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Preparation of monodisperse large-porous silica microspheres with polymer microspheres as the templates for protein separation

Hongjun Xia, Guangping Wan, Fan Yang, Jianshan Wang, Quan Bai *

Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of Ministry of Education, Institute of Modern Separation Science, Key Lab of Modern Separation Science in Shaanxi Province, Northwest University, Xi'an 710069, China

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ABSTRACT

In this study, the monodisperse large-porous silica microspheres were successfully synthesized with polymer microspheres as the templates and characterized. The functionalization of the $P_{GMA\text{-}EDMA}$ microspheres was carried out with TEPA to generate amino groups. Subsequently, TEOS was readily hydrolyzed to form Si-containing low molecular weight species with ammonia as a catalyst. The low molecular weight species could be attracted by the amino groups and deposited into the functionalized $P_{GMA\text{-}EDMA}$ microspheres to form polymer/silica hybrid microspheres. Finally, the monodisperse large-porous silica microspheres were obtained with controllable morphology and structure after calcination to remove the templates. Using this method, the agglomeration of the hybrid microspheres was overcome, and the yield of 95.7% of the monodisperse large-porous silica microspheres was achieved with high concentration of polymer templates. The resulting large-porous silica microspheres were modified with ODS and the chromatographic evaluation was performed by separating the proteins and the digest of BSA. The high column efficiency and good reproducibility suggested that the large-porous silica microspheres could be used as a matrix for protein separation.

1. Introduction

High performance liquid chromatography (HPLC) is known as a good kind of efficient separation technology and has been used widely in many fields. As the most popular matrix, the porous silica microspheres have been used for fast separation and analysis of complex samples in HPLC [1]. The methods, such as molecular template method [2], spray drying method [3] and polymerization induced colloid aggregation method [4], are often employed to synthesize nano-sized silica particles [5]. However, these methods are not easy to be used to synthesize micro-size monodisperse porous silica microspheres from 3 to 10 μm [6].

Recently, Meyer et al. [7] proposed a new method to prepare porous inorganic monodisperse microspheres with polymer microspheres as a template. However, during the removal of the template, the spheres suffered much shrinkage and the yield was very low. He et al. [6] improved the method to overcome the shortcomings above. The porous silica microspheres were obtained with controllable morphology and structure, particle yields of around 98% was achieved. Unfortunately, the agglomeration could not be avoided with tetrabutylammonium bromide (TBAB) as a dispersant if the concentration of the template was increased under the same condition. Only 0.13 g silica particles could be obtained from 0.2 g of polymer microspheres and 5 mL of TEOS solution (10% isopropyl alcohol solution). Obviously, the production of the porous silica microspheres was very low. In this work, the agglomeration of the hybrid microspheres was overcome under high concentration of polymer templates, and the yield of 95.7% of the monodisperse large-porous silica microspheres was achieved. The production of the silica microspheres was increased significantly comparing with He's method [6]. The synthesized large-porous silica was used as the matrix for the separation and analysis of biological macromolecules and shown good performance.

2. Experimental

The silica microspheres were synthesized using a template method [6] with some modifications. Typically, 5 g of the poly (glycidyl methacrylate-*co*-ethyleneglycol dimethacrylate) microspheres ($P_{GMA/EDMA}$) was dispersed in 120 mL of water, then 7.5 mL of tetraethylenepentamine (TEPA) was added, the temperature was elevated to 80 °C for 24 h. The resulting TEPA-functionalized microspheres were washed repeatedly with water and dried at 80 °C. 5 g of the TEPA-functionalized microspheres, 200 mL of

* Corresponding author.
E-mail address: baiquan@nwu.edu.cn (Q. Bai).

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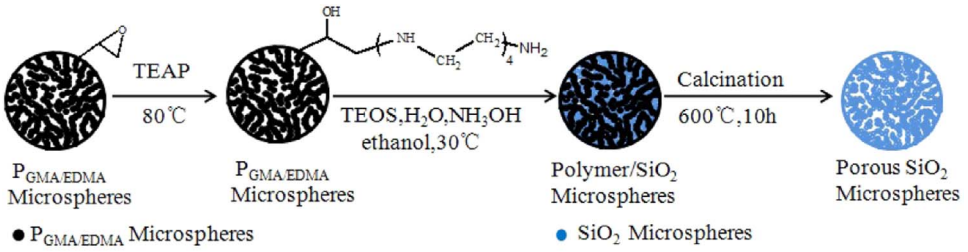

Scheme 1. Schematic diagram of synthesis of monodisperse silica microspheres.

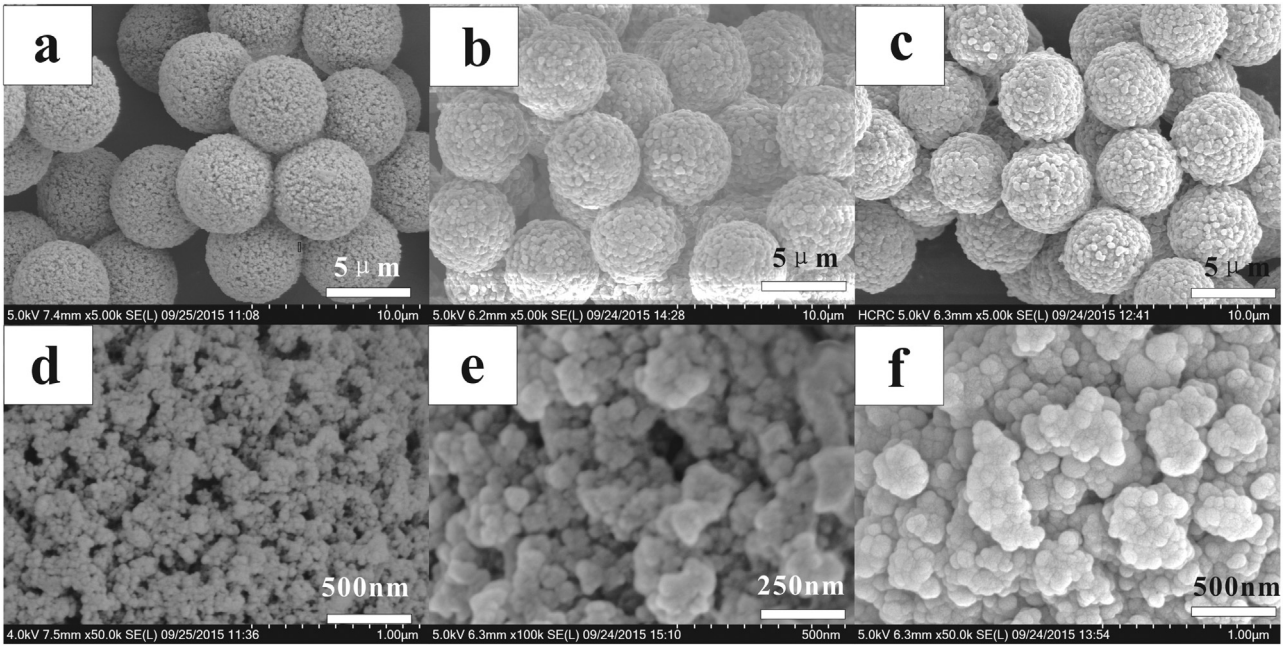

Fig. 1. SEM images of the $P_{GMA/EDMA}$ microspheres (a and d), $P_{GMA/EDMA}$/silica hybrid microspheres (b and e), and porous silica microspheres (c and f).

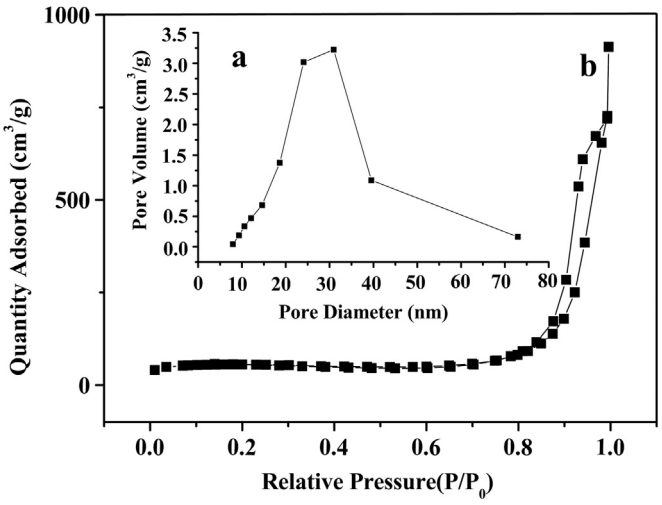

Fig. 2. Pore-size distribution (a) and the N_2 absorption isotherms (b) of SiO_2 microspheres.

isopropyl alcohol, 40 mL of water and 1 mL of ammonia hydroxide were mixed and sonicated for 5 min, subsequently, 125 mL of tetraethylorthosilane solution (TEOS, 10% isopropyl alcohol solution) was added in the mixture with 0.2 mL/min and stirred for 24 h at 30 °C. The microspheres obtained were washed with ethanol and water before being dried at 60 °C for 12 h. Finally, the products were calcinated at 600 °C for 10 h at a heating rate 10 °C/min. 2 g of the synthesized silica microspheres was dispersed in dry toluene, 1 mL octadecyltrichlorosilane (ODS) was added and refluxed at 125 °C for 6 h. The product was washed with ethanol and dried at 65 °C.

Microspheres morphology was observed by scanning electron microscopy (SEM, ZEISS EVO18, Germany), The particle size analysis was carried out on dynamic laser scattering (Malvern Instruments, UK). Specific surface area and pore size distribution of particle were obtained by physical adsorption experiments (Tristar-3020, USA). All chromatographic tests were carried out using a HPLC system (LC-20A, Shimadzu, Japan).

3. Results and discussion

3.1. Preparation and characterization of monodisperse large-porous silica microspheres

The synthetic procedures of the monodisperse large-porous silica microspheres are shown in Scheme 1. The $P_{GMA/EDMA}$ microspheres were synthesized firstly by a single-step swelling and polymerization method [8]. Fig. 1(a) and (d) indicate that the polymer microspheres are uniform in size and have macroporous structure. The mean diameter of them is 5.12 ± 0.22 μm.

The $P_{GMA/EDMA}$ microspheres were functionalized with TEPA to generate amino groups as catalyst for TEOS hydrolysis. Subsequently, the TEPA-functionalized polymer microspheres were dispersed in alcohol-water solution, TEOS was readily hydrolyzed