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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).

2.4. Reusability of immobilized α-amylase

Immobilized α-amylase was exposed in 1% starch (w/v) solution for 30 min at 50 °C and the released reducing sugar was measured. The enzyme immobilized microspheres were collected by external magnet and were washed with phosphate buffer for repeated use. For measuring the reducing sugar, 1 mL of enzyme treated starch (w/v) solution was taken and mixed with 1 mL of DNS (3,5-dinitrosalicylic acid) reagent. The resulting solution was incubated in a boiling water bath for 5 min. After dilution with DI water, the amount of hydrolysis products (reducing sugar) was measured spectrophotometrically at 540 nm with maltose as the standard. All the data points are an average of duplicated measurements.

2.5. Preparation of Reactive Black 5 modified magnetic chitin microspheres (RMCM) and methylene blue adsorption test

RMCM were obtained via a nucleophilic substitution reaction (Wang et al., 2013). Briefly, 2.0 g magnetic chitin microspheres (MCM) were dispersed in 100 mL Reactive Black 5 aqueous solution (1 mg/mL), then the pH of the mixture was adjusted to 10 with Na_2CO_3 aqueous solution (2 M), and the reaction mixture was shaken for 3 h at 30 °C. RMCM were washed with deionized water until the eluent was colourless, then freeze dried for further use.

As for the methylene blue adsorption test, 10 mg RMCM and 20 m/L l methylene blue (MB) with various concentrations (5–80 mg/L) were mixed and shaken at room temperature for 1 h. The dye loaded RMCM was separated by an external magnet. The concentrations of MB remaining in the solutions were determined by measuring UV absorbance at 662 nm. The amount of MB adsorbed onto the microspheres was calculated using the difference between solution concentration before and after adsorption.

2.6. Synthesis of chitin/Au nanocomposite microspheres and catalytic study

Chitin microspheres (0.1 g) were placed into 15 mL solution of 2 mM $HAuCl_4$ and incubated for 3 h. Chitin/Au nanocomposite microspheres were obtained via in situ reduction of chloroauric acid by quick addition of $NaBH_4$ (1×10^{-2} M) as reducing agent. Then the microspheres were washed with distilled water, treated with liquid nitrogen and then freeze dried for catalytic reduction. The catalytic 4-nitrophenol reduction was carried out as follows. Ten milliliters of fresh $NaBH_4$ aqueous solution (0.25 M) was mixed with 10 mL of freshly prepared 4-nitrophenol aqueous solution (2.5×10^{-4} M), then (10 mg) chitin/Au nanocomposite microspheres were added. As a result, the concentration of 4-nitrophenol and $NaBH_4$ in the reaction solution was 1.25×10^{-4} and 0.125 M, respectively. UV/vis absorption spectra were recorded to monitor the change in the reaction mixture after the removal of the composite microspheres. After the completion of the reduction process, the catalysts were separated from the mixture and rinsed with distilled water for reuse in the next cycle, and this process was repeated for 10 times.

3. Results and discussion

3.1. Preparation of chitin microspheres

Microspheres with an average diameter of 1010 μm, 750 μm, 490 μm, 280 μm were obtained when the initial microspheres were filtered with a 30, 40, 60 and 80 mesh sieve in turn. They take up about 9 ± 0.5%, 11 ± 0.6%, 26 ± 1% and 30 ± 1.5% of the initial content of chitin, respectively. Because chitin solution was stirred in a 250 round-bottom flask, it was difficult to obtain microspheres with a uniform size by this sol–gel method, especially when the stirring speed, oil–water ratio and the content of emulsifier were low. Meanwhile, it was commonly recognized that the microsphere size decreased rapidly with increasing the dispersant dosage, oil–water ratio and stirring speed (Biró et al., 2009; Patil & Sawant, 2011), so microspheres with smaller diameter and uniform size would be obtained if the dispersant dosage, oil–water ratio and stirring speed increased. Moreover, it has been reported that there was no evaporation of any chemical agent during dissolution of chitin in NaOH/urea aqueous at low temperature (Duan et al., 2013), so the preparation method for CM is simple and safe. Therefore, this is a "green" process, and the chitin microspheres with various sizes will be of great importance to both fundamental researches in laboratory and industrial applications for various purposes. Fig. 1 shows SEM of the chitin microspheres (CM) with diameter of 1010 μm, 750 μm, 490 μm and 280 μm. Microspheres with a diameter of 280 μm are used for the subsequent experiments.

3.2. Microspheres used for immobilization of α-amylase

X-ray diffraction has been used to study the structure and interaction between the inorganic particles and Chitin matrix. The powder X-ray diffraction patterns of chitin microsphere (CM), magnetic chitin microsphere (MCM), Chitin (C) and Fe_3O_4 were shown in Fig. 2c. The XRD peaks of the Fe_3O_4 nanoparticles agreed well with those of Fe_3O_4 and no other crystalline phases were observed. The diffraction peaks at 9.3°, 12.6°, 19.2°, 23.3° and 26.3° for (0 2 0), (0 2 1), (l 1 0), (1 3 0), (0 3 1) planes, respectively, are the characteristic peaks for alpha chitin crystal. The diffraction peaks of the chitin microspheres were much broader and weaker than that of the chitin powder, indicating a decrease in crystallinity and crystallite size (Duan et al., 2013). Moreover, the diffraction peaks of Fe_3O_4 at 29.91°, 35.41°, 43.11°, 57.21°, and 62.81° could be observed in MCM. The results indicated that the Fe_3O_4 nanoparticles were embedded in the chitin microsphere, and the structure of Fe_3O_4 was hardly changed. This clearly verified that the Fe_3O_4 nanoparticles in the cavity of the porous chitin microsphere were protected because of the existence of a matrix to prevent the removal. The content of Fe_3O_4 in MCM is 15.6%.

Fig. 2 shows the SEM images of morphology and surface structure of CM (Fig. 2a) and the enlarged view (Fig. 2b). The microspheres exhibited good spherical shape and homogeneous surface with porous structure (the pore diameter is about 1 μm) when the microspheres in the wet state were first frozen in liquid nitrogen and then freeze-dried. When microspheres in the wet state were first frozen in a common refrigerator at −30 °C and then freeze dried, they exhibited a less porous structure, which is not shown here. This is because ice crystals grew much faster and more evenly when the microspheres were immediately frozen under liquid nitrogen condition, while bigger ice crystals slowly formed in the frozen process under the common refrigerator (Jiang & Hsieh, 2014). Taking advantage of this porous structure, it is a good idea to immobilize catalyst on this microsphere. But chitin lacks active groups that can be used for the immobilization of enzyme, while solid supports modified with polydopamine have been demonstrated to be an effective matrix for immobilization of biomolecules (Chao et al., 2013; Ko et al., 2013) since Messersmith and coworkers pioneered the single-step formation of polydopamine films on various substrates. Meanwhile, this method does not involve complex linkers and is free of organic solvents, making it suitable for a wide range of applications. Quinone groups generated by self-oxidation of dopamine in the biomolecule layer will actively take part in nucleophilic addition reaction with —SH and —NH_2 of amino acid residues of protein that leads to biomolecules being immobilized (Chao et al., 2013). So in this study, polydopamine was coated on the porous magnetic chitin microspheres to immobilize

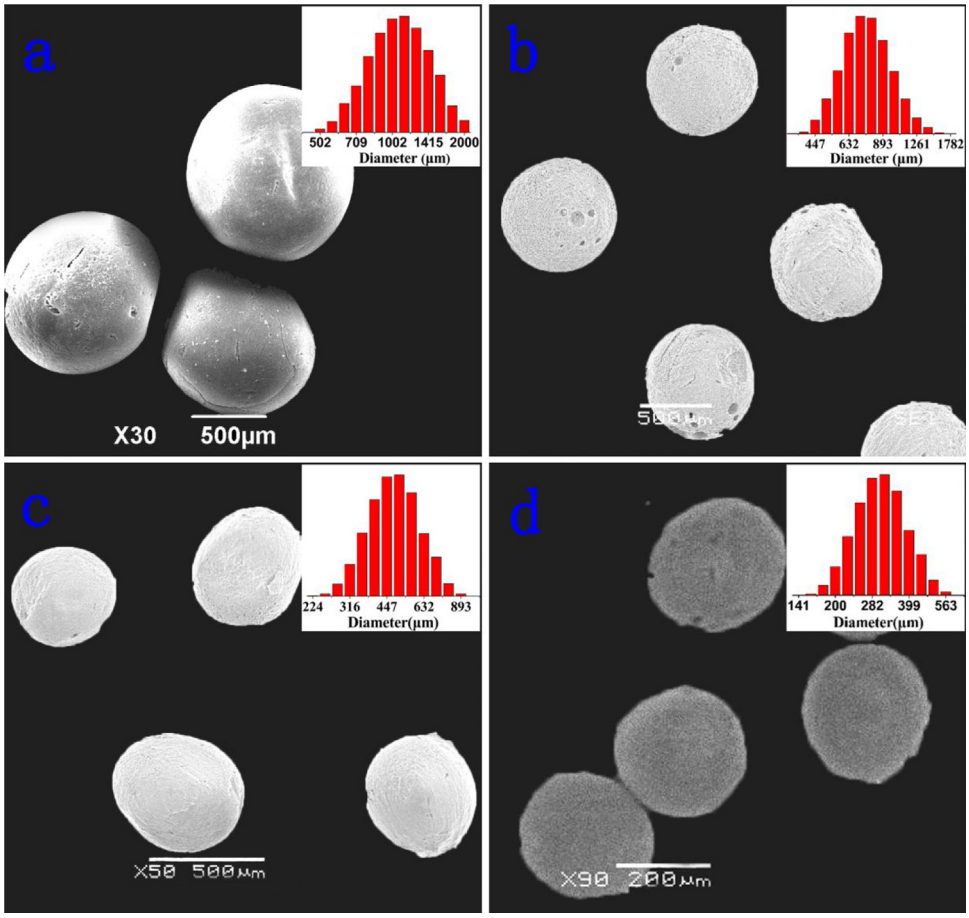

Fig. 1. SEM images of the CM microspheres with different particle diameters: (a) (1010 μm); (b) (750 μm); (c) (490 μm); (d) (280 μm).

α-amylase, a model biocatalyst. Meanwhile, glutaraldehyde (GA), the most common cross-linking agent for enzyme immobilization, was applied to further improve the immobilization efficiency of α-amylase. The amount of the covalently bound enzyme was found as 49.6 ± 2.6 mg per gram of PMCM. Unlike free enzyme, it is easy to quickly remove enzyme immobilized magnetic particles from the reaction medium solution using an external magnet. To investigate the reusability, the immobilized α-amylase was used for starch hydrolysis in pH 6.5 at 50 °C for 30 min. The relative activity of the first cycle was taken to be 100% and the hydrolysis reaction was repeated for 10 consecutive cycles. Fig. 3 shows the change of the activity of immobilized enzymes after multiple reusing by magnetic separation. It was concluded that the enzyme immobilized on PMCM particles was active and 95% of α-amylase activity was retained after 10 repeated cycles. Evidently, glutaraldehyde (GA) could bind α-amylase on PMCM more securely and leads to a stable performance in repeated use. Furthermore, the magnetic chitin microspheres loaded with enzyme could be removed and recovered easily by introducing a magnetic field, leading to an acceptable reusability. The results indicated that polydopamine coated magnetic chitin microspheres exhibited great potential for enzyme immobilization.

3.3. *Microspheres used as scaffod for gold nanoparticles*

Inspired by this unique porous structure, these microspheres were further loaded with noble metal gold to explore their application in catalyst system. Chitin/Au nanocomposite microspheres could be prepared by a convenient in-situ synthesis of Au nanoparticles. The successful preparation of 5 nm Au NPs in the porous chitin microspheres was confirmed by TEM images of ultrathin section of chitin/Au nanocomposite microspheres and XRD analysis (Fig. 3b), indicating the ability of chitin swollen polymer to control the growth of gold nanoparticles. Actually, chitin microspheres can be regarded as supramolecular chelating ligand for gold complex because chitin has long been demonstrated as an effective chelating agent for heavy metals (Zhou, Zhang, Zhou, & Guo, 2004). Upon contact with chitin microspheres, gold metals are scavenged by amino pendant chains due to the aurophilicity of these ligands. In this study, the immobilized amount of gold nanoparticles is 42.8 mg/g and the porous structure not only offered bulks of loading sites for the Au particles, but also protected the Au particles from aggregation, which may remarkably decrease the catalytic efficiency (Ning et al., 2013). Meanwhile, the freeze dried chitin/Au microspheres with perfect dispersion of gold nanparticles showed high surface-area mesoporous structure as mentioned above, which was beneficial for the catalytic reaction. The chitin/Au nanocomposite microspheres prepared by a convenient in situ synthesis of AuNPs in chitin microsphere showed good stability and this preparation method is energy-saving. Fig. 3a apparently demonstrated the Au nanoparticles are uniformly distributed in the meshy skeleton. The gold-catalyzed reduction of *o*-nitrophenol by $NaBH_4$ to *o*-aminophenol was chosen as a model reaction to evaluate the catalytic capability of the synthesized Au/chitin microspheres and the results are shown in Figs. 4c and 3d. The reduction did not proceed without the presence of Au/chitin microspheres catalyst, as evidenced by a constant absorption peak at 400 nm. When the free gold nanaparticles were added into the solution, the reduction of *o*-nitrophenol into *o*-aminophenol was completed in 1 min. But the free gold

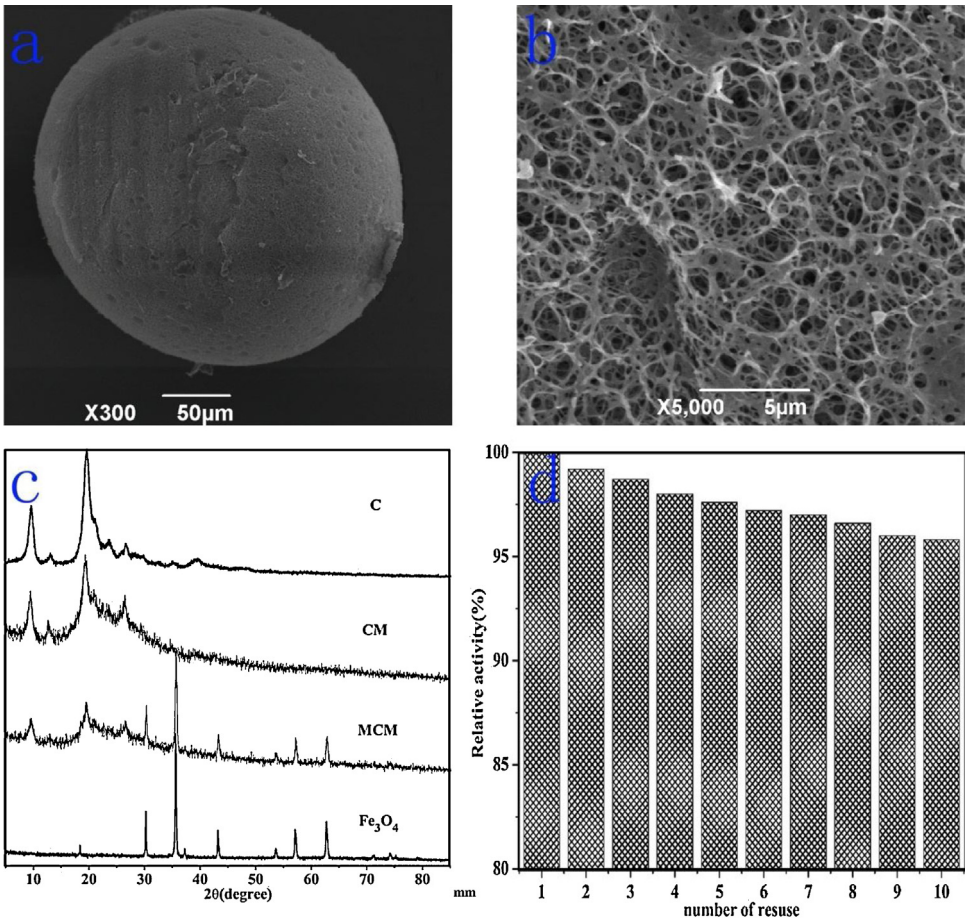

Fig. 2. (a) SEM images of CM. (b) Surface structure of CM. (c) Powder X-ray diffraction pattern of magnetic chitin microsphere (MCM) chitin microspheres (CM) native chitin(C) Fe_3O_4 nanoparticles. (d) Operation stability of immobilized α-amylase.

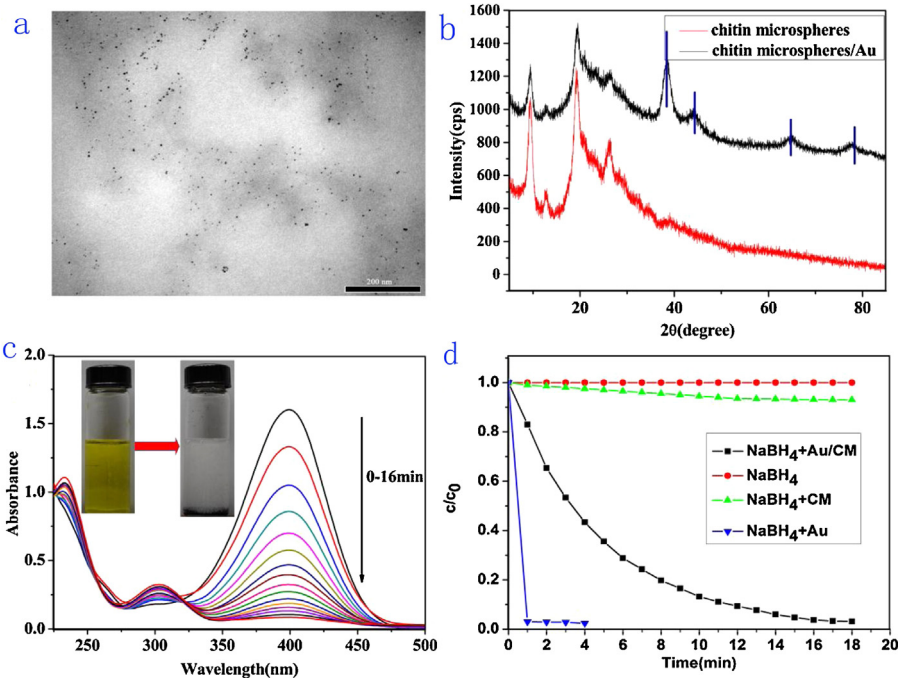

Fig. 3. (a)TEM images of ultrathin section of chitin/Au nanocomposite microspheres. (b) X-ray diffraction spectra of gold–chitin microspheres. (c) Illustration change of color and UV/vis spectra of *o*-nitrophenol after the addition of chitin/Au nanocatalyst. (d) Plots of C/C_0 versus time for different aerogels

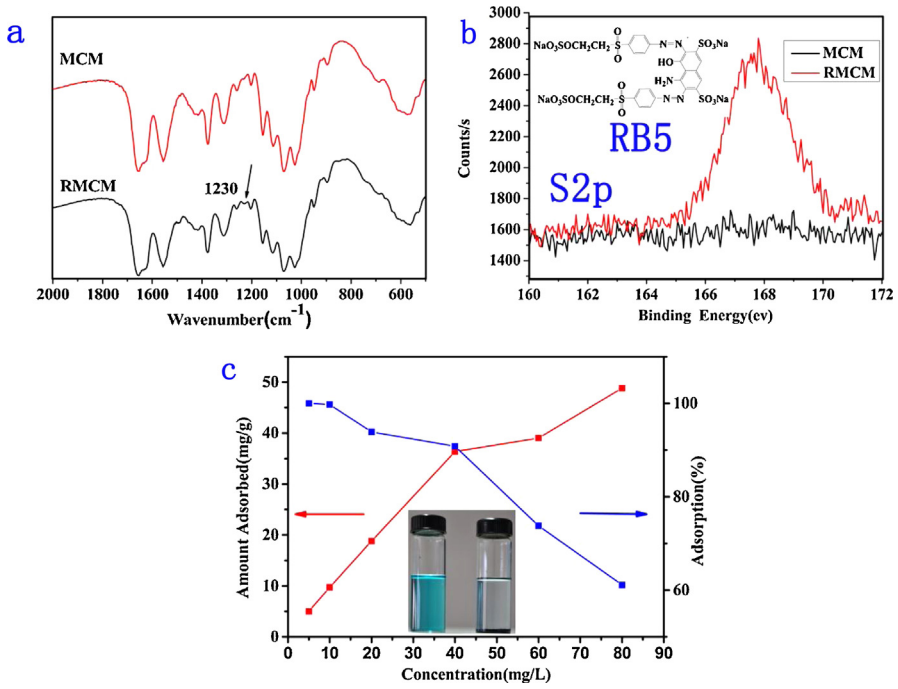

Fig. 4. (a) FT-IR spectra of the magnetic chitin microspheres (MCM) and Reactive Black 5 modified magnetic chitin microspheres (RMCM). (b) XPS spectrum of S 2p for MCM and RMCM. (c) Adsorption isotherm of methylthionine chloride (MB) on RMCM as a function of concentration at equilibrium. Inset: A digital graph shows an aqueous solution of MB (10 mg/L) and separation of dye loaded RMCM with a magnet in MB solution (right).

nanoparticles cannot be recoveried for reusage and will bring pollution to environment. However, when Au/chitin microspheres catalyst was introduced into the solution, the absorption at 400 nm slowly decreased while the absorption at 295 nm increased. The reduction of *o*-nitrophenol into *o*-aminophenol was completed in 18 min. The complete conversion of onitrophenol could also be visually appreciated by the color change of the solution from yellow to clear. The catalyst showed high activity after 10 successive cycles of reaction, with conversion rate close to 100% within 20 min of reaction time. Clearly, this kind of chitin microsphere was efficient in working as a capping agent to stabilize the nanoparticles by preventing their aggregation. The catalyst can be recovered conveniently from the reaction solution using external magnetic field for many cycles without significant loss of activity (in TEM images and XRD images, we did not use magnetic chitin microspheres because Fe_3O_4 and Au nanoparticles will interfere with each other, but in practical application, we can use magnetic chitin/Au nanocomposite microspheres for convenient recovery), making the catalyst reusable after multiple cycles of reaction. And recyclability of chitin microspheres makes it an attractive platform for nanocatalyst.

3.4. Microspheres used for removal of cationic pollutants

Magnetically separable microspheres are highly attractive for the adsorption and enrichment of organic pollutants associated with liquid-phase processes. The magnetic chitin microspheres could not effectively adsorb cationic dyes, which are widely used in chemical industry but harmful to human beings. It was found that nucleophilic substitution occurred between chitin and reactive dyes under alkaline condition, which incorporated groups with negative charge to chitin (Wang et al., 2013). So in this study, magnetic chitin microspheres modified with Reactive Black 5 were used as an adsorbent for cationic dyes. The FTIR spectra of magnetic chitin microspheres and Reactive Black 5 modified magnetic chitin microspheres are shown in Fig. 4a. The absorption band at 1230 cm^{-1} refers to characteristic asymmetric stretching of $-SO_2$, and this indicated that Reactive Black 5 has been successfully covalently attached to the magnetic chitin microspheres (Tüzmen et al., 2012). Moreover, the SO_3 group in RMCM was further confirmed by the peak at 167.64 eV from XPS measurement in Fig. 4b (Yuan et al., 2004). The utilization of magnetic chitin microspheres as an adsorbent was illustrated by demonstrating the removal of methylene blue from aqueous solution. As shown in Fig. 4c inset, after the addition of magnetic chitin microspheres, the color of methylene blue solution (10 mg/L) changed from blue to colorless within minutes, indicating a quick uptake of methylene blue by RMCM. The dye loaded RMCM could be easily separated from the solution by an external magnet. The adsorption ratio was 100% below the initial concentration of 10 mg/L, and the adsorption capacity of methylene blue on RMCM was determined to be 46 mg/g when the initial concentration was 80 mg/L (see Fig. 4c). It should be noted that the adsorption capacity of dye on polymer materials is related to the structural properties, pH, and temperature (Liu, Chung, Oh, & Seo, 2012) and this is beyond the focus of the current study.

4. Conclusion

In summary, a simple and green method was developed to prepare chitin microspheres from NaOH/urea solvent without any toxic chemicals and this is our advantage. Commonly, microspheres were prepared from chitosan which is obtained by deacetylation of chitin and toxic glutaraldehyde or expensive genipin is used as the crosslinker. Developing microspheres directly from chitin will minimize at least the costly chitosan synthesis step and does not involve any chemical cosslinkers. Moreover, chitin microspheres were funtionlized via three different ways to broden its application in the field of food, energy and environment, which will provide guidance for future research.

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References

Biró, E., Németh, A. S., Feczkó, T., Tóth, J., Sisak, C., & Gyenis, J. (2009). Three-step experimental design to determine the effect of process parameters on the size of chitosan microspheres. *Chemical Engineering and Processing: Process Intensification*, *48*(3), 771–779.

Chao, C., Liu, J., Wang, J., Zhang, Y., Zhang, B., Zhang, Y., et al. (2013). Surface modification of halloysite nanotubes with dopamine for enzyme immobilization. *ACS Applied Materials & Interfaces*, *5*(21), 10559–10564.

Chung, J. W., Guo, Y., Kwak, S.-Y., & Priestley, R. D. (2012). Understanding and controlling gold nanoparticle formation from a robust self-assembled cyclodextrin solid template. *Journal of Materials Chemistry*, *22*(13), 6017–6026.

Das, S., & Subuddhi, U. (2014). Controlled delivery of dexamethasone to the intestine from poly(vinyl alcohol)–poly(acrylic acid) microspheres containing drug-cyclodextrin complexes: Influence of method of preparation of inclusion complex. *RSC Advances*, *4*(46), 24222–24231.

Deng, H., Lin, P., Xin, S., Huang, R., Li, W., Du, Y., et al. (2012). Quaternized chitosan-layered silicate intercalated composites based nanofibrous mats and their antibacterial activity. *Carbohydrate Polymers*, *89*(2), 307–313.

Duan, B., Chang, C., Ding, B., Cai, J., Xu, M., Feng, S., et al. (2013). High strength films with gas-barrier fabricated from chitin solution dissolved at low temperature. *Journal of Materials Chemistry A*, *1*(5), 1867–1874.

Hu, B., Ting, Y., Zeng, X., & Huang, Q. (2012). Cellular uptake and cytotoxicity of chitosan–caseinophosphopeptides nanocomplexes loaded with epigallocatechin gallate. *Carbohydrate Polymers*, *89*(2), 362–370.

Jiang, F., & Hsieh, Y.-L. (2014). Super water absorbing and shape memory nanocellulose aerogels from TEMPO-oxidized cellulose nanofibrils via cyclic freezing–thawing. *Journal of Materials Chemistry A*, *2*, 350–359.

Ko, E., Yang, K., Shin, J., & Cho, S. W. (2013). Polydopamine-assisted osteoinductive peptide immobilization of polymer scaffolds for enhanced bone regeneration by human adipose-derived stem cells. *Biomacromolecules*, *14*(9), 3202–3213.

Li, G., Du, Y., Tao, Y., Deng, H., Luo, X., & Yang, J. (2010). Iron(II) cross-linked chitin-based gel beads: Preparation, magnetic property and adsorption of methyl orange. *Carbohydrate Polymers*, *82*(3), 706–713.

Li, W., Xu, R., Zheng, L., Du, J., Zhu, Y., Huang, R., et al. (2012). LBL structured chitosan-layered silicate intercalated composites based fibrous mats for protein delivery. *Carbohydrate Polymers*, *90*(4), 1656–1663.

Liang, S., Liu, L., Huang, Q., & Yam, K. L. (2009). Preparation of single or double-network chitosan/poly(vinyl alcohol) gel films through selectively cross-linking method. *Carbohydrate Polymers*, *77*(4), 718–724.

Liu, S., Zhou, J., Zhang, L., Guan, J., & Wang, J. (2006). Synthesis and alignment of iron oxide nanoparticles in a regenerated cellulose film. *Macromolecular Rapid Communications*, *27*(24), 2084–2089.

Liu, T. G., Li, B., Huang, W., Lv, B., Chen, J., Zhang, J. X., et al. (2009). Effects and kinetics of a novel temperature cycling treatment on the *N*-deacetylation of chitin in alkaline solution. *Carbohydrate Polymers*, *77*(1), 110–117.

Liu, F., Chung, S., Oh, G., & Seo, T. S. (2012). Three-dimensional graphene oxide nanostructure for fast and efficient water-soluble dye removal. *ACS Applied Materials & Interfaces*, *4*(2), 922–927.

Liu, D., Zhu, Y., Li, Z., Tian, D., Chen, L., & Chen, P. (2013). Chitin nanofibrils for rapid and efficient removal of metal ions from water system. *Carbohydrate Polymers*, *98*(1), 483–489.

Liu, H., Wei, Z., Hu, M., Deng, Y., Tong, Z., & Wang, C. (2014). Fabrication of degradable polymer microspheres via pH-responsive chitosan-based Pickering emulsion photopolymerization. *RSC Advances*, *4*(55), 29344–29351.

Mi, F.-L., Shyu, S.-S., Lin, Y.-M., Wu, Y.-B., Peng, C.-K., & Tsai, Y.-H. (2003). Chitin/PLGA blend microspheres as a biodegradable drug delivery system: A new delivery system for protein. *Biomaterials*, *24*(27), 5023–5036.

Muzzarelli, R. A., Boudrant, J., Meyer, D., Manno, N., DeMarchis, M., & Paoletti, M. G. (2012). Current views on fungal chitin/chitosan, human chitinases, food preservation, glucans, pectins and inulin: A tribute to Henri Braconnot, precursor of the carbohydrate polymers science, on the chitin bicentennial. *Carbohydrate Polymers*, *87*(2), 995–1012.

Muzzarelli, R. A. A. (1980). Immobilization of enzymes on chitin and chitosan. *Enzyme and Microbial Technology*, *2*(3), 177–184.

Muzzarelli, R. A. (1996). Chitosan-based dietary foods. *Carbohydrate Polymers*, *29*(4), 309–316.

Ning, Y., Yang, Y., Wang, C., Ngai, T., & Tong, Z. (2013). Hierarchical porous polymeric microspheres as efficient adsorbents and catalyst scaffolds. *Chemical Communications*, *49*(78), 8761–8763.

Oh, J. S., Dang, L. N., Yoon, S. W., Lee, P. C., Kim, D. O., Kim, K. J., et al. (2013). Amine-functionalized polyglycidyl methacrylate microsphere as a unified template for the synthesis of gold nanoparticles and single-crystal gold plates. *Macromolecular Rapid Communications*, *34*(6), 504–510.

Patil, S. B., & Sawant, K. K. (2011). Chitosan microspheres as a delivery system for nasal insufflation. *Colloids and Surfaces B: Biointerfaces*, *84*(2), 384–389.

Setoguchi, T., Yamamoto, K., & Kadokawa, J.-i. (2012). Preparation of chitin nanofiber-graft-poly(L-lactide-*co*-ε-caprolactone) films by surface-initiated ring-opening graft copolymerization. *Polymer*, *53*(22), 4977–4982.

Silva, S. S., Duarte, A. R. C., Mano, J. F., & Reis, R. L. (2013). Design and functionalization of chitin-based microsphere scaffolds. *Green Chemistry*, *15*(11), 3252–3258.

Sun, Y., Li, Y., Nie, J., Wang, Z., & Hu, Q. (2013). High-strength chitosan hydrogels prepared from LiOH/urea solvent system. *Chemistry Letters*, *42*(8), 838–840.

Tajiri, R., Mihata, A., Yamamoto, K., & Kadokawa, J.-i. (2014). Facile preparation of chitin gels with calcium bromide dihydrate/methanol media and their efficient conversion into porous chitins. *RSC Advances*, *4*(11), 5542–5546.

Tüzmen, N., Kalburcu, T., & Denizli, A. (2012). α-Amylase immobilization onto dye attached magnetic beads: Optimization and characterization. *Journal of Molecular Catalysis B: Enzymatic*, *78*, 16–23.

Wang, Y., Wang, S., Lei, J., Sun, H., Jin, W., Ye, T., et al. (2013). Removal of reactive dyes by a solid waste product from food processing: Crayfish carapace. *Desalination and Water Treatment*, *52*(28–30), 1–12 (ahead-of-print).

Yan, H., Li, H., Yang, H., Li, A., & Cheng, R. (2013). Removal of various cationic dyes from aqueous solutions using a kind of fully biodegradable magnetic composite microsphere. *Chemical Engineering Journal*, *223*, 402–411.

Yuan, Y., Ai, F., Zang, X., Zhuang, W., Shen, J., & Lin, S. (2004). Polyurethane vascular catheter surface grafted with zwitterionic sulfobetaine monomer activated by ozone. *Colloids and Surfaces B: Biointerfaces*, *35*(1), 1–5.

Zhou, D., Zhang, L., Zhou, J., & Guo, S. (2004). Cellulose/chitin beads for adsorption of heavy metals in aqueous solution. *Water Research*, *38*(11), 2643–2650.