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Dendritic core-shell silica spheres with large pore size for separation of biomolecules

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ABSTRACT

Monodispersed core-shell silica spheres with fibrous shell structure and tunable pore size were prepared by using a one-pot oil-water biphase method. The pore size could be tuned from 7 nm to 37 nm by using organic solvents with different polarities as oil phase. The spheres synthesized by using benzene as organic solvent had the maximum pore size of 37 nm and possessed a surface area of 61 $m^2 g^{-1}$. The obtained wide pore core-shell silica spheres were applied for rapidly separating small molecules, peptides, small proteins, and large proteins with molecular weight up to 200 kDa. Since the pore size of the core-shell silica spheres was sufficiently large for the free access of all the solutes, sharp and symmetric peaks were obtained. The separation performance was as high as 264,531 plates m^{-1} for fluorene. The great efficient separation demonstrates that the wide pore core-shell silica spheres have a great potential for rapid analysis of both small and large solutes with high performance liquid chromatography.

1. Introduction

The idea of using core-shell silica particles to improve separation performance was pioneered by Horvath and Kirkland [1,2]. In the beginning, the particles size was as large as 50 μm. The particles were made with a solid glass bead core and a ca. 1 μm thin layer of fine silica particles. Their main drawback was the low loading capacity. Fifty years later, 2.7 μm Halo core-shell particles were introduced by Kirkland [3]. Compared with the low surface area of the earlier particle design, modern core-shell particles contain a 0.5 μm thick high surface area porous shell and a 1.7 μm core. Consequently, the drawback of the low loading capacity is eliminated. In addition, it is found that these new generation core-shell particles can exhibit much higher separation efficiency than those of totally porous particles with same particle size. The reason for that can be attributed to the limited solute penetration depth in the shell, thereby improving mass transfer. Because of the exceptional properties of the modern core-shell particles, considerable attention has been paid to them [4–10].

Normally, the core-shell particles are made from silica and the porous shell is built up by a layer-by-layer (LBL) approach. The LBL is a time-consuming method, in which 40–50 steps are required to achieve a 0.5 μm shell [11]. To overcome this shortcoming, coacervation method was developed. However, 2–3 coating steps were still needed to achieve a porous shell [11]. In addition, fines and aggregates were generated in the coating process. Therefore, additional size classification is unavoidable.

Pore size is critical to the chromatographic process. Large pores minimize the effect of reduction in diffusion coefficient, leading to the enhanced separation performance. Compared with the LBL method, template method is a simple method to obtain core-shell particles. However, the pore sizes of these particles are normally smaller than 10 nm. These pore sizes are not sufficiently large to allow free movement of large molecules such as peptides or proteins within the pore channel, although great success for the separation of small molecules has been achieved. Therefore, additional chemical etching process should be applied to expand the pore size. The pore size can be enlarged by chemical etching at the expense of weakened mechanical strength. Moreover, the chemical etching process must be controlled very carefully to avoid the adhesion of the particles.

Recently, we reported a one-pot biphase method to prepare core-shell silica particles with unique dendritic center-radial oriented pore structure and tunable pore size [12]. The pore sizes

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were tuned from 5 to 16 nm by simply changing the stirring speed. Since the pore channels were perpendicular to the surface of silica particles, mass transfer was further reduced compared to the core shell particles with worm-like pore structure. Consequently, separation efficiency up to 2.25×10^5 plates m^{-1} for naphthalene was obtained. The pore size of 16 nm was sufficiently large for the separation of peptides and small proteins [13]. However, to be used for the separation of large biomolecules such as proteins or monoclonal antibodies, the pore sizes of these core-shell particles are still too small. Therefore, in this work, a modified one-pot oil-water biphase method was developed to manipulate the pore size. By adjusting the types of the organic solvents, core-shell silica particles with pore size ranged from 7 to 37 nm were synthesized. The core-shell particles with pore size of 37 nm were used successfully for high performance liquid chromatography (HPLC) separation of peptides, small proteins, and large proteins with molecular weight up to 200 kDa.

2. Experimental

2.1. Materials

Peptides, proteins, and hexadecyltrimethylammonium bromide (CTAB, for molecular biology, ≥99%) were obtained from Sigma-Aldrich, Corp. (St. Louis, MO). *n*-Octadecyltrichlorosilane (95%) was purchased from Alfa Aesar. Chlorotrimetylsiane and tetraethyl orthosilicate (TEOS) were purchased from Aladdin Chemistry Co. (Shanghai, China). All other reagents (analytical reagent grade) were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). All chemicals were used as received without purification.

2.2. Synthesis of SiO_2@dSiO_2 spheres

Silica cores were prepared according to the procedure reported in literature [14,15]. Briefly, SiO_2 spheres with an average diameter of ~580 nm were prepared by mixing 3.4 mL of TEOS with 3.3 mL of $NH_3 \cdot H_2O$, 9.9 mL of H_2O, and 33.4 mL of ethanol under vigorous stirring. After 1-h reaction, one-eighth of the mixture was taken from the solution and used as seeds for the subsequent growth of 1.0 μm SiO_2 spheres. In turn, one-fourth of the 1.0 μm SiO_2 sphere sample was removed and used as seeds for the growth of 2.2 μm SiO_2 spheres. The obtained nonporous silica particles were washed with H_2O three times and dried at 60 °C for 3 h.

The core-shell silica particles were prepared via a biphase process in the presence of CTAB as a structure-directing agent. The solution composition of CTAB:H_2O:urea:iso-propanol:organic solvents:TEOS (molar ratio) varied in a range of 0.625:372:2.23:2.68:(53.0–75.3):1. In a typical process for forming a shell on silica core, 0.5 g dried silica particles, 0.5 g CTAB, 0.46 mL (6.0 mmol) of iso-propanol, and 0.3 g (5.0 mmol) urea were dissolved in 15 mL of H_2O. Subsequently, 15 mL of benzene and 0.5 mL of TEOS were added to the solution. The mixture was magnetically stirred at a rate of 200 rpm. After stirring for 5 min at room temperature, the reaction mixture was heated to 70 °C for 16 h. The products were collected by centrifugation and washed with ethanol and subsequently dried at 60 °C for 3 h. The particles were calcined at 550 °C for 2 h in air to remove the surfactant and the residual reactants and produce the mesoporous SiO_2@dSiO_2 spheres for characterization. To be used as stationary phase, the spheres were further derivatized with *n*-octadecyltrichlorosilane (C18).

2.3. Characterizations

The structure and morphology of the particles were examined by D8 Advance X-ray powder diffractometer (Bruker, Germany) using CuKα radiation (λ = 1.5406 Å), field emission scanning electron microscopy (FESEM, Hitachi S-4800 II, 15 kV), and Philips Tecnai 12 transmission electron microscope (TEM, 120 keV). The surface area, pore volume, and pore size of the particles were determined by nitrogen adsorption-desorption measurements using a Quantachrome autosorbiQ instrument. The Brunauer-Emmett-Teller (BET) surface area was obtained by applying the BET equation to the adsorption data. The pore size distribution was calculated from the adsorption branch of the sorption isotherms using the Barrett-Joyner-Halenda (BJH) method. The carbon loading of C18 modified SiO_2@dSiO_2 spheres was analyzed with Elementar vario EL III (Elementar Co. Germany).

2.4. Preparation of SiO_2@dSiO_2 packed columns

The dried SiO_2@dSiO_2 spheres (3 g, 2.4 μm) were dispersed in 100-mL toluene under a N_2 steam. A volume of 6 mL of *n*-octadecyltrichlorosilane was added. The mixture was refluxed for 12 h under a N_2 stream. Then, the product was filtered and washed three times with toluene and methanol, respectively. The obtained C18-modified SiO_2@dSiO_2 spheres were dried in vacuum at 60 °C for 5 h and then packed into stainless steel columns (4.6 mm I.D. × 100 mm) using an SSI HPLC packer. The columns were packed using 10% (*m*/*v*) suspensions of the C18-modified SiO_2@dSiO_2 spheres in a mixture of toluene and acetone (2:3, *v*/*v*) at 60 MPa using methanol as propulsion solvent. In order to make sure that the mechanical strength of the SiO_2@dSiO_2 spheres is strong enough, some of the particles were taken from the packed column. The SEM images of SiO_2@dSiO_2 spheres before and after packing were shown in Fig. S1. It can be seen from Fig. S1 that the structure of the core-shell particles after packing was almost unchanged indicating that the mechanical strength of the spheres was strong enough to withstand the high pressure. The columns were fitted into an Elite EClassical 3100 HPLC system (Elite, Dalian, China). The plate numbers were calculated by the equation $N = 5.54(t_R/W_{1/2})$, where t_R is the retention time and $W_{1/2}$ is the half height peak width of the peak concerned.

3. Results and discussion

In our previous work, we have found that the presence of two phases is the key factor to obtain fibrous morphology [12]. The function of organic solvent was to control the hydrolysis rate of TEOS to the aqueous phase, consequently to control the assembly and growth of the silica fibrous structure. According to the "like dissolves like" principle, the closer the polarity between TEOS and organic solvent, the bigger the solubility of TEOS and the slower of the hydrolysis rate of TEOS from oil to the aqueous. Therefore, it is expected that nonpolar solvents with different polarities might affect the pore structure of the shell. To confirm this view, organic solvents with different polarities were selected to explore the effect of polarity of the solvents on the pore size. The properties of selected organic solvents are shown in Table S1.

Fig. 1 shows the TEM images of the SiO_2@dSiO_2 spheres prepared by using different organic solvents as oil phase. It can be seen that silica cores were covered with shells when cyclohexane, toluene, benzene, or *N,N*-dimethylaniline were used separately as oil phase (Fig. 1A–D). The mesopore channels were perpendicular to the curved silica surface. However, no shells were found on the silica cores when ethyl acetate was used as oil phase (Fig. 1E). Fig. 1 shows that the density of the silica fibers varies with the polarities of the organic solvents, indicating that the pore sizes of these shells were different.

To further study the effect of polarity of organic solvents on the pore size, all the SiO_2@dSiO_2 spheres were tested by nitrogen

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