



Synthesis of fibrous and non-fibrous mesoporous silica magnetic yolk–shell microspheres as recyclable supports for immobilization of *Candida rugosa* lipase

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ABSTRACT

The mesoporous yolk–shell nanoparticles (MYSNs), with a movable Fe₃O₄ core inside the hollow capsules, with two different morphologies (1) Fe₃O₄@SiO₂@hollow@fibrous SiO₂ (Yolk Shell-1) (2) Fe₃O₄@SiO₂@hollow@mesoporousSiO₂ (Yolk Shell-2) were fabricated as carriers for *Candida rugosa* lipase (CRL) immobilization, through a template-assistant selectively etching method. Firstly the hydrophilic Fe₃O₄ nanoparticles were synthesized according to the solvothermal method. Then, SiO₂ shell was coated on the Fe₃O₄ nanoparticle surface by a sol–gel reaction. Subsequently, the resorcinol formaldehyde (RF) shell was covered on the surface of as-prepared Fe₃O₄@SiO₂ composites and sandwiched Fe₃O₄@SiO₂@RF composites were formed, which were finally provided with mesoporous fibrous (KCC) in the Yolk Shell-1 and mesoporous Silica in the Yolk Shell-2(Non fibrous). After selectively etching CTAB and the middle RF layer, the two kinds of yolk/shells composites were obtained. By utilization of their large and open mesopores (20–50 nm) with good surface area (457.78–625.54 m²/g,) CRL was immobilized after amino functionalization using glutaraldehyde as a linker. The ICRL on Y.S-1, Y.S-2 showed good loading (797–501 mg/g) and enzyme activity of (1503–837 U/g) respectively. Thermal stability, resistance to pH, recycling and storage capacity were improved as compare to free lipase.

1. Introduction

The industrial utility of enzymes is majorly dependent on their physiochemical stabilities, and enzymes [1] that are stable at a wide range of pH and temperature are very important from the biotechnological point of view. To enhance the biological activity, life time, pH and thermal stabilities of enzymes [2], these biological catalysts are immobilized on suitable solid supports that provide mechanical strength against pH and heat inactivation for a long time uses [3].

Nanoscaffolds such as nanoparticles, nanofibers, nanotubes, and nanocomposites have been recently used for enzyme immobilization. In this regard, chemically modified magnetic nanoparticles have gained more widely attention as lipase carrier, due to their high surface area, high stability, and easy separation [4–6].

The majority of chemical modifications are related to silica coated magnetic nanoparticles and their functionalization by suitable groups that can link to enzyme by formation of chemical bond [7]. Regarding to the recent development of nanoscience and nanotechnology, a great variety of nanomaterials with interesting morphologies has been used. Surface modification of nanomaterials, not only are important for

engineering their surface energy and interfacial properties, but also for providing active surfaces to attach designated molecules that are immobilized on the surfaces of the nanostructures [8,9].

Mesoporous silica has emerged as one of the most promising nanomaterials owing to its outstanding peculiarities, such as facile synthesis, tunable pore and particle size, good mechanical and thermal stability, easy modification, low toxicity, high biocompatibility, excellent affinity with other materials, and extended capacity of loading guest species [10–12]. Enzyme immobilization on mesoporous materials has been widely reported. The immobilization of the isolated heme domain of P450, BM-3 on two mesoporous molecular sieves indicated that the matching between pore diameter and protein dimensions plays a key role in the operational stability and activity of the immobilized enzyme [13]. Ajitha et al. [14] immobilized α -amylase over well-ordered mesoporous molecular sieve SBA-15 with different pore diameters; SBA-15 with the optimum pore size showed the maximum adsorption of the enzyme. However, a significant leakage was observed from the support under the reaction conditions [15].

Core–shell magnetic mesoporous spheres comprising magnetic core and mesoporous shell with relatively larger mesopores (> 4 nm at

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least) are supposed to be an ideal candidate for enzyme immobilization in proteolysis, because the spheres have a good dispersibility in solution, with a short diffusion distance of mass transport [16].

Most of previous reports about magnetic mesoporous silica microspheres (abbreviated as MagnMSMs) were synthesized by using surfactant as a pore forming agent, which led to the pore size less than 2.5 nm [17]. Small mesopores are not suitable for enzyme immobilization for proteolysis, because most proteins have a hydrodynamic diameter of larger than 3.2 nm and thus they cannot enter into the pore channels to interact with the immobilized enzyme [18].

Ever since the first demonstration of yolk-shell Au@mesoporous polymer shell and carbon shell by Hyeon et al. [19], (Au@Ag)@polypyrrole(PPy) [20] a large number of mesoporous Yolk Shell nanoparticles (MYSNs) with different core and shell compositions and in wide range of particle sizes, shapes, and structures have been successfully synthesized [21].

Yolk Shell nanoparticles (YSNs) have been considered as promising candidates for emerging applications, such as drug delivery biomedicine and catalysis [22,23]. It is not hard to image that the YSNs with magnetic cores should be essential nano adsorbents for removal of various contaminants from aqueous solution. The magnetic core allows easy separation while the porous shell provides desired functionality. Various magnetic YSNs, Fe_3O_4 @Au–polyaniline (PANI) [24] and Fe_3O_4 @PMAA, Fe_3O_4 @MS, Fe_3O_4 @ SiO_2 , Fe_3O_4 @HHSS have been employed as a novel adsorbent for heavy metal removal, due to their stability in a wide pH range (2–14), high surface area and a large number of surface hydroxyls (–OH) [25].

Compared with conventional silica nanomaterials, the radially oriented mesochannels in mesoporous silica spheres cause increased surface area and pore volume, which facilitate porous wall functionalization and interaction with various species [26]. Meanwhile, radially oriented mesochannels allow for the easy penetration of molecules into or out of the pore [27]. Efficient methods have been developed to synthesize MSNs with radially oriented mesochannels [28].

YSNs with different chemical compositions have been reported such as polyaniline@ SiO_2 [29], Fe_2O_3 @PAA@ TiO_2 [30] metal NPs@ SiO_2 , metal oxide@ SiO_2 , metal NPs@C, metal NPs@metal oxide, metal NPs@polymer, SiO_2 @metal oxide, SiO_2 @C and polymer@polymer, by using different synthetic methods [31].

Recently, the extension of the Stöber method for the synthesis of resorcinol-formaldehyde (RF) polymer nanospheres opens a novel pathway for synthetic strategies in the facile preparation of RF based core-shell nanospheres [32]. For example, Fuertes and coworkers reported a one-step Stöber method to synthesize RF@silica and carbon capsule structures [33]. Furthermore, Liu et al. reported one-step Stöber approach to produce uniform Au (Ag)-silica-polymer spheres with a core-shell-shell structure as templates for Au (Ag)@void@C yolk-shell nanostructures [34,35]. Shao synthesized the magnetic rattle-type carbon nanospheres using the similar strategy [36].

Herein, we report the synthesis of two kinds of radially oriented mesochannels yolk-shell microspheres with comparable porosity for lipase encapsulation. Y.S-1 and Y.S-2 consisting of silica coated magnetite (Fe_3O_4 @ SiO_2) materials, which were subsequently coated with RF and then finally with mesoporous silica and mesoporous fibrous silica (KCC). The RF and CTAB were removed at higher temperature (550 °C) selectively by etching method. These yolk-shell mesoporous supports were constructed through a controllable stepwise interface deposition and surfactant-templating co-assembly process. The obtained multifunctional microspheres, denoted as Fe_3O_4 @ SiO_2 @hollow KCC (Y.S-1), Fe_3O_4 @ SiO_2 @hollow m SiO_2 (Y.S-2), microspheres, possess a movable silica-protected magnetic core (300 nm), tunable hollow spaces (850–650 nm in diameter respectively), highly accessible mesoporous channels (20–50 nm), high surface area (457.78–625.54 m²g⁻¹) and large magnetization (25–29.5 emu g⁻¹). After amino functionalization, lipase was immobilized on the microspheres using glutaraldehyde as a linker. Through a simple magnetic separation, the ICRL on the yolk-

shells can be easily recycled, and reused without significant reduction of the activity even after 15 times of use. Of the two supports (Y.S-1 & 2), the ICRL on Y.S-1, showed good loading, high activity, thermal and pH stability and longer storage capability as compare to the free lipase.

2. Experimental

2.1. Chemicals

Anhydrous FeCl_3 , trisodium citrate, sodium acetate, tetraethyl orthosilicate (TEOS), resorcinol, formaldehyde, ethanol, ethylene glycol, concentrated ammonia solution (28 wt%) are of analytical grade (Shanghai Chemical Corp.). γ -Aminopropyltriethoxysilane (APTES, Sigma-Aldrich Companies), Cetyltrimethyl ammonium bromide (CTAB) n-butyl hydroperoxide was supplied by Sigma-Aldrich. All the other chemicals were used as received. Candida rugosa lipase (CRL, 739 U/mg, Sigma-Aldrich Company), glutaraldehyde (GA, Morning Chemical Reagent Factory in Tianjin). Deionized water was ultrapure produced by an apparatus for pharmaceutical purified water (Aquapro Co. Ltd.).

2.2. Synthesis of Fe_3O_4 particles

The hydrophilic Fe_3O_4 particles were synthesized according to the method reported previously with a minor modification. Briefly, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (3.25 g), trisodium citrate (1.3 g), and sodium acetate (NaAc, 6.0 g) were well dissolved in ethylene glycol (100 mL) with magnetic stirring. The obtained yellow solution was sealed into a Teflon-lined stainless-steel autoclave (200 mL in capacity). The autoclave was heated at 200 °C for 10 h, and then allowed to cool down to room temperature. The black product was washed with deionized water and ethanol several times.

2.3. Preparation of Fe_3O_4 @ SiO_2 nanoparticles

The Fe_3O_4 @ SiO_2 nanoparticles were synthesized as follows [37]. 0.1 g of Fe_3O_4 nanoparticles were dispersed in 20:80 mL of deionized water and ethanol in a 250 mL three-necked flask. Concentrated ammonia aqueous solution (1 mL, 28 wt %) & 100 μL of TEOS were added by stirring for 6 h at room temperature. The product was separated using external magnetic field and washed with ethanol and deionized water several times.

2.4. Synthesis of Fe_3O_4 @ SiO_2 @RF microspheres

200 mg of Fe_3O_4 @ SiO_2 microspheres were dispersed in a mixture solution of (40:20 mL) ethanol and deionized water. Dispersed, ammonium hydroxide aqueous solution (0.1 g, 28 wt %), resorcinol (0.1 g, 0.09 mM), and formaldehyde (0.2 g, 37 wt %), stirred for 2 h at 30 °C, forming a layer of resorcinol-formaldehyde (RF) resin on the seed. After polymerization, the obtained Fe_3O_4 @ SiO_2 @RF microspheres were collected by a magnet and washed three times with deionized water and alcohol [38].

2.5. Synthesis of Fe_3O_4 @ SiO_2 @Hollow@mesoporous fibrous SiO_2 (KCC) microspheres (Y.S-1)

0.2 g of the Fe_3O_4 @ SiO_2 @RF was added to 30 mL of water containing 3.6 g of urea and sonicated for 30 min. Then CTAB 2.00 g, n-butyl alcohol 0.2 g and cyclohexane 40 g were added. After 30 min stirring 2.00 g of TEOS was added drop wise. Finally stirring for 24 h at 70 °C, it was washed with ethanol and water several times. The obtained sample was dried in vacuum at 40 °C for 12 h and calcined at 550 °C in air for 5 h to remove CTAB and RF, and yolk-shell magnetic mesoporous silica (Fe_3O_4 @ SiO_2 @hollow mesoporous fibrous SiO_2) microspheres were

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