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Hard-templating synthesis of mesoporous carbon spheres with controlled particle size and mesoporous structure for enzyme immobilization

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ABSTRACT

Mesoporous carbon spheres (MCSs) with controlled particle size and pore structure were synthesized via a combined hard templating and sol–gel processing within water-in-oil emulsions, using resorcinol–formaldehyde polymer as carbon precursor and colloidal silica nanoparticles as hard templates. The addition of silica nanoparticles in polymer sol not only served as the pore structure reagents but also shortened the gelation time, making it easy to control the emulsion process. The sphere size of MCSs can be controlled in the range from 10 to 500 μm by changing the emulsification conditions. The pore structure of MCSs can be tuned by adjusting the mass ratio of resorcinol–formaldehyde polymer to silica nanoparticles and the diameter of silica nanoparticles. The as-prepared MCSs possessed large surface area (>600 $m^2 g^{-1}$), large pore volume (>1 $cm^3 g^{-1}$) and a narrow pore size distribution replicated from the silica nanoparticles used. These MCSs exhibited extraordinary high adsorption capacities (ca. 1100 $mg g^{-1}$) for α-Chymotrypsin (Chy) in solution. Due to their well-developed pore structure and the controllable pore size, as well as the unique shape and good affinity to biomolecules, the as-prepared MCSs should have a good potential in enzyme immobilization.

1. Introduction

Enzyme immobilization has attracted continuous attention in the fields of fine chemistry, biomedicine, and biosensor [1]. The use of immobilized enzymes can allow repeated use, facile separation from reaction mixtures, and prevent enzyme contamination in products as well as enhance the stability [2,3]. Numerous supports have been employed for enzyme immobilization, including polymers and resins, silica and silica–alumina composites, and carbonaceous materials [4–6]. Among these supports, porous carbon materials as carrier matrices exhibit some significant advantages, such as biocompatibility, highly developed pores and chemical stability as well as low cost and accessible porosity [7,8]. It has been shown that the pore size and pore size distributions of porous carbon materials have a strong effect on the immobilization of bulky biochemical molecules [9]. Generally, mesopores are required to host enzymes and allow an easy transport of substrate into its active site, and importantly, create an environment most favorable for the expression of enzyme activity [10–13]. A considerable amount of research on the immobilization of enzymes and adsorption of biomolecules on mesoporous carbon materials can be found in the literature [14–16].

The ability to control pore size and characters of mesoporous carbons is of immense advantage since these factors can exhibit direct impacts on activity and stability of immobilized enzymes. As such, tremendous recent interests have been triggered in the controllable synthesis of mesoporous carbons. Three main ways are effective to introduce mesopores in carbon materials, including activation to high burn-off degrees or combination of physical and chemical activation [17–19], replica synthesis with templates [20–22], and carbonization of organic gels issued from sol–gel processing [23,24]. By employing the first method, it is difficult to achieve mesoporous carbons with strictly controlled pore structures. The template approach has been proved to be very feasible to prepare mesoporous carbons with well controlled pores [25–28]. However, the resulting mesoporous carbons are generally in the form of fine powder, demonstrating poor mechanical integrity. Sol–gel processing could be effective to introduce 3-D interconnected mesopores and keep high mechanical properties; nevertheless, the furry and complicated supercritical drying makes the resulting materials high cost and limited availability. Recently, several new synthesis routes for the preparations of mesoporous carbons using colloidal silicas as templates and resorcinols as carbon precursors have been demonstrated [29,30]. Such surface-templating routes normally lead to carbon replicas with large pore volumes and multi-modal pore sizes. Unfortunately, the pores of these mesoporous carbons are mostly randomly distributed, and that their adsorptive properties are not well understood. Therefore, a facile synthesis procedure for mesoporous carbon with controlled

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pore structures and accessible pore is much in demand for biomedical applications.

For enzyme immobilization, the morphology and size of mesoporous carbons are also important factors to be considered. The mesoporous carbon powders cannot be used in most biomedical applications due to difficulties of separating them from the biofluid. The spherical morphology with appropriate pore size is an attractive feature to open up possibilities for the creation of supports that are expected to find practical applications in enzyme immobilization and biomedical materials [31]. Thus, many synthesis strategies have been developed to form mesoporous carbon spheres so far. Horikawa et al. [32] prepared spherical carbon aerogels by an emulsion polymerization and carbon dioxide supercritical drying method. Lei et al. [33] reported the synthesis of spherical mesoporous carbons by chemical vapor deposition (CVD) method using ferrocene as the concurrent iron/carbon precursor and silica arrays as template. More recently, carbon microspheres with a uniform size of 1 μm were prepared through a nanocasting technique using uniform spherical mesoporous silicas as sacrificial templates [34]. However, how to control and tailor the shape of mesoporous carbons and their pore structures for biomedical applications is still a challenging work.

In this paper, we combined the advantages of hard templating and sol–gel processing and developed a robust method to prepare mesoporous carbon spheres (MCSs). This method employed the inverse emulsion sol–gel polymerization of RF monomers and colloidal silica in a paraffin oil medium, followed by the ambient drying, carbonization and silica template removal. The primary advantages for this method included giving up the troublesome supercritical drying and preserving the 3-D rigid carbon network in spherical shape. Moreover, the addition of colloidal silica nanoparticles into resorcinol–formaldehyde sol caused the gelation time to be very short (ca. 5 min at 85 °C), making it very easy to control the particle size by changing the emulsification conditions. The pore structure of MCSs was reversely replicated from the silica network, which can be tuned by changing the mass ratio of resorcinol–formaldehyde polymer/silica nanoparticles and the diameter of silica nanoparticles. To the best of our knowledge, it was the first trial that the mesoporous carbons with controlled spherical shape were prepared via a cost effective hard-templating method. A preliminary study on the physical adsorption of α-Chymotrypsin suggested that the as-prepared MCSs have a good potential in enzyme immobilization. Also this kind of MCSs can be used in other application fields such as column packing, fillers, adsorption/separation and catalyst support.

2. Experimental

2.1. Preparation of MCSs

The MCSs were prepared via a combined hard templating and sol–gel method within water-in-oil emulsions with resorcinol–formaldehyde polymer as carbon source and colloidal silica sol (LUDOX® SM-30 colloidal silica, 30 wt.%, DuPont Co.) as hard template. In a typical synthesis, 4.85 g of resorcinol and 7.16 g of formalin (37 wt.% formaldehyde) were dissolved in 15 ml deionized water. Next, 25 g of colloidal silica sol was dropwise added to the above solution under stirring. The solution was then diluted to 50 ml. After pre-polymerization at 45 °C for 20 min, the solution was transferred to a 500 ml glass reactor containing a mixture of 300 ml paraffin oil and 1 ml sorbitan monooleate (Span 80). Then, the mixture was stirred with a speed of 200 rpm at 85 °C for 30 min and aged at 85 °C for 2 days. The as-made products, hybrid hydrogel spheres, were separated from the solvent by filtration and washed with ethanol and water. The spheres were dried in an oven at 80 °C for 24 h to obtain hybrid silica–polymer spheres. The silica–carbon spheres were obtained by pyrolysis of the silica–polymer spheres at 800 °C for 3 h in the nitrogen atmosphere. After carbon-silica spheres were immersed in 10 wt.% HF solutions for 24 h, silica was removed and crake-free MCSs were obtained.

In this work, to control particle size of MCSs, we changed the emulsification conditions such as the pre-polymerization period from 0 to 40 min, the concentration of Span 80 from 0.3 to 10 g L^{-1} and the stirring speed from 100 to 500 rpm. Three different mass ratios of resorcinol–formaldehyde polymer to silica (R) and three kinds of colloidal silica particles with average particle sizes of 7, 12 and 22 nm were used to control the pore structure under the same emulsion conditions (20 min of pre-polymerization time, 200 rpm of stirring speed and 1 ml of Span 80). The obtained MCSs are denoted as MCS(x)-R, where x is the silica particle size used and R is the mass ratio of resorcinol–formaldehyde polymer to silica. Some hybrid hydrogel spheres (7)-20/15 were dried under supercritical petroleum ether (boiling range 30–60 °C) conditions operating at 240 °C and 7.0 MPa [35].

2.2. Characterization

The particle size distributions of MCSs were measured with a dynamic light scattering analyzer (DLS, Malvern Mastersizer 2000) covering a wide size range of 0.02 and 2000 μm. The DLS was operated under the following experimental conditions: dispersant of water, particle concentration of 0.0634 vol%, temperature of 25 °C. The morphologies of MCSs were characterized by a scanning electron microscopy (FEI, Qunta 300). The microstructures were observed under transmission electron microscopy (TEM, JEOL 2100F) operated at 200 kV. The samples were prepared by dispersing the powder products as slurry in ethanol, which was then deposited and dried on a holy carbon film on a Cu grid. Nitrogen adsorption/desorption isotherms were measured at 77 K with a Micromeritcs ASAP2020 analyzer. Before the measurements, the samples were degassed in vacuum at 473 K for 12 h. The Brunauer–Emmett–Teller (BET) method was utilized to calculate the specific surface areas. The total pore volume was estimated from the adsorbed amount at a relative pressure of $PP^{-1}{}_0 = 0.985$. The micropore volume was calculated by the t-plot method. The pore size distributions were derived from desorption branch by using the Barrett–Joyner–Halenda (BJH) model.

2.3. Adsorption experiment

α-Chymotrypsin (Chy) was purchased from Merck Company. The molecular dimension and molecular weight for Chy are about 2.9 nm × 2.0 nm × 4.0 nm and 25,000, respectively. The isoelectric point (pI) of Chy is 8.3. Ultrapure water (18 MΩ station Millipore Milli-Q Plus) was used for all adsorption experiments. The pH of solution was adjusted using different buffer solutions.

To determine the adsorption rate, test samples (500 mg) were suspended in 250 ml of 400 mg L^{-1} Chy aqueous solution at the initial pH value of 8.3 (Tris–HCl buffer) and 6.1 (sodium phosphate buffer), respectively. The mixture was continuously shaken in a water bath with a speed of 160 rpm at 25 °C. Three milliliter samples was taken from the reactor at pre-determined time intervals, including equilibrium time. The adsorption isotherms were measured at 25 °C by shaking 100 ml of each Chy solution at different initial concentrations for 48 h with 50 mg of the carbons. The suspensions were filtered, and the filtrates were analyzed by a UV–vis spectrometer (UV-3150, Purkinje General Instrument Co. Ltd.). Calibration experiments were done separately before each set of measurements. The wavelength of spectrometer for Chy is 280 nm. The adsorption capacities (q) were determined according to the following formula:

$$q = \frac{(C_0 - C) \times V}{m}$$

wherein C_0 is the initial concentration, C is the residual concentration, V is the volume of the solution and m is the mass of the adsorbent.

3. Results and discussion

3.1. Particle size evolution during the preparation process

MCSs were synthesized by a combined hard templating and sol–gel processing within water-in-oil emulsions, which involved two intermediates named the silica–polymer spheres and the silica–carbon spheres. To understand the morphology and particle size evolution during the preparation process, Fig. 1 gives the typical SEM images and particle size distributions for the three different forms of spheres. It can be seen that all spheres are uniform in size and retain the high monodispersity. Moreover, their surfaces are smooth without obvious cracks or contaminations, suggesting the effectivity of this preparation method. The particle size distributions of the three spheres derived from DLS are shown in Fig. 1d. The average size decreases gradually from 336 to 168 and then to 117 μm during the preparation process. Huge shrinkage (ca. 50%) takes place during the carbonization, as a result of the formation of compact carbon network [36]. The spheres experience ca. 30% shrinkage in diameter after the silica removal because of the collapse of their nanoparticle network arising from the large capillary pressures during the drying.