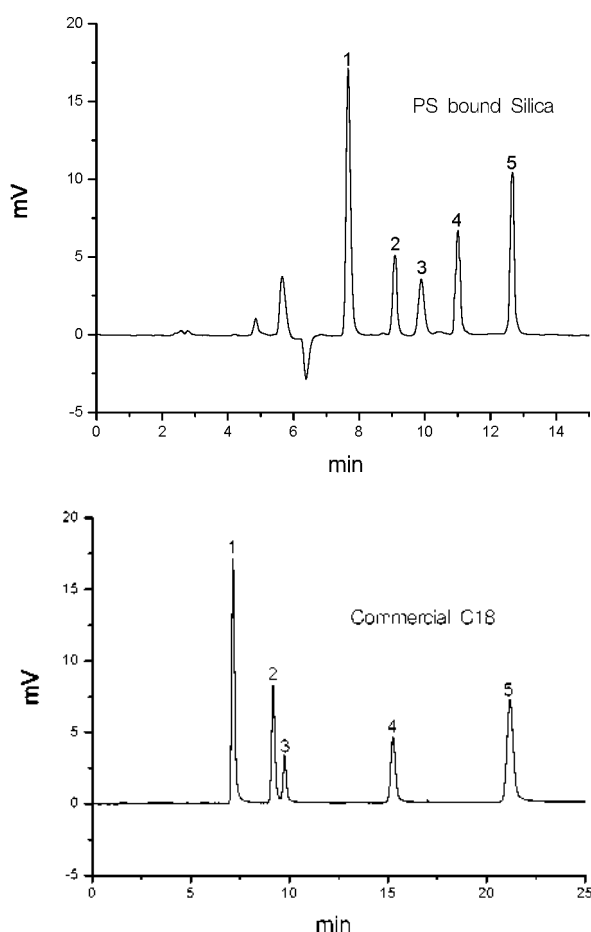

column appeared to be significantly shorter than those of the commercial C18 phase.

The above observation means that there is not only faster mass transfer kinetics but also smaller accessible stationary phase surface area in the polystyrene bound silica phase than in the commercial C18 phase. However, the carbon load of the polystyrene bound phase was higher than that of the C18 phase (Table 1).

The decrease in retention time can be explained as the surface area of the PS bound stationary phase accessible by sample components was significantly decreased due to the blocking of intraparticle pores by PS polymerization. However, the increase of column efficiency despite the decrease of retention time can be explained by the enhanced mass transfer kinetics due to the difference in sample partition between the two stationary phases and the reduction of intraparticle pores, in which the latter effect reduced the additional band bro-adening caused by the randomness of molecular paths inside pores.

The chromatographic resolution of the polystyrene bound silica was found to be considerably lower than that of the C18 phase in spite of the improved column efficiency owing to congestion of peaks due to reduced retention times. The chromatographic resolution might be improved by extension of retention times with a different eluent.

Thus it is reasonable to conclude that the performance of the new phase is comparable to that of the commercial phase. It is uncertain if such stationary phase architecture is good although some promising results were observed in this study. It is rather reasonable to form a uniform and thin polystyrene layer over the whole porous surface (outer and inner) of silica to obtain further improved separation efficiency and selectivity, which is subject to future study.

Conclusion

A new stationary phase has been prepared by synthesizing new initiator bonded silica and forming polystyrene on the silica surface. The performance of the polystyrene bound initiator silica was estimated to be comparable to that of a commercial C18 phase.

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Figure 2. Comparison of the chromatograms obtained by the poly-styrene combined initiator silica and the commercial C18 phase in 60/40 acetonitrile/ 50 mM ammonium acetate at a flow rate of 10 μL/min at 254 nm. The sample was composed of 0.5 μL phenol, 0.3 mg 2-nitroaniline, 0.1 μL acetophenone, 2.0 μL benzene, and 1.0 μL toluene dissolved in 1 mL solvent. 1;phenol, 2;2-nitroaniline, 3;acetophenone, 4;benzene, 5;toluene.

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