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Porous Silica Particles As Chromatographic Separation Media: A Review

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Porous silica particles are the most prevailing raw material for stationary phases of liquid chromatography. During a long period of time, various methodologies for production of porous silica particles have been proposed, such as crashing and sieving of xerogel, traditional dry or wet process preparation of conventional spherical particles, preparation of hierarchical mesoporous particles by template-mediated pore formation, repeated formation of a thin layer of porous silica upon nonporous silica core (core-shell particles), and formation of specific silica monolith followed by grinding and calcination. Recent developments and applications of useful porous silica particles will be covered in this review. Discussion on sub-3 μm silica particles including nonporous silica particles, carbon or metal oxide clad silica particles, and molecularly imprinted silica particles, will also be included. Next, the individual preparation methods and their feasibilities will be collectively and critically compared and evaluated, being followed by conclusive remarks and future perspectives.

Key Words : Fully porous, Nonporous, Core-shell, Hierarchical mesoporous, Ground silica monolith

Introduction

There was a comprehensive review in 2000 on synthesis of spherical porous silicas in the micron and submicron size range and their application in high performance liquid chromatography (HPLC) and capillary electrochromatography (CEC) with a special emphasis on small silica particles with sizes of 2 μm or less.[1] There have been more review articles on various silica particles. A couple of reviews on most recent core-shell and very fine particles were presented in 2012[2] and 2011.[3] A review appeared in 2012 on hierarchical mesoporous silica and organosilica materials such as MCM-41, M41S, HMS, MSU, SBA series, and periodic mesopororous organosilicas (PMOs).[4] A similar prior review[5] was reported in 2008 on hierarchical mosoporous silica materials as adsorbents for the separation. There was also a devoted review on only MSU-X silicas.[6] A variety of sub-2 μm porous silica materials were reviewed in 2012.[7] The influences of silanol groups on solute retention and the determination methods of residual silanol groups were reviewed in 2012.[8] An extensive review on development of silica based stationary phases for HPLC by various modifications was presented in 2011.[9] In addition, a review on numerous recent developments of HPLC stationary phases was given in 2013 including small particles (including core-shell), monoliths, hydrophilic interaction chromatography (HILIC) phases, and mixed mode phases.[10] Use of silica-hydride stationary phases in aqueous normal phase liquid chromatography was reviewed in 2013.[11]

In this review, the most recent progresses in preparation and application of specific silica particles such as core-shell particles, sub 3-μm particles, hierarchical mesoporous particles, carbon or metal oxide clad particles, ground monolith particles, and molecularly imprinted particles will be presented. A brief summary for the prior ages will be added in each section if necessary. Especially in a separate section (Critical overview on feasibilities of various types of silica particles), the individual preparation methods of specific types of silica particles and their feasibilities will be collectively and critically compared and evaluated. The discussion will be based on factors governing the chromatographic performances, strong and weak points, and perspectives in the future, *etc.* In the concluding section, brief summary of all the above discussions and some suggestions of future study will be presented.

Core-shell Silica Particles

The recently commercialized core-shell silica phases have attracted a great interest. Their merits are the lowest reduced plate height (h) among the present chromatographic phases and the superior capability for rapid HPLC analysis. Core-shell particles are composed of nonporous silica core and porous silica shell, and they are also called superficially porous particles. The layer-by-layer self assembly technique is in general incorporated to make core-shell particles[2,3,12] while spray-drying was adopted in the preparation of old type commercialized core-shell particles.[13] Concerning the

Abbreviations: BPA, bisphenol A; BTEE, 1,2-bis(triethoxysilyl)ethane; BTME, 1,2 bis(trimethoxysilyl) ethane; 4-CPI, 4-chloromehtylphenylisocynate; CTAB; cetyltrimethylammonium bromide; DPA, Diphenolic Acid; HETP, height equivalent to theoretical plate; HILIC, hydrophilic interaction chromatography; MIP, molecularly imprinted polymer; pCEC, pressurized capillary electrochromtography; PES, poly-ethoxysilane; PMOs, periodic mesopororous organosilicas; PSS, poly(sodium-*p*-styrenesulfonate); RAFT, reversible addition fragmentation transfer; SDS, sodium dodecyl sulfate; SEC, size exclusion chromatography; TBBPA, tetrabromobisphenol A; TEOS, tetraethoxysilane; UHPLC, ultra high pressure liquid chromatography.

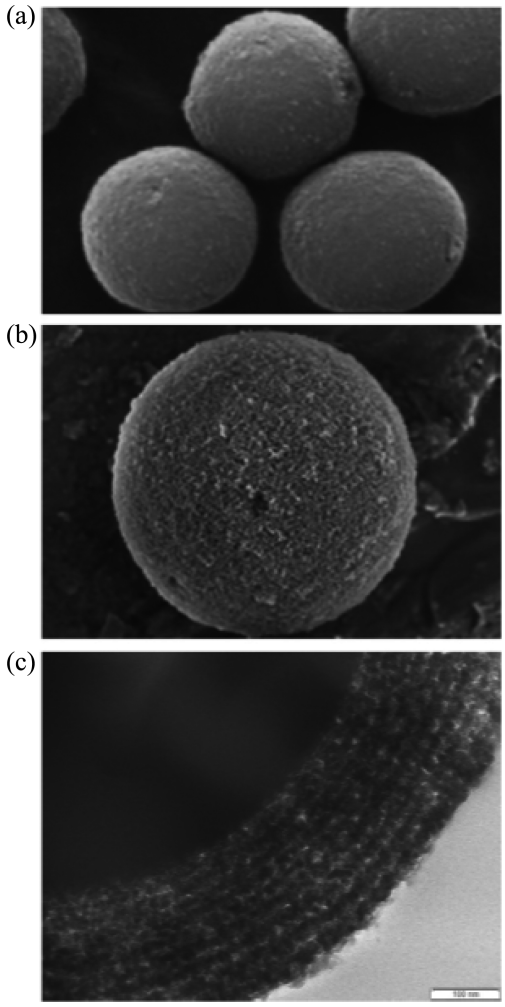

Figure 1. Scanning electron microscopy (SEM) photographs of 2.6 μm particles (a and b). (c) Zooming at the external surface of the particles. Note the apparent smoothness of the external surface area, the homogeneity of the thickness of the shell, and the structure (10–11 overlaid layers) within the porous shell. Reproduced with permission of Phenomenex, Torrance, CA.[23]

reasons of improved performance, some different opinions were suggested.[10]

Nonporous sub-2 μm silica particles are required as the raw material for cores. Such nonporous particles were first made in the study of Stöber *et al*..[14] Some modifications of such method were made to improve productivity.[15-18]

Core-shell silica particles fulfill the short diffusion paths (fast mass transfer kinetics) and the need for use of rather cheap conventional HPLC system instead of ultra high pressure liquid chromatography (UHPLC) system.[2,3,10] Such good performances of core-shell particles can be obtained only with nonporous core. The core-shell particles made of porous cores showed poor separation performance[12]. The superiority of core-shell particles over fully porous particles was more evident for analytes of high molecular weights.[19-22]

In the study where two different brands of C18 modified core-shell particles were compared, the particles with a very narrow particle size distribution, nearly spherical geometry, a smooth external surface and a uniform shell thickness (Fig. 1), were found to show better chromatographic performances.[23]

Very recent applications of core-shell particles are summarized below. Fast HPLC separations of proteomic samples were reported.[24] Comparison of separation efficiency was made between the core-shell and fully porous particles ODS bonded phases where the core-shell bonded particles showed better results as expected.[25] A comparative HPLC study was made in chiral separation on the stationary phases that were made by coating a polysaccharide ligand on core-shell and fully porous silica particles, and the former showed clearly better separation performance.[26] Using a high pressure was proposed to re-equilibrate columns faster as the simplest method in the study on re-equilibration time of core-shell based columns in gradient elution.[27]

The 1.3 μm core/shell particles have been very recently commercialized, and some relevant studies have been reported.[28-30] The achievements of H_{min} of 1.95 μm (corresponding to 500,000 plates/m) and the shortest analysis time were reported, but limitations based on current instrumentations (in view of pressure limit) and extra-column band broadening were also indicated.

Sub 3 μm Silica Particles

The importance of sub-3 μm particles has been recently enhanced owing to the advent of ultra high pressure liquid chromatography for fast analysis. Superficially porous (core/shell) sub-3 μm silica particles have also been prepared recently.[2,3,7,10,19-30]

Nonporous Silica Particles. Nonporous silica particles have been used for quick separation of peptides and proteins.[1] A couple of later studies are introduced below. A 43-cm-long capillary column of 10 μm I.D. packed with C18 1.0 μm nonporous silica particles was used to get 730,000 plates/m under 6800 bar.[31] Home-made 1 μm nonporous silica particles were C18 modified and packed electrokinetically into fused-silica capillaries with 100 μm I.D. for a length of 20 cm to get 200,000 plates/m under 500 psi in CEC.[32] No more studies of such long columns (20 cm or longer) packed with such small nonporous silica particles have been reported afterwards either in HPLC or CEC probably because of the required pressure for such HPLC beyond the limit of acceptable common instruments and poor stability and reproducibility of such pressured CEC (pCEC) systems.

Porous Silica Particles. The problems of nonporous silica particles such as low sample loading capacity and too high column back pressure may be lessened when porous silica particles are used instead. However still quite high pressure is required up to 15,000 psi (1,000 bar) compared to the common pressure limit of 6,000 psi (400 bar) of the conventional HPLC, and this new system is called ultra high pressure liquid chromatography. Much faster analysis is possible in UHPLC than in conventional HPLC if similar separation efficiency is pursued for both systems.[33]

A test sample was injected at overload concentrations onto

three separate C18 columns containing 1.7, 3.5, and 5.0 μm porous particles to compare the performances in preparative LC,[34] and both the number of theoretical plates and resolution were consistently superior for the 1.7 μm particles.

In an interesting study, a systematic evaluation of the possibilities and limitations of the separations obtained with 5 cm columns packed with 1.5-3.0 μm fully porous particles was carried out.[35] The same efficiency could be obtained with columns packed with 1.9-2.1 μm particles as with smaller particles. Moreover, the lowest reduced plate height minimum (h = 2.2) was achieved for the column packed with 3 μm particles. In another study, a systematical evaluation of various phases was carried out, and the authors concluded that among the considered columns (sub-3 μm core-shell silica particles, fully porous sub-2 μm silica particles, and monoliths), the column packed with the 2.6 μm Kinetex core-shell particles gave the best chromatographic performance.[36] A commercial C18 modified phase based on 1.7 μm hybrid silica particles having bridged ethyl/siloxane silica structures was explored as a promising phase for UHPLC.[37] The manufacturing method of ethyl-bridged hybrid particles having an empirical formula $(SiO_2)(O_{1.5}SiCH_2CH_2SiO_{1.5})_{0.25}$ was previously introduced in the literature.[37,38] This phase showed better resistance to basic environments than conventional silica particles.

Hierarchical Mesoporous Silica Particles

The structure of hierarchical silica is characterized with periodically arrayed uniform pores. The preferred format for chromatographic application is particles with mesopores. Mesopores are defined as pores with sizes in the range of 2-50 nm. The typical size of mesopores of hierarchical mesoporous particles has been less than 5 nm, and progresses have been achieved only recently to make hierarchical silica particles of larger mesopores.

The commonly adopted synthetic method of various mesoporous silica materials is to couple the "sol-gel" process to supramolecular self-assembly. Surfactant-silicate species are formed by the hydrolysis and condensation reactions of silica precursors in the presence of surfactant and their micelles, and gel particles are formed and precipitated as the reactions proceed. The filtered solid materials are calcined to make pores. The factors of reaction conditions for determination of structural features of the final mesoporous materials are pH, reaction temperature, type of surfactant or silica source, *etc.* More details of the history of development of various mesoporous silica materials, different synthetic mechanisms proposed by different research groups for various mesoporous silica materials with different structural features, and their surface modifications can be found in some review articles.[4-6]

Most of the above-mentioned studies have been for particles of rather smaller mesopores (2-5 nm), and such particles are not appropriate for chromatographic applications. In this review, only some meaningful developments of hierarchical mesoporous silica particles for efficient chromatographic separation will be discussed.

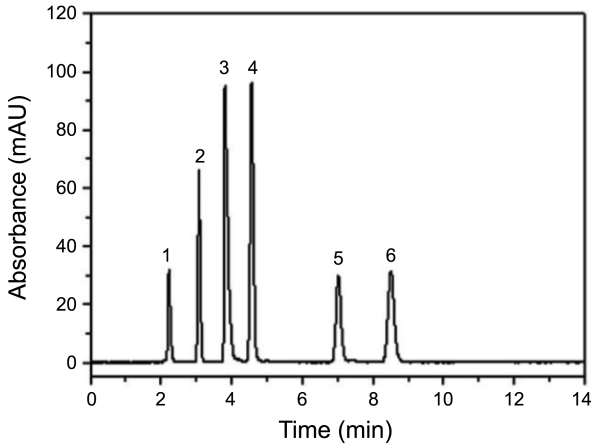

Figure 2. Separation of mixtures on the column packed with C18-SBA-15-1 silica. Solutes: (1) uracil; (2) phenol; (3) *o*-methylaniline; (4) nitrobenzene; (5) toluene; (6) naphthalene. Column: 150 mm × 4.6 mm I. D. Mobile phase: acetonitrile/H_2O mixture (60/40, v/v) at 0.8 mL/min. Column temperature: 30 °C. UV detection at 254 nm. Reproduced with permission of Ref. [46].

The first meaningful chromatographic separation might be in the study of Thoelen *et al.* where spherical MCM-41 particles covalently linked with R-naphthylethylamine as selector were used to show good chiral resolution (R) of racemic mixtures.[40] Spherical MSU-1 particles of 7.6 μm radius and 2.5 nm pores were used to separate aromatic hydrocarbons, but the solute bandwidths were rather broad as easily predicted by the too small pore size (2.5 nm).[41] Spherical SBA15-type particles (radius > 3 μm, 5-10 nm pore size) were prepared with TEOS as silica source, triblock copolymer Pluronic P123 poly(ethyleneoxide)-poly(propyleneoxide)-poly(ethyleneoxide) ($EO_{20}PO_{70}EO_{20}$) as surfactant and cetyltrimethylammonium bromide (CTAB) as co-surfactant, then C18 modified, end-capped, and used for separation of aromatic hydrocarbons.[42] Spherical micrometer-sized silica particles of ultra-large pore volumes (1.6-2.4 mL/g) and large tunable pores (26-50 nm) with narrow pore size distribution, were prepared for fast size exclusion chromatography (SEC) of polystyrene only to give incomplete separation performance.[43]

In recent years, some progresses in separation performance have been obtained. Specific spherical MCM-41 particles (5 μm, *ca* 3 nm pore) were prepared by stirring commercial silica particles (5 μm, 10-15 nm pore) in a NaOH solution containing a surfactant at room temperature followed by cooking at 100 °C.[44] Those particles were C18 modified and used for HPLC separation, and their separation efficiency was found to be similar to that of the C18 modified phase of the original commercial silica particles. A specific study was reported for chromatographic application of organic-inorganic hybrid silica particles,[45] where such particles were prepared by using 1,2 bis(trimethoxysilyl) ethane (BTME) and alkyltrimethylammonium-chloride or -bromide surfactant. The C18 modified particles (6-8 μm) resulted in slightly inferior separation performance to the commercial C18

phase (5 μm). Micrometer-sized (2-4.5 μm, 20-30 nm pore size) SBA-15 silica spheres were prepared using triblock copolymer ($EO_{20}PO_{70}EO_{20}$), 1,3,5-trimethylbenzene and KCl.[45] The separation performance of the particles (4.5 μm) after C18 modification was found quite good (N~ up to 79,240/m) (Fig. 2).[46] Silica spheres with periodical large-pore size (up to 20 nm) and uniform small particle size (1-1.7 μm) were synthesized by utilizing a new kind of surfactant to show great potential application in HPLC.[47]

A series of spherical hybrid MSU-1 with homogeneously distributed ethane groups in a silica framework were prepared using 1,2-bis(triethoxysilyl)ethane (BTEE) and alkyl polyethylene oxide (Tergitol 15-S-12) surfactant where hydrolysis of BTEE was executed in acidic condition and condensation of silicate species was activated by F^- catalyst.[48] The materials had wormhole-like mesopore structures. The C8 modified hybrid MSU-1 (3-4 μm, 2.7 nm pore size) was packed in a microcolumn to demonstrate a good performance that was still inferior to that of commercial C8 columns.[48] Spherical aminopropyl-functionalized ethane-bridged periodic mesoporous organosilicas (6-7 μm, 4.1nm pore size) of ordered cubic mesostructure were prepared by a one-step co-condensation of BTEE and 3-aminopropyltriethoxysilane using cetyltrimethyl ammonium chloride with the aid of a co-solvent (methanol) in basic medium,[49] and the product was found a potential stationary phase with the good chemical stability in basic mobile phases.

Monodisperse phenylene-bridged organosilica spheres were synthesized by co-condensation of 1,4-BTEE and TEOS under basic conditions with an aid of dodecylamine and CTAB as templates.[50,51] Then pore expansion was carried out by two-step hydrothermal treatments in a Teflon-lined autoclave using *N,N*-dimethyldecylamine/dodecylamine and tris-(hydroxymethyl)-aminomethane (TRIS) in sequence to give particles of 3.0-3.5 μm size and 8.5 nm pores.[51] After C18 modifiaction, the phase was examined for chromatographic performance in comparison with the C18 phase based on regular silica spheres (5 μm, 10nm). The results showed similar separation efficiencies (*ca* 95,000 plates /m based on the data obtained with columns of 50 mm × 2.1 mm I.D.) but much greater chemical stability of the former than that of the latter.[51]

The sorbents based on hierarchical mesoporous silica have not proved comparable chromatographic efficiency to conventional sorbents yet in general. Thus it seems that the pores in the common amorphous structure are much better structured (well-connected pores) in view of mass transfer kinetics than the hierarchical pores (rather isolated pores). Improvement of connectivity of pores may be the key issue of future developments.

C or Metal Oxide Clad Silica Particles

A serious weak point of silica based chromatographic phases is decomposition of the silica skeleton upon exposure in basic environments. Coating the porous surface of silica with a layer of material having durability against basic environments is the method to solve the problem. Carbon and metal oxides have been used for such a purpose.

C or Carbon Nanotube Clad Silica Particles. The single wall carbon nanotubes were functionalized to have acyl chloride (-COCl) groups, and reacted with 3-aminopropyl-silica particles, and the product (the first carbon nanotube modified silica phase) was packed in a column and used for separation of polynuclear aromatic hydrocarbons.[52] This study opened the possibility of carbon nanotube clad silica particles as chromatographic separation media. Hydroxy carbon nanotubes (1.5-2 μm length, O.D. < 8 nm) were layer-by-layer adsorbed onto porous silica particles (7 μm, 28.1 nm pore size) with the aids of sonication, stirring, and a specific surfactant poly(sodium-*p*-styrenesulfonate) (PSS).[53]

Carr and coworkers developed carbon clad silica phases by treating the silica particles (5 μm) with a monolayer (or less) of Al (III) cation for binding to deprotonated silanols followed by chemical vapor deposition at 700 °C using hexane as the carbon source without damaging the silica's native pore structure.[54] The new carbon clad phase behaved as a reversed phase and had higher retentivity than carbon clad zirconia particles with reasonable separation efficiency (50,000-79,000 plates/m). Next, the same research group adopted the above method to develop a novel carbon phase on superficially porous silica (2.7 μm), and the new packing maintained the good mass transfer properties of core/shell particles as demonstrated by the effect of velocity on HETP, and exhibited efficiencies up to 5.6-fold higher than carbon clad zirconia particles especially for polar compounds.[55]

Metal Oxide Clad Silica Particles. Core/shell type ZrO_2/SiO_2 (ZrO_2 shell, SiO_2 core) spheres were made by dispersing silica particles (3.49 μm, 13 nm pore size) in a sodium dodecyl sulfate (SDS) solution to allow formation of SDS monolayer on the silica particles followed by depositing zirconia sol on them, and such steps (alternate adsorption of surfactant SDS and zirconia sol) were repeated (Fig. 3).[56] The product exhibited high surface area and pore volume compared to commercial ZrO_2 particles while showing similar excellent chemical stability to ZrO_2. They did not report any chromatogram obtained by the C18 bonded ZrO_2/SiO_2 phase. Instead, they showed chiral separation performance of cellulose derivative modified ZrO_2/SiO_2, which was comparable to that of cellulose derivative modified SiO_2. The same

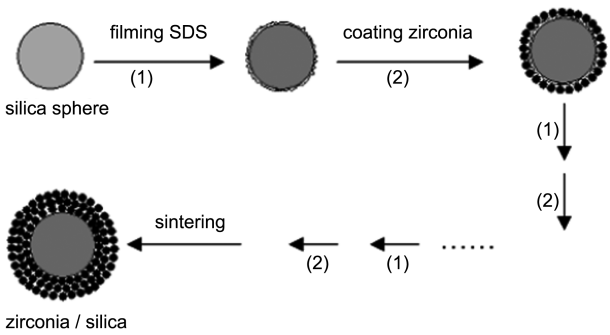

Figure 3. Preparation steps for ZrO_2/SiO_2 spheres. Reproduced with permission of Ref. [56].

group of the above study[56] reported chromatograms obtained with the C18 ZrO_2/SiO_2 in another study.[57] Next, the same group prepared the C18 TiO_2/SiO_2 phase similarly.[58] The chromatographic performance of the above studies was inferior to that of conventional C18 silica phases. The reason seems to be the improper choice of the porous core silica particles (13 nm pore) instead of non-porous core.

Core/shell type Al_2O_3/SiO_2 (Al_2O_3 shell, SiO_2 core) spheres were made by dispersing silica particles (7 μm, 30.9 nm pore size) in a PSS solution with stirring to allow formation of PSS monolayer on the silica particles followed by depositing alumina sol on them then drying/ calcination, and such steps (alternate adsorption of surfactant PSS and alumina sol followed by calcination) were repeated 10 times to give Al_2O_3/SiO_2-10.[59] The thickness of the alumina particle multilayers was 4.8 nm, and the diameter of the alumina particle was about 0.25 nm. The pore size of Al_2O_3/SiO_2-10 was 21.3 nm.

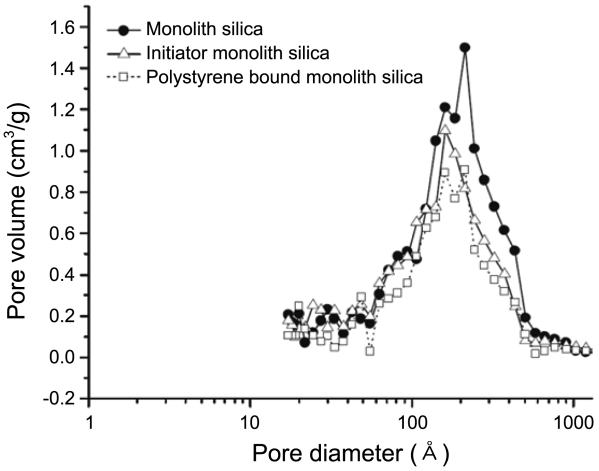

Figure 4. The BJH differential pore volume (dV/dlogD) plots against pore size for the ground monolith silica, initiator bound monolith silica, and polystyrene bound monolith silica. Reproduced with permission of Ref. [63].

Ground Silica Monolith Particles

A silica monolith is a one-body silica structure having through-flow channels (macropores) and mesopores. The silica monolith is made by a sol-gel process with a reaction mixture composed of silica precursor, porogen, catalyst, and solvent (water in general). The formed gel is calcined to remove organics and to form pores. The calcined monolith is modified (typically C18) and entrapped in a polymer column.[60] Some commercial C18 monolith columns are available. The monolith columns are of a very low column back pressure, and are useful for fast analysis at a high flow rate. However, their separation efficiency is in general inferior to that of conventional C18 particles probably owing to the internal void volume (too large total volume of macropores). The void volume can be minimized by adoption of a reaction mixture with a higher content of silica precursor, but then a trouble may be encountered in the modification step for the reaction rate will be slow and the reaction yield will be low. Ground silica monolith particles may be used to solve both problems.

A series of very unique studies have been carried out in our laboratory[61-65] where silica monolith was first made and ground into particles, and used as the raw material for stationary phases of HPLC. In the first study of the series, a hard silica monolith was made, ground, washed, and sieved to give a major portion of 5-10 μm particles which were then C18 modified.[60] The micro-column (30 mm length, 0.5 mm I.D.) packed with this phase showed reasonable column separation efficiency (column plates of 15,000-20,000).

Next, some modification of the formulation of the reaction mixture for monolith formation was made and coupled to the same post-procedure to yield a major portion of 3-5 μm particles, and the C18 modified product showed improved column separation efficiency of 24,000-35,000 plates[62] when packed in a column of 0.5 mm I.D. and 30 mm length.

The production process was further improved to enable removal of the sieving step resulting in particles with rather large pores (Fig. 4) that were modified with polystyrene by reversible addition fragmentation transfer (RAFT) polymerization (Fig. 5), and the product showed column separation efficiency of 26,000-32,000 plates.[63] The ground silica monolith particles were reacted with 3-chloropropyl trimethoxysilane followed by sodium diethyldithiocarbamate to give the initiator attached particles (S2) (Fig. 5). The above process was further improved to result in particles of smaller sizes (partially sub 2 silica monolith particles) and wider pores (34.3 nm) by adoption of the subtle formulation of reaction mixture and specific multiple heating steps, and the polystyrene modified product exhibited column separation efficiency of average 49,500 plates.[64] The same silica particles of Ref. 64 were reacted with 4-chloromehtylphenylisocynate (4-CPI) *via* catalyzed isocyanate-hydroxyl reaction followed by sodium diethyldithiocarbamate to give the S1 type initiator attached particles that were modified with polystyrene by RAFT polymerization (Fig. 5).[65] The column (300 mm length, 1 mm I.D.) packed with this new phase showed column separation efficiency of average 56,500 plates (Fig. 6), which is better than that (N per column) of any commercial HPLC or UHPLC column so far.[65] The catalyzed isocyanate-hydroxyl reaction enabled formation of S1 with a high density of initiator moiety to cause simultaneous and uniform growth of polymer chains on individual initiator ligands followed by termination upon congestion of the grown chains. Reduced average particle size, partially monolithic architecture after packing, formation of homogenous and uniformly distributed thin polymer film, and pseudo core-shell type behavior were claimed to be the factors contributing to such enhanced separation efficiency.[64,65] In addition, the silica monolith particles produced by being formed as soft bulk monolith, smashed into particles, and calcined, may be subject to mass production at a low cost in a small space, thus the stationary phases based on these silica particles are likely to enable realization of disposable micro-columns in the future.

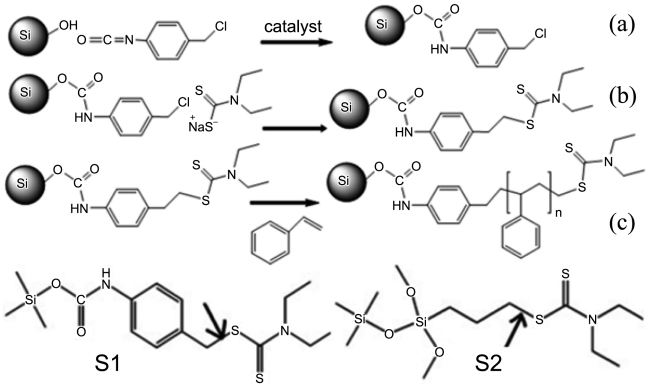

Figure 5. Schematic pathways for synthesis of silica modified with chlorine-terminated ligand (A), initiator attached silica (B), and polystyrene bound silica (C), and the initiator silica structures prepared with 4-CPI (S1) and 3-chloropropyltrimethoxysilane (S2). The arrows denote the bond where the polymer chain is introduced and grown. Reproduced with permission of Ref. [65].

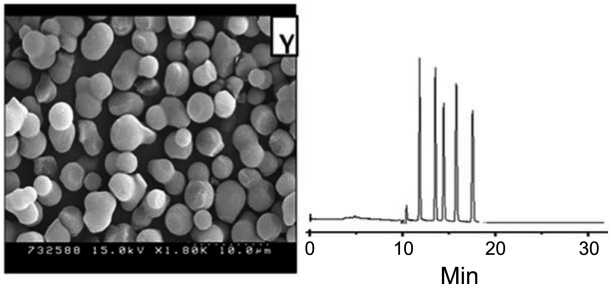

Figure 6. The SEM image of the polystyrene modified silica monolith particles and the chromatogram obtained with the column (1 mm × 300 mm) packed with the phase. <Eluent> 60/40 (v/v) ACN/water with 0.1% TFA, The elution order of analytes was phenol, acetophenone, 4-methyl-2-nitronaniline, benzene and toluene. Reproduced with permission of Ref. [65].

Molecularly Imprinted Silica Particles

Molecular imprinting technique has been employed in a wide scope of applications such as chemical sensing, separation science, drug delivery and catalysis.[66] Molecular imprinting is usually carried out by polymerization of a reaction mixture composed of a template, a functional monomer, a cross-linking monomer, an initiator, and porogenic solvents followed by removing the trapped template molecules to leave cavities complementary to the template in size, shape, and molecular interactions.[67] A single monomer may be used as both functional and cross-linking monomer. Molecularly imprinted polymer (MIP) can be used as separation media. If an optical isomer is used as the template out of the two enantiomers, then the resulting MIP can be used for chiral separation.

The most desirable format would be direct inclusion of the template cavities in the silica matrix. No study has been applied to mass separation yet, and their applications have been in the stage of exploring increased binding capacity, SPE, or preliminary chromatography. Some recent studies will be introduced below.[68-72] The typical method was to simply mix the template in the reaction mixture and carry out the sol-gel process followed by removal of template *via* thorough washing.[69,70,72] Another method was to incorporate the template-modified precursor in the reaction mixture of sol-gel process and to remove the template by a specific reaction.[68,71] The molecular imprinting was made either in the bulk monolith[68,70,72] or in the surface layer on silica particles (surface imprinting).[69,71] The employed precursors were TEOS,[68-71] 3-aminopropyl triethoxysilane,[69,71] phenyl trimethoxysilane,[69] and 3-(trimethoxysilyl) propylmethacrylate.[72]

Modification of Silica Particles

Spherical porous silica particles are still the most frequently used raw material for preparation of stationary phases of liquid chromatography. The traditional C18 modified silica particles have been the prototype of modern stationary phases although other various types of modifications have also been continually tried.[9,10] They include other nonpolar and medium polar phases for reversed phase liquid chromatography (RPLC), polar phases for hydrophilic interaction chromatography (HILIC), ionic phases for ion exchange chromatography, ionic-liquid modified phases, polymer modified phases, and chiral stationary phases. Such a variety of stationary phases have been commercialized, and the C18 phase is still the item of a major market, which may reflect the fact that better separation efficiencies have been hardly observed in other stationary phases than the C18 phase. Another item of a large market is chiral stationary phases on which many review articles have been published.[73-79] Chiral stationary phases bear a great importance in pharmaceutical fields. A variety of chiral ligand modified silica particles have been commercialized. Interestingly, the majority of the chiral ligands are founded on either one of the natural biological enantiomer units, that is, D-glucose and L-amino acids, or both.

Critical Overview on Feasibilities of Various Types of Silica Particles

The preparation methods of various types of silica particles are comparatively overviewed in Figure 7. For a conventional phase, a rather simple and effective method is given as a representative one where a silica precursor (such as TEOS) and a long chain alkylamine are mixed in water/2-propanol phase of basic pH (containing ammonia) to result in precipitation of monodispersed sub 3-μm particles in a single-step reaction. Core/shell particles are prepared by the following steps: 1) Formation of nonporous core particles, 2) Suspension of the core particles; 3) Formation of a thin silica layer by adding a silica precursor, a basic catalyst, and a surfactant followed by filtration; 4) Repeating the steps 2) and 3) until the desired shell thickness is obtained. The typical hybrid porous particles can be prepared by the co-condensation of 1,2-bis(triethoxysily)ethane with TEOS, and the particle size can be controlled in the range of 1.5-5 μm with a large pore size. The preparation procedure of MCM-41 is shown as the

representative hierarchical mesoporous particles in Figure 7. The silica sol skeleton is formed by impregnating the hexagonal array of micelle rods with the catalyzed solution of silica precursor followed by removal of the organic micelle rods by calcination. Appropriate control of reaction parameters enables the gathering of hexagonal structures into spherical particles. The schematic procedures for preparing the diphenolic acid-imprinted polymers (a) and bisphenol-A-imprinted polymers (b) on the surfaces of silica particles are illustrated in Figure 7 as an example of molecularly imprinted silica particles. The template is first dissolved in 3-aminopropyltrimethoxysilane and mixed with TEOS, a catalyst, and silica particles suspension. After surface imprinting, the template is removed by solvent washing. The last part of Figure 10 illustrates the procedure of synthesis of silica monolith particles, that is, preparation of reaction mixture of a well controlled formulation (tetramethoxysilane, porogen, and catalytic solvent), formation of soft monolith by multi-stage heating, drying, grinding, and calcination.

Some questions may arise in selecting appropriate silica particles for given specific chromatographic tasks. Different conclusions may be derived depending upon what factor is primarily considered. What type and size should be chosen if short analysis time is of the first concern?; What about the case of looking for high separation efficiency?; What particle shape is suitable?; Is a phase of narrow particle size distribution mandatory?; What particle type is the most suited for good column packing?; What about cost and working convenience?

When short analysis time is wanted, a short column packed with small (sub-3 μm) particles is generally used at a high flow rate. Core/shell particles are certainly the first choice in this case. The disadvantages of core/shell particles are the lower sample loading capacity and presumably the higher production cost, which could be a major barrier to application in preparative scale separation or production of disposable microcolumns.

There may be some limitations for actual fast analysis. If a gradient elution is adopted, there should be an enough interval time preserved for complete re-equilibration between consecutive runs. The re-equilibration time will be longer than the analysis time for the runs of fast analysis. If the virtual analysis time is defined as the sum of the actual LC run time and the re-equilibration time, then the significance of fast analysis is faded away much. For most of the biological applications such as proteomic analysis, not only good separation but also identification of each component should be secured, thus LC/MS (or LC/MS/MS) is generally required. The general trend of LC/MS is towards nano-LC/MS for enhanced detection sensitivity, which is incompatible with fast analysis operated under a high flow rate. In addition, an enough interval time is required between runs to eliminate the memory effect of the previous run. Moreover, the column packed with core/shell particles may not be strictly demanded when a sample with a few components separable with minimum separation efficiency is routinely analyzed. In conclusion, the phase based on core/shell particles is the item of the first consideration for fast analysis, but the other phases may be good enough depending upon analytical requirements and sample situations.

Is it really required to use spherical particles with narrow particle size distribution for desirable column packing quality and good separation efficiency as has been believed until very recent days? Such belief may be altered now. Ground silica monolith particles made of bulk soft monolith have shown even better separation efficiency than the fully porous spherical silica particles although the average size and distribution of the ground monolith particles are larger and broader than those of the conventional spherical particles. The column packing efficiency has also been improved with monolith particles, thus a longer column can be packed compared to the case of spherical particles. Thus the column packed with this phase has shown the higher number of theoretical plates per column than any commercial HPLC or UHPLC column so far. Silica monolith particles are expected to enable realization of disposable microcolumns owing to the low production cost. The disadvantages of this phase are the higher reduced plate height and the more reduction of separation efficiency at a fast flow rate in comparison to core/shell particles. If fast analysis is the primary concern, then core/shell particles may be adopted. Otherwise, monolith particles or fully porous spherical particles may be employed instead.

As for particle size, there is a simple basic principle in view of column separation efficiency and column back pressure. The column separation efficiency is enhanced and the column back pressure is increased with a decrease of particle size. In other words, the better separation performance is obtained at the price of increased cost (a more expensive LC system for the higher pressure). A variety of UHPLC systems are commercially available now. Nevertheless, none of them is compatible with sub-1 μm particles yet. Limitations related to particle size were also discussed in a recent review.[10] The upper limit of packable column length is also decreased with the decrease of particle size. Thus the net effect of decreasing particle size is to reduce the analysis time with the same separation efficiency under an increased operation pressure. The column packed with the smaller particles is also subject to easier column clogging. In the analytical tasks where fast analysis is of no significance, it is unnecessary to reduce the particle size. If only column pressure drop is considered, the order of preference may be bulk monolith > ground monolith > spherical fully porous > spherical core/shell > spherical nonporous. Excluding bulk monolith, it is also the order of column packing quality.

If operation under wider range of pH (usually basic environments) is required, carbon or metal-oxide clad silica particles or hybrid organo-silica particles (either conventional or hierarchical) can be considered. Molecularly imprinted silica particles have shown only limited application in chromatographic separation so far although their potential for analytical and preparative scale separation is great. Development of advanced surface imprinting techniques will be the crucial issue to solve for practical application of molecularly

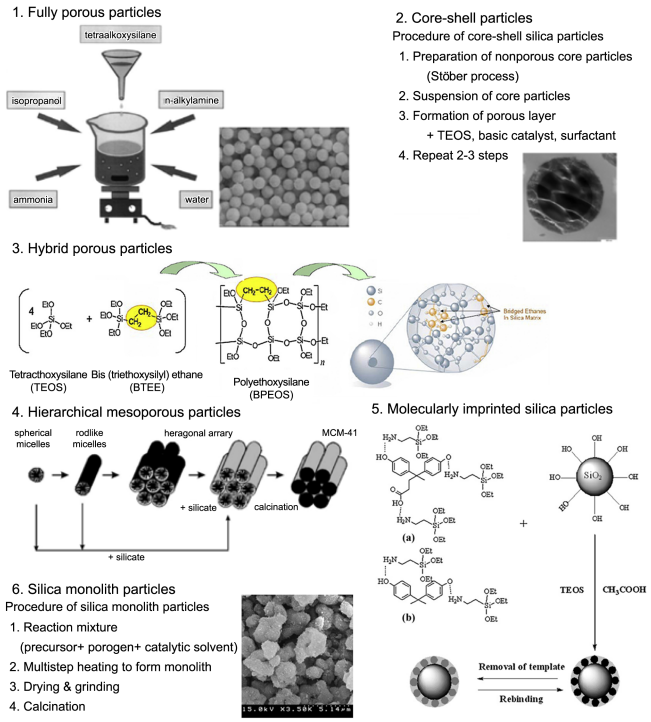

Figure 7. An overview of preparation of various silica particles. **1**. Fully porous spherical particles. A rather simple and effective method is shown (refer to the text) among the many developed methods. Reproduced with permission of Ref. [1]. **2**. Core-shell particles. The brief description of the preparation procedure is given. Reproduced with permission of Ref. [3]. **3**. Hybrid porous particles. This material was prepared by the co-condensation of 1,2-bis(triethoxysily)ethane with TEOS. Reproduced with permission of Ref. [7]. **4**. Hierarchical mesoporous particles. The preparation procedure of MCM-41 is shown (refer to the text). The hexagonal structures can be gathered into spherical particles by appropriate control of reaction parameters. Reproduced with permission of Ref. [5]. **5**. Molecularly imprinted silica particles. Schematic procedures for preparing the diphenolic acid-imprinted polymers (a) and bisphenol-A-imprinted polymers (b) on the surfaces of silica gel particles. Reproduced with permission of Ref. [71]. **6**. Silica monolith particles. The brief description of preparation procedure is given. Reproduced with permission of Ref. [64].

imprinted silica particles in the future. On the other hand, their application in SPE has been relatively successful.

It should be noted that the column separation performance is not just related to the type of stationary phase. Additional factors may include physical design of column, packing structure after packing, column packing procedure, and fritting techniques. These factors have not been studied well compared to the stationary phases and more relevant studies are required in the future. Miniaturization of column dimension is the general trend, which is against the efforts for improving packing quality. Fritting also becomes an important factor owing to miniaturization. Future advances in column packing techniques may be helpful to lessen such burden.

Concluding Remarks

The stationary phases based on core-shell type spherical silica particles have been recently developed and commercialized, and regarded to be the chromatographic media of choice. Nevertheless, the stationary phases (especially C18) based on 3-5 μm fully porous spherical silica particles are likely to be the major item prevailing in the market. Nonporous spherical silica particles are inferior in view of sample loading capacity or column back pressure, thus the phases have been used only for fast HPLC analysis of large and polar molecules using short and wide bore columns packed with typically 1 μm particles. Such a trend of limited use will continue. The separation efficiencies of the stationary phases based on hierarchical mesoporous silica particles have never been better than those of traditional C18 phases probably due to the feature of rather isolated pores, and efforts to improve the connectivity among pores are required if they are considered as chromatographic media. It has been difficult for the separation performances of the phases based on carbon clad or metal oxide clad silica particles to be equal to or better than those of the conventional C18 phases. Nevertheless more relevant studies are expected in the future since such phases show dramatically improved stability in basic eluents enabling a wide scope of feasibility. Another type of stationary phase usable in basic environments is hybrid silica particles prepared by incorporating a silica precursor bridged with an organic moiety. They are not compatible with calcination while the conventional silica particles can be calcined to enhance the particle strength and adjust the pore size. Hybrid silica particles may be directly used for chromatography without further modification, thus related research will continue for such a merit. Molecularly imprinted silica particles can be made by just incorporating a template in the reaction mixture with some modification of silica precursor, but cannot be calcined to enhance the mechanical strength for the risk of rupture of the template cavities. A dummy molecule whose molecular structure is similar to that of the analyte can be used as the template to reduce the production cost. Development of this phase is only at the early stage, thus more extended studies are expected in the future. A new generation of silica particles has appeared recently, *i.e.*, ground silica monolith particles. A soft cake of bulk silica monolith is formed, smashed into particles, and calcined to give particles of irregular shapes and wide but proper size distribution. The particles may be subject to C18 modification or RAFT polymerization to have polymer ligands. Especially, when the particles were allowed to have a high density of initiator moieties *via* catalyzed isocyanate-hydroxyl reaction and modified with polystyrene by RAFT polymerization, the resultant phase showed superb column separation efficiency. The stationary phases based on them are expected to enable realization of disposable microcolumns.

Regardless of choice of particle types (core/shell, fully porous, nonporous), using particles of 3 μm or less is subject to column packing problem. Nevertheless, core/shell, nonporous, and porous particles of 3 μm or less are commercially available. It seems that sub-3 μm core/shell particles will be more extensively used owing to their superior

chromatographic performance and future development of more advanced UHPLC systems. Development of a simplified process of low production cost in the formation of multilayer shell will be helpful for realization of accelerated spread of core/shell particles in chromatography. Other types of silica particles will certainly maintain some popularity in specific analytical requirements and sample situations. In addition, progresses in column packing techniques as well as studies regarding influences of various column packing parameters on packing quality are also required.

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