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Fabrication of chitin microspheres and their multipurpose application as catalyst support and adsorbent

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ABSTRACT

In this study, novel chitin microspheres (CM) with diameters of 1010 μm, 750 μm, 490 μm, 280 μm were fabricated by employing the sol–gel transition method. Then the chitin microspheres served as the enabling platform for a range of applications including recyclable catalyst support and adsorbent. First, the freeze dried porous chitin microspheres were coated with dopamine to enhance the loading efficiency of a model biomacromolecule, α-amylase. The immobilized enzyme (49.6 mg/g) retained more than 95% of relative activity after 10 repeated cycles and exhibited easy recovery ability. Then porous magnetic chitin microspheres could be prepared, and the swollen porous polymer successfully controlled the growth of gold nanoparticles. The chitin/Au nanocomposite microspheres were a good recyclable catalyst due to the porous structure and a reduced dimension of the metal particles ($r \leq 5$ nm). Finally, the magnetic chitin microspheres were modified into an adsorbent for enhanced removal of a typical cationic compound, methylene blue from aqueous solution.

1. Introduction

Polymer microspheres have been widely used as sorbents for separation science, packing material for chromatogram technology, and biocatalyst carriers (Das & Subuddhi, 2014; Liu et al., 2014; Mi et al., 2003; Patil & Sawant, 2011). However, the widespread applications of synthesized polymer microspheres have posed a threat to the environment due to the non-biodegradability of most of these materials, so microspheres made from native polymer are of great importance. Chitin is the second most abundant biopolymer on earth after cellulose with very attractive properties, such as biocompatibility, biodegradability, as well as thermal and chemical stability (Liu et al., 2009; Muzzarelli et al., 2012). The deacetylated product of chitin, chitosan, has been widely used in a wide range of fields such as environment, energy, medicine (Deng et al., 2012; Hu, Ting, Zeng, & Huang, 2012; Li et al., 2012; Liang, Liu, Huang, & Yam, 2009; Muzzarelli, 1980, 1996; Sun, Li, Nie, Wang, & Hu, 2013). However, the direct utilization of chitin is not extensive due to the lack of a benign solvent for effective dissolution and processing into a final product (Setoguchi, Yamamoto, & Kadokawa, 2012; Tajiri, Mihata, Yamamoto, & Kadokawa, 2014). Simone S. Silva designed chitin-based microsphere scaffolds using the ionic liquid, 1-ethyl-3-methylimidazolium acetate (EMI Ac) as the solvent (Silva, Duarte, Mano, & Reis, 2013). But the size of the microspheres they synthesized cannot be regulated and the solvents are relatively less "green" compared with the aqueous solvent containing NaOH/urea/H_2O (Liu, Zhou, Zhang, Guan, & Wang, 2006), which successfully dissolved chitin under low temperature and opened a new vista for chitin (Duan et al., 2013). However, to the best of our knowledge, there is no report on the direct synthesis of size controllable chitin microspheres based on this solvent. Therefore, the most widely used micro emulsion template method was adopted to fabricate chitin microspheres based on this solvent, with which the size of the microspheres can be regulated and microspheres for both laboratory research and industrial application scale can be obtained (Biró et al., 2009; Patil & Sawant, 2011). Moreover, this is a simple and environmentally friendly pathway.

Polymer microspheres have long been employed as adsorbents for heavy metals, various dyes, or as scaffold for Pd, Au, Pt, Ag nanocatalysts and enzyme (Ning, Yang, Wang, Ngai, & Tong, 2013; Tüzmen, Kalburcu, & Denizli, 2012). Meanwhile, chitin microspheres have advantage over most inorganic microspheres in terms of these applications because of their low cost and good biodegradability. Further, the large surface area, porosity, hydrophilicity, abundant hydroxyl and acetamido groups made it feasible for modification of chitin microspheres for broadening its applications (Li et al., 2010). So first, taking advantage of porous structure of these microspheres, it is a good idea to immobilize catalyst

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on chitin microspheres. Meanwhile, Since Polydopamine coated supports have been proved to a good system for immobilization of biomacromolecules (Chao et al., 2013; Ko, Yang, Shin, & Cho, 2013), expecially proteins, so a model biocatalyst, α-amylase, was immobilized on the porous polydopamine coated magnetic chitin microspheres for repeated utilization and easy recycling. Second, since chitin has long been proved as an effective chelating agent for heavy metals (Liu et al., 2013), it is an interesting idea to take advantage of the swollen porous microspheres to immobilize and control the growth of gold nanoparticles, which has been used extensively as a typical example of inorganic catalyst, as the reduced dimension of the metal particles ($r \leq 10$ nm) and a favorable interaction with the support are the two most important factors affecting the catalytic activity of gold nanoparticles (Chung, Guo, Kwak, & Priestley, 2012; Oh et al., 2013).

Third, magnetic microspheres are gaining widespread use in water treatment due to its great significance in accelerating separation speed and improving water treating efficiency, but the adsorption efficiency of unmodified chitin related materials for cationic pollutants is usually unsatisfactory (Wang et al., 2013; Yan, Li, Yang, Li, & Cheng, 2013). So in this study, magnetic chitin microspheres were modified with Reactive Black 5 to incorporate negative charged groups and the obtained porous magnetic chitin microspheres exihibited high removal efficiency for a typical example of cationic pollutants, methylthionine chloride.

In this paper, we have prepared chitin microspheres through a simple and green way, and then extend the application of chitin microspheres to the field of food, energy and environment by immobilizing α-amylase, immobilizing and controlling the growth of gold nanoparticles and modification with Reactive Black 5 to incorporate negative charged groups.

2. Materials and methods

Chitin ($M_w = 1.31 \times 10^6$ Da) was provided by Yuhuan Ocean Biochemical Co., Ltd (Taizhou, China). Dopamine was supplied by Yuancheng Technology Development Co., Ltd (Wuhan, China). Coomassie brilliant Blue G-250 and Bovine serum albumin (BSA) were purchased from Solarbio Company (Beijing, China). A-amylase (Bacillus sp.) was obtained from Sigma-Aldrich (St. Louis, MO). Magnetic Fe_3O_4 nanoparticles were obtained from Nanshangle chemical plant (Beijing, China). GA (25% water solution, m/v) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals were used without further purification as analytical grade.

2.1. Preparation of chitin microspheres and magnetic chitin microspheres

First, Chitin was purified before use by treating with 5 wt% NaOH for 10 h and 7% (v/v) hydrochloric acid aqueous solution for another 24 h. The chitin solution was prepared according to the previous method (Duan et al., 2013). Four grams of purified chitin powder was dispersed into a 96 g mixture of NaOH, urea, and distilled water in the ratio of 11:4:85 by weight and the suspension was frozen at −30 °C for 4 h, and then stirred vigorously at room temperature. The freezing/thawing cycle was repeated twice to obtain transparent chitin solution, with chitin concentration of 4 wt%. The chitin solution was degassed by centrifugation at 4000 rpm for 10 min at 0 °C. Then 30 mL of the chitin solution was dropped in 100 mL paraffin oil containing 1 g Span-80 slowly and stirred at 100 rpm with mechanical agitation at 0 °C for 3 h. Then, the generated chitin microspheres were formed when the pH value of suspension was adjusted to 7.0 by the addition of dilute hydrochloric acid with stirring. The suspension was allowed to stand until it was separated into two layers. The upper organic phase layer was recovered and the lower aqueous phase layer was rinsed with deionized water, acetone and alcohol three times to obtain chitin microspheres, coded as CM. Span-80 was extracted from the microspheres with alcohol at 60 °C for 24 h and three times. To prepare magnetic chitin microspheres (MCM), a certain amount of Fe_3O_4 (20 wt%, according to the weight of chitin) was added to the chitin solution and homogenized by continuous agitation at 200 rpm for 20 min, then the same steps were followed as above.

2.2. Characterization of microspheres

The definite size distribution of the wet CM microspheres was determined with a Malvern Mastersizer 2000 laser particle size analyzer (Malvern, UK).The morphology of the microspheres was observed with scanning electron microscope (SEM) (SEM, Hitachi X-650 microscope, Japan). The MCM samples at wet state were frozen in liquid nitrogen and freeze-dried using a lyophilizer (CHRIST Alpha, Germany). Then the microspheres were coated with Pt for SEM observation. X-ray diffraction for the microspheres was carried out on the X-ray diffractometer (Rigaku D/MAX-2400 XRD with Ni-filtered Cu Ka radiation). The XRD patterns were recorded in the region of 2θ from 5 to 85°. Samples were ground into powders and dried in a vacuum oven at 60 °C for 48 h before characterization. The FTIR spectra of the samples were measured by a Fourier transform infrared spectrometer (IR300, Nocolet). X-ray photoelectron spectra (XPS) were recorded on a Thermo VG ESCALAB250 X-ray photoelectron spectrometer. TEM analysis was conducted with a JEM-100CX II operated at 200 kV. The microspheres were firstly embedded in resin, and then ultrathin section (100 nm) was obtained with a microtome (Leica UC).

2.3. Preparation of polydopamine coated magnetic chitin microspheres (PMCM) and immobilization of α-amylase

MCM in the wet state were first frozen in liquid nitrogen and freeze-dried, then the obtained porous microspheres were continuously over-night stirred in dopamine solution (2 mg/mL, 10 mM, pH 8.5 Tris buffer). Self-oxidized or polymerized dopamine was expected to form adherent polydopamine coating on the surface of the MCM particles. The unadhered polydopamine particles were removed by rinsing the resulted microspheres with water for several times. MCM and PMCM were stored at 4 °C. About 1 g of PMCM was added to the α-amylase solution (1 mg/mL in 20 mL pH 6.5 50 mM phosphate buffer containing 0.5% glutaraldehyde) and the immobilization reaction was carried out at 4 °C for 6 h in a rotary shaker. The particles were collected by an external magnet and the unbound enzyme was removed thoroughly with phosphate buffer. The amylase immobilized particles were stored in phosphate buffer at 4 °C until use. The Bradford method was used to determine the loading amount of enzyme on PMCM by measuring of the initial and final concentration of protein within the immobilization medium solution. A calibration curve was plotted using Coomassie Brilliant Blue G-250 solutions as standards (0–24 mg/L). The enzyme concentration in the solution can be determined with ultraviolet–visible spectrophotometry by measuring the absorbance at 595 nm. The amount of enzyme immobilized onto the PMCM was calculated by mass balance with the following equation:

$$\text{Enzyme loading (mg/g)} = \frac{(C_0 - C_1) \times V}{W_S} \times 100\%$$

where C_0 is the initial enzyme concentration (mg/mL), C_1 is the final enzyme concentration (mg/mL), V is the enzyme volume (mL) and W_s is the support dose added (g).