



Preparation and characterization of monodisperse large-porous silica microspheres as the matrix for protein separation



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ABSTRACT

High performance liquid chromatography (HPLC) is a kind of efficient separation technology and has been used widely in many fields. Micro-sized porous silica microspheres as the most popular matrix have been used for fast separation and analysis in HPLC. In this paper, the monodisperse large-porous silica microspheres with controllable size and structure were successfully synthesized with polymer microspheres as the templates and characterized. First, the poly(glycidyl methacrylate-co-ethylene glycol dimethacrylate) microspheres ($P_{GMA-EDMA}$) were functionalized with tetraethylenepentamine (TEPA) to generate amino groups which act as a catalyst in hydrolysis of tetraethyl orthosilicate (TEOS) to form Si-containing low molecular weight species. Then the low molecular weight species diffused into the functionalized $P_{GMA-EDMA}$ microspheres by induction force of the amino groups to form polymer/silica hybrid microspheres. Finally, the organic polymer templates were removed by calcination, and the large-porous silica microspheres were obtained. The compositions, morphology, size distribution, specific surface area and pore size distribution of the porous silica microspheres were characterized by infrared analyzer, scanning-electron microscopy, dynamic laser scattering, the mercury intrusion method and thermal gravimetric analysis, respectively. The results show that the agglomeration of the hybrid microspheres can be overcome when the templates were functionalized with TEPA as amination reagent, and the yield of 95.7% of the monodisperse large-porous silica microspheres can be achieved with high concentration of polymer templates. The resulting large-porous silica microspheres were modified with octadecyltrichlorosilane (ODS) and the chromatographic evaluation was performed by separating the proteins and the digest of BSA. The baseline separation of seven kinds of protein standards was achieved, and the column delivered a better performance when separating BSA digests comparing with the commercial one currently available. The high column efficiency and good reproducibility present that the large-porous silica microspheres obtained can be used as a matrix for peptide and protein separation.

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1. Introduction

High performance liquid chromatography (HPLC) has been used widely in many fields, such as biological medicine, petrochemical, environmental analysis and food safety [1–5]. As the most popular matrix, the micro-sized porous silica microspheres have been used for fast separation and analysis in HPLC because of their high mechanical stability, a wide variety of derivatization and relatively higher column efficiency [6]. Especially, mesoporous silica spheres with diameter in the range of 3–10 μm are widely-used HPLC supports in order to obtain high column efficiency [7].

In the late 1960s, Stöber and co-workers reported the preparation of uniform colloidal silica spheres based on the sol-gel chemistry [8]. This method is often employed in the preparation of nano-sized silica particles without much difficulty. On the basis of sol-gel process, K. Nozawa et al. [9] synthesized the monodisperse silica particles up to micron-size by the progressive addition of a diluted tetraethyl orthosilicate (TEOS) solution into ethanol. Furthermore, highly monodisperse silica particles with an average size of 6.6 μm were successfully produced by adding an electrolyte into the reaction system [10]. Unfortunately, those products obtained were all nonporous based on these methods.

Mesoporous silica spheres of several micrometers in size have been synthesized by various methods. The molecular template method [11–16] made it possible to produce mesoporous size of less than 4.0 nm with a small cationic surfactant as the template,

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e.g. cetyltrimethylammonium bromide (CTAB). Although the pore size could be enlarged to 10.6 nm when using block copolymers as the templates, the problems of agglomeration and secondary nucleation of the particles had to be solved. The spray drying method [17–19] could be used to overcome the above problems to produce mesoporous silica spheres. However, the particle size distribution is so wide that it must undergo the size classification leading to a higher cost. For the silicon stack method [20–22], it was believed that the particle pore structure and the particle size could be adjusted by using TEOS sols with different sizes and different polymerization-induced colloid aggregation conditions, respectively. However, the methods for preparing mesoporous silica spheres with uniform particle size and good dispersibility are still required [23].

Recently, Meyer et al. [24] proposed a new method to prepare porous inorganic monodisperse microspheres with polymer microspheres as a template for the formation of inorganic materials such as silica, titania, gold and other metal oxides. However, during the removal of the template, the spheres suffered much shrinkage rendering a precise control of desired particle size impossible. Moreover, the yield of the porous silica microspheres also turned out to be very low. He et al. [25] developed a method to overcome the shortcomings above, the porous silica microspheres were obtained with controllable morphology and structure. However, the agglomeration could not be avoided when the concentration of the template was increased in the reaction system. Thus, it was very difficult to enlarge the production scale with the method mentioned above.

In this study, a new method was developed to synthesize micro-sized monodisperse large-porous silica microspheres with polymer microspheres as templates. The obtained silica microspheres with a porous inner structure maintain the same size dimensions as the initial polymer microspheres, and the yield of 95.7% of the monodisperse large-porous silica microspheres was achieved under high concentration of polymer templates. The production of the silica microspheres was increased significantly comparing with He's method [25]. The synthesized monodisperse large-porous silica microspheres with controllable size and structure can be used as the matrix for the separation and analysis of biological macromolecules and shows good performance.

2. Experimental

2.1. Reagents and chemicals

Styrene (St), ammonium hydroxide (28% aqueous solution) and tetraethylenepentamine (TEPA) were bought from Chengdu Kelong Chemical Reagent Co. Ltd (Chengdu, China). Octadecyltrichlorosilane (ODS), poly-(vinylpyrrolidone) (PVP), ethylene dimethacrylate (EDMA), glycidyl methacrylate (GMA) and all protein standards including ribonuclease A (RNase A), lysozyme, insulin, transferrin, bovine serum albumin (BSA), myoglobin and ovalbumin were purchased from Sigma-Aldrich (St. Louis, MO, USA). GMA was distilled under vacuum to remove polymerization inhibitor. Benzoyl peroxide (BPO) was bought from Shanghai Shanpu Chemical Reagent Co. Ltd (Shanghai, China). Cyclohexanol and butyl phthalate (DBP) were purchased from Shanghai Chemical reagent Company (Shanghai, China), Sodium dodecyl sulfate (SDS) and polyvinyl alcohol (PVA) were obtained from Beijing Chemical Reagent Co. Ltd (Beijing, China). Tetraethyl orthosilicate (TEOS) was obtained from Tianjin Kemiou Chemical Reagent Co. Ltd (Tianjin, China). All chemical reagents are of analysis grade.

2.2. Characterization

Microspheres morphology was observed by scanning electron microscopy (SEM, ZEISS EVO18, Germany). The particle size analysis was carried out on dynamic laser scattering (Mastersizer 2000, Malvern Instruments, UK). Intrusion volumes and pore-size distributions were analyzed by the mercury intrusion method using an Autopore IV 9510 porosimeter (Micromeritics Instrument Corporation, USA). The composition of different microspheres was characterized by Fourier transform spectrometer (TENSOR 27, Bruker, Germany). Thermal gravimetric analysis (TGA) was performed on a thermal analyzer (Perkin-Elmer, STA 6000, USA) at a heating rate of 10 °C/min. All chromatographic tests were carried out by using a LC-20A chromatographic system (Shimadzu, Japan), including two LC-20AT *vp* pumps, a SCL-20A *vp* system controller, a SPD-20A *vp* UV–vis detector, and a CLASS-VP chromatography workstation. Samples were injected through a Rheodyne 7725 valve and detected at 280 nm. A commercial C₁₈ column (EF-C18M, particle size 5.0 μm, pore size 300 Å, surface area 60 m²/g, carbon loading 8%, 50 mm × 4.6 mm i.d.) was purchased from Wuxi Galak Chromatography Technology Co. Ltd (Wuxi, China).

2.3. Preparation of monodisperse P_{GMA-EDMA} microsphere

Monodisperse P_{GMA-EDMA} microspheres were prepared in our laboratory by modified seed swelling polymerization. In accordance with the method reported by Pane et al. [26], monodisperse polystyrene seeds with low molecular weight were prepared by dispersion polymerization of styrene in alcohol media in the presence of the inhibitor (BOP) and the stabilizer (PVP) under a nitrogen atmosphere. Typically, 20 mL of styrene, 80 mL of ethanol, 1.0 g of PVA and 0.5 g of BPO were mixed and sonicated for 5 min, the mixed solution was stirred for 0.5 h with nitrogen purging. Then, the reaction solution was stirred continuously for 24 h with a speed of 120 rpm in a preheated water bath at 70 °C. The obtained polystyrene seeds were repeatedly washed by centrifugation (5000 rpm for 10 min) with ethanol and distilled water before being dried at 60 °C overnight.

The polystyrene seeds were swollen by emulsifying mixture of glycidyl methacrylate, ethylene dimethacrylate, benzyl peroxide and diluents in an aqueous solution containing PVA and SDS at room temperature [27,28]. Typically, 1.0 g of polystyrene seeds was dispersed in 30 mL of water (0.2% SDS) and sonicated for 5 min, then the solution was stirred at 30 °C. 7.0 mL of glycidyl methacrylate, 7.0 mL of ethylene dimethacrylate, 14.0 mL of cyclohexanol, 0.56 g of BPO, 0.3 g of SDS, 3.0 g of PVA and 250 mL water were mixed and emulsified for 30 min. Subsequently, the emulsifying mixture was dropped in the suspension of polystyrene seeds. After the monomer mixture was completely absorbed by the seed beads, the temperature was elevated to 70 °C for 24 h. The beads obtained were washed with water and methanol. Then the porogens were removed by extraction with toluene. The beads were washed with methanol and dried at 50 °C for 6 h. The mean diameter of the polymer microspheres is 5.12 ± 0.22 μm carried on dynamic laser scattering, and the average pore size is 105.6 nm measured by mercury intrusion porosimetry.

2.4. Preparation of monodisperse porous silica microspheres

The monodisperse porous silica microspheres were synthesized using a template method [25] with some modifications. Typically, 6 g of P_{GMA-EDMA} microspheres were dispersed in 240 mL of deionized water and sonicated for 5 min, then 9.0 mL of tetraethylenepentamine (TEPA) was added in the solution. Temperature was elevated to 80 °C for 24 h. Finally, the product was washed with water and ethanol before drying at 60 °C for 12 h.

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