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Introduction

Nowadays, energy, resources and climate problems have drawn serious attention in view of sustainability.^{1,2} Enhanced environmental consciousness has promoted the fusion of green chemistry with nanotechnology.^{3–5} The purpose of improving efficiency and lightening the environmental burden has made nanoscience and nanotechnology one of the most burning issues. In the ever-expanding field of nanomaterials research, noble metallic nanoparticles (Au, Ag, Pt, *etc.*) have garnered extensive attention due to their fascinating properties and applications in catalysis,^{6,7} sensors,^{8,9} imaging,^{10,11} photonics.^{12,13} Undoubtedly, catalysis is certainly among the most intensely studied problems.¹⁴ Recently, the efficiency of noble metallic catalysts in organic synthesis has been significantly improved by employing nano-sized catalysts due to their high ratio of surface area to volume, leading to dramatic enhance-

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Ag–Fe₃O₄ nanocomposites@chitin microspheres constructed by *in situ* one-pot synthesis for rapid hydrogenation catalysis†

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The fabrication of reusable and biodegradable materials from renewable resources such as chitin is essential for a sustainable world. In the present work, chitin was dissolved in 11 wt% NaOH–4 wt% urea aqueous solution *via* freezing–thawing, and then chitin microspheres (RChS) were prepared by a sol–gel transition method. Subsequently, novel magnetic $Ag-Fe_3O_4$ @chitin microspheres (MRChS) were constructed successfully by an *in situ* one-pot synthesis of $Ag-Fe_3O_4$ nanoparticles onto the RChS surface. The magnetic chitin microspheres displayed a spherical shape with a 3D-mesh structure, and had a narrow size distribution (150–400 µm). There were many micro- and nano-pores existing in MRChS, and the $Ag-Fe_3O_4$ nanoparticles were immobilized through anchoring with the acetyl amine groups of chitin in these pores. The MRChS microspheres were used as a chromatography column packing material for a "catalytic reaction column", and exhibited highly effective catalytic activity in the rapid transformation from 4-nitrophenol to 4-aminophenol. Moreover, the microspheres displayed a small hysteresis loop and low coercivity, as well as high turnover frequency (at least 10 times) without any loss of catalytic activity. Thus, MRChS could be quickly removed from the water under a magnetic field, leading to easy recycling and reuse. Therefore, this is an environmentally friendly process, and would be highly beneficial to address industrial requirements.

> ments in the contact between reactants and catalysts during the catalysis.^{15,16} However, the difficult recovery and reuse of these expensive nano-sized catalysts from the catalytic reaction systems by using traditional methods such as filtration and centrifugation results in environmental and economic barriers.^{17,18} To overcome this issue, many efforts have been made to synthesize magnetically retrievable nanocatalysts which possess high activity, selectivity, as well as meet the requirements of high accessibility with improved reusability.^{19,20} Unfortunately, they very easily aggregate to minimize their surface, leading to a remarkable reduction in their catalytic activities.^{21,22} To solve the problem of agglomeration, the immobilization of magnetic nanocatalysts on a solid support has been regarded as one of the most efficient ways to overcome this drawback.23,24 Moreover, many solid supported catalysts have been successfully utilized for catalytic applications.²⁵ Materials with high surface area and good mechanical stability, such as carbon-based materials (carbon nanotubes,²⁶ graphene oxide,²⁷ and carbon-based nanofibers²⁸), soluble and insoluble polymers,^{29,30} mesoporous silica,³¹ metal oxide,³² titania,³³ etc., have proved to be popular candidates for nanocatalyst supports. Furthermore, as far as the call of the world for energy and sustainability is concerned,



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bio-inspired templating techniques using nature polymers (protein,³⁴ chitosan,³⁵ cellulose,^{36,37} *etc.*) for catalyst supports has become a valuable strategy.

It is worth noting that chitin is the second most abundant natural biopolymer after cellulose, existing mainly in the exoskeletons of crabs and shrimps.³⁸ Chitin has been reported to be a nature polysaccharide with significant biofunctionality.^{39,40} However, chitin remains perhaps the least exploited biomass source due to its intractable molecular structure which results in its recalcitrance towards dissolution in most common solvent systems.⁴¹ To date, only a few solvents have been employed for chitin dissolution to prepare materials, including hexafluoroisopropanol (HFIP),42 a dimethylacetamide (DMAc)-LiCl mixture,⁴³ CaCl₂-MeOH⁴⁴ and ionic liquids.45 In our laboratory, chitin was dissolved in an 11 wt% NaOH-4 wt% urea aqueous solution by using freezing (-30 °C)-thawing cycles to prepare chitin hydrogels,⁴⁶ aerogels⁴⁷ and films.⁴⁸ However, chitin microspheres fabricated directly from a chitin solution have been scarcely reported. Noticeably, chitin has been extensively investigated as an adsorbent for metal extraction due to its large amount of acetyl amino groups, which serve as chelation sites for metal ions.⁴⁹ Therefore, chitin microspheres are a potential support for binding metal ions as well as for fabricating and stabilizing metal nanocatalysts. Thus, we attempted to construct an easily retrievable chitin microsphere through a low-cost and energyeffective pathway by using the sol-gel transition (SGT) method. Subsequently, Ag-Fe₃O₄ nanocomposites were in situ one-pot synthesized onto chitin microspheres to construct magnetic nanocatalysts. Furthermore, the catalytic activity and retrievable properties of the nanocomposite microspheres were measured to evaluate their application in adsorption and catalyst fields. It is not hard to imagine that the regenerated chitin microspheres with external and internal surface area, porosity, hydrophilicity, and acetyl amino groups would be easy for fabricating and stabilizing functional nanoparticles for separation or catalysis thus showing potential application in chromatographic separation and nanocatalysis. The utilization of chitin from a renewable resource to directly construct new microspheres via an environmentally friendly process would be meaningful for chemistry for a sustainable world.

Experimental section

Materials

Chitin powder was purchased from Golden-Shell Biochemical Co. Ltd (Zhejiang, China). The chitin powder was purified by a procedure described previously.⁴⁸ Firstly, 100 g of chitin powder was treated with 400 g of 5 wt% NaOH solution for 10 h under vigorous stirring. This suspension was then filtered and washed with distilled water. Subsequently, the resulting chitin powder was treated with 400 g of 7% (v/v) hydrochloric acid aqueous solution for 1 day to remove the residual protein. After filtration and rinsing with distilled water, the treated sample was dispersed in 400 g of 5 wt% NaOH solution for

1 day. The pigments were then removed from the sample by using 1.7 wt% sodium chlorite in 0.3 M sodium acetate buffer (400 g) for 6 h at 80 °C, followed by washing with distilled water and drying to obtain the purified chitin powder. The degree of acetylation (DA) of the original and purified chitin was calculated to be 90% and 94%, respectively, from the FT-IR spectra according to

$$A_{1560}/A_{2875} = 0.0125 \times \mathrm{DA} + 0.2 \tag{1}$$

where A_{1560}/A_{2875} is the ratio of the absorption bands at 1560 cm⁻¹ and 2875 cm⁻¹.⁵⁰ The weight-average molecular weight (M_w) was determined to be 53.4 × 10⁵ in 5% (w/v) LiCl-DMAc by dynamic light scattering (DLS, ALV/GGS-8F, ALV, Germany). All of the chemical reagents were purchased from commercial sources in China, and were of analytical-grade.

Fabrication of regenerated chitin microspheres

The sol-gel process means the transition of a system from a liquid ("sol") into a solid ("gel") phase. In a colloidal suspension, the microspheres can aggregate into a new phase to form a gel.^{51,52} In this work, the regenerated chitin microspheres were fabricated by a sol-gel transition method as follows. 7 g of purified chitin powder was dispersed into 93 g of a mixture of NaOH, urea, and distilled water (11:4:85, by weight) with stirring to obtain a suspension. Subsequently, the suspension was frozen at -30 °C for 4 h, and then thawed at room temperature. The freezing-thawing cycle was repeated twice to obtain a transparent chitin solution, with a chitin concentration of 7 wt%. The chitin solution was degassed by centrifugation at 7200 rpm for 15 min at 0 °C. A well-mixed suspension containing 100 mL of paraffin oils, 1.5 g of Tween 80 and 0.5 g of Span 80 were dispersed in a reactor. The resulting suspension was stirred at 500 rpm for 30 min, and then 20 mL of the chitin solution was dropped into the suspension within 5 min. The suspension was kept stirring for 2 h at the same stirring speed at 0 °C, and then was heated to 25 °C with the same stirring speed for 0.5 h to form the regenerated chitin gels. Subsequently, 2 ml of epichlorohydrin (ECH) was dropped into the suspension within 10 min, and then was stirred at the same speed and temperature for an additional 1.5 h to completely crosslink the chitin gel. To the resultant suspension, dilute hydrochloric acid (10%) was added until pH = 7. After removing the liquid paraffin, about 20 ml of regenerated chitin microspheres in the substratum were obtained, coded as RChS. The RChS microspheres were washed with deionized water, and then ethanol successively three times to remove the residual paraffin oils, Tween 80 and Span 80. Finally, the microspheres were freeze-dried or stored in 20% ethanol at 5 °C for characterization.

Fabrication of Ag-Fe₃O₄@chitin nanocomposite microspheres

Magnetic nanocomposite chitin microspheres were *in situ* onepot synthesized by redox reaction between Ag_2O and $Fe(OH)_2$ in the RChS microspheres as follows. Desired amounts of $FeCl_2 \cdot 4H_2O$ were dissolved in 140 mL of water, to which 15 mL

of hydrated RChS (water content 80 wt%) was added, and then the suspension was stirred for 1 h with the protection of nitrogen at room temperature. Subsequently, the mixture was heated to 90 °C and a certain amount of 1 M NaOH aqueous solution was mixed with the solution rapidly. The mixture was maintained at 90 °C in air with stirring after the addition of 10 mL of an aqueous solution containing a desired amount of AgNO₃ for 2 min to obtain the magnetic chitin microspheres, coded as MRChS The MRChS microspheres were coded as M15, M30 and M45, according to the different concentrations of FeCl₂ at 15 mmol, 30 mmol, 45 mmol, corresponding to the amounts of 1 M NaOH/AgNO3 being 10 mL/0.212 g, 20 mL/ 0.424 g and 30 ml/0.636 g, respectively. The MRChS microspheres were washed with deionized water until pH = 7. Finally, the MRChS microspheres were freeze-dried or stored in 20% ethanol at 5 °C for characterization. The optical photomicrographs of the chitin microspheres were taken using a biological microscope (EX20, Sunny, China).

Characterization

Fourier-transform infrared (FT-IR) spectra of the microsphere samples were recorded on a Perkin-Elmer FT-IR spectrometer (model 1600, Perkin-Elmer Co. USA). The tested samples were prepared by the KBr-disk method. Wide-angle X-ray diffraction (XRD) was carried out on an XRD instrument (XRD-6000, Shimadzu, Japan) with Cu K α radiation ($\lambda = 0.154$ nm). The XRD data were collected from $2\theta = 10$ to 90° at a scanning rate of 2° min⁻¹. X-ray photoelectron spectra (XPS) were recorded on a Kratos XSAM800 X-ray photoelectron spectrometer, using Mg Kα radiation as the excitation source. Thermo-gravimetric analysis (TGA) of the dry samples was carried out on a Pyris TGA linked to a Pyris diamond TA Lab System (Perkin-Elmer Co., USA) at a heating rate of 10 K min⁻¹ from 30 to 700 °C under an air atmosphere. Scanning electron microscopy (SEM) was performed on a FESEM (SEM, SIRION TMP, FEI) by using an accelerating voltage of 12 kV. The microspheres in the wet state were frozen in liquid nitrogen and freeze-dried under vacuum. The freeze-dried microspheres were sputtered with gold before observation. The high-resolution transmission electron microscopy (HRTEM) images were taken on a JEOL JEM 2010 FEF (UHR) microscope at 200 kV. The imbibed water of MRChS was exchanged to acetone, and then the microspheres were embedded with epoxy resin Epon812 (Shanghai Bioscience, Shanghai, China). After that, the embedded specimen was sectioned by a Leica Ultracut-E using a diamond knife to prepare approx. 80 nm-thick sections. These samples were sputtered with carbon before HRTEM. The definite size distribution of RChS and MRChS was determined with a Mastersizer 2000 laser particle size analyzer (Malvern, UK).

Nitrogen physisorption measurements at 77 K were performed by a Micromeritics AsAp2020 (USA), and Brunauer– Emmett–Teller (BET) and Barrett–Joyner–Halendar (BJH) analyses were done by software. The microspheres were degassed at 105 °C in vacuum to remove all the adsorbed species. BET analysis was performed for relative vapor pressures of 0.05–0.3. The BJH analysis was performed from the desorption branch of the isotherms.

The magnetic properties of the composite microspheres were measured with a vibrating sample magnetometer (VSM, Lake Shore, 7304, USA) at 25 °C, and the hysteresis loop was obtained in a magnetic field that varied from -0.6 to +0.6 T.

Catalytic activity assays

The microspheres used for catalytic reduction were obtained via exchanging the water in the hydrated microspheres to acetone and then drying under vacuum at ambient temperature for 24 h. The catalytic activity of Ag-Fe₃O₄@chitin microspheres was evaluated by using the reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP) in a quartz cell at 25 °C, monitored using UV-visible spectroscopy (UV-6, Shanghai Meipuda instrument Co., Ltd, Shanghai, China). More specifically, 0.75 mL of fresh NaBH₄ aqueous solution (0.4 M) was mixed with 1.5 mL of an aqueous dispersion of MRChS (Ag-Fe₃O₄(a) chitin microspheres) at 50 mg L^{-1} . Subsequently, 0.75 mL of 4-NP aqueous solution $(4 \times 10^{-4} \text{ M})$ was added. As a result, the concentration of 4-NP and NaBH₄ in the reaction solution was 1×10^{-4} and 0.1 M, respectively. The catalytic reaction was monitored at decided time intervals. A control experiment was carried out under identical conditions except that the Ag-Fe₃O₄@chitin microspheres aqueous dispersion was replaced with 50 mg L^{-1} chitin microspheres. At the end of the reaction, the catalyst was separated from the reaction system using an external magnet, washed three times with ethanol and dried at room temperature for the next cycle. Additionally, the MRChS microspheres suspended in distilled water were packed in an injector (1 ml) which acted as a reaction column to form a 1.5 cm long gel bed. The column was equipped with a glass collector, and a piece of cotton wool was placed at the bottom of the injector to prevent the outflow of the MRChS. The mixture solution of NaBH₄ (0.1 M) and 4-NP (1×10^{-4} M) was added in the injector from the top and squirted through the MRChS packing. The product was gathered in the glass collector.

Results and discussion

Green process of the preparation of the magnetic chitin microspheres

The process for the preparation of the chitin microspheres (RChS) is described in Scheme S1.[†] Firstly, the chitin solution was prepared by using aqueous NaOH–urea, *via* a freezing–thawing method, which is an environmental friendly solvent. Moreover, it was very easy to induce a sol–gel transition for the chitin solution directly through heating up to 20 °C.⁴⁷ Secondly, the chitin droplets were heated to 25 °C to proceed the "sol–gel" transition. Unfortunately, the chitin microspheres regenerated directly with heating displayed a non-homogeneous distribution and nonspherical shape (see Fig. S1a[†]). This might result from their poor mechanical properties. To construct chitin microspheres with a stronger network

Paper

Green Chemistry

structure, further crosslinking was performed. As expected, the chitin microspheres crosslinked with ECH (see Scheme S2†) displayed a perfectly well-defined spherical shape with a porous structure (see Fig. S1b and S2†). The preparation of RChS was a green process, which included the direct utilization of a renewable resource, dissolution in the environmental friendly NaOH–urea aqueous solvent, no evaporation of any chemical agents, as well as low cost and a clean emulsification pathway. It was noted that the dissolution and regeneration of the chitin was a physical process through non-covalence, and the DA value hardly changed.⁴⁸

The FTIR spectra of the regenerated chitin microspheres (see Fig. S3[†]) showed there were large amounts of acetyl amino groups (DA = 91%, calculated with eqn (1)), which could serve as anchoring sites for metal ions.49 Thus, the Fe2+ can be easily absorbed and stabilized onto the chitin microspheres through strong interactions with these acetyl amino groups of chitin. Moreover, by adding alkaline and AgNO₃, the Fe(OH)₂ reacted with Ag₂O to give magnetic Ag-Fe₃O₄ nanocomposites in the chitin microspheres, leading to the formation of the magnetic MRChS microspheres. Herein, the entire preparation of the MRChS composite in aqueous solution was finished under clean and moderate conditions, and was also an environmentally friendly method. Moreover, the original feature (such as metal ion absorbent) of chitin was maintained in the magnetic microspheres, leading to them being an excellent candidate for nanoparticle support.

Structure and morphology of the magnetic chitin microspheres

The optical photomicrographs and size distribution observed with the laser particle size analyzer for the RChS and MRChS microspheres are shown in Fig. 1. RChS and MRChS (M30) in their swollen state had average diameters about 251 μ m and 253 μ m, respectively, and displayed a narrow size distribution

which fitted Gaussian distribution on the whole (Fig. 1b and d). It was noted that both RChS and MRChS exhibited the same spherical shape and size distribution. This could be explained since the synthesis reaction of Ag-Fe₃O₄ occurred in the pores of the chitin microspheres, so the morphology of the composite microspheres hardly changed compared to the original one. From the analysis of the XRD patterns (see Fig. S4[†]), the Ag-Fe₃O₄@chitin composite microspheres displayed a typical α -chitin structure.⁴⁸ Distinct peaks of MRChS at 2θ = 38.1, 44.3, 64.4, 77.5 and 81.6° were assigned to the (111), (200), (220), (311) and (222) planes of Ag, whereas the peaks at $2\theta = 30.2, 35.6, 57.2, \text{ and } 62.6^{\circ}$ were ascribed to the (220), (311), (511) and (440) planes of Fe₃O₄, respectively. The diffraction peaks of Ag appeared to be much stronger than those of Fe₃O₄, suggesting that the crystallization of Fe₃O₄ nanoparticles was not as perfect as that of Ag.¹⁷ In view of the results in Fig. 1 and S4,[†] the MRChS consisted of chitin, Ag and Fe₃O₄.

To clarify the morphology of the chitin microspheres and the distribution of Ag-Fe₃O₄ nanoparticles on their surfaces, the microspheres were observed by SEM. The SEM images of the surfaces of RChS and MRChS are shown in Fig. 2, S2 and S5.† The morphology of RChS and MRChS displayed a spherical shape (Fig. 2) and 3D network structure with interconnecting pores (see Fig. S2 and S5[†]). Obviously, there was no essential change in the MRChS morphology during the growth of the Ag-Fe₃O₄ nanoparticles compared with RChS. The surface of RChS exhibited a microporous structure with an apparent mean diameter of 3-20 µm for the micropores (measured from Fig. 2). The pore formation was a result of the phase separation induced by the occupying H₂O during the sol-gel process, where the solvent-rich regions contributed to the pore formation.⁵³ However, as shown in Fig. 2b-d, the MRChS composite microspheres (M15, M30 and M45) exhibited relatively denser surfaces than that of RChS. It was further



Fig. 1 Optical photomicrographs of RChS (a) and MRChS (c), the scale bar is 300 μm and the size distribution of RChS (b) and M30 (d) was observed with a laser particle size analyzer.



Fig. 2 SEM images of the RChS (a), M15 (b), M30 (c) and M45 (d). The scale bar is 50 $\mu m.$



Fig. 3 Nitrogen adsorption and desorption isotherms (a) and Barrett–Joyner–Halendar (BJH) pore size distribution (b) of RChS, M15, M30, and M45.

confirmed that Ag–Fe₃O₄ nanoparticles could be readily impregnated into the pores and/or channels of the chitin matrix through bounding to the chitin macromolecules as a result of the chelation of acetyl amino groups. The Ag–Fe₃O₄ nanoparticles were extensively and homogeneously dispersed on the surface of the chitin microspheres (see Fig. S5b–d†). This suggested that the unique 3D-porous structure and abundant acetyl amino groups of RChS provided a good platform for the immobilization of Ag–Fe₃O₄ nanoparticles, which were deposited and anchored in the chitin matrix during a redox reaction process between Fe(OH)₂ and Ag₂O through being immersed in salt and alkaline aqueous solution.

The SEM could only be used to confirm the micro-sized porous structure appearing on the surface of the chitin microspheres. Thus, the surface area and corresponding nano-sized porous structure of the Ag-Fe₃O₄ nanocomposites@chitin microspheres was determined by using N2 adsorptiondesorption. Fig. 3 shows the nitrogen adsorption and desorption isotherms and Barrett-Joyner-Halendar (BJH) pore size distribution of RChS, M15, M30, and M45. Both RChS and MRChS exhibited a type I H3 hysteresis loop according to the IUPAC and BDDT classification, as the adsorption branch is parallel to P/P^0 over a large extent.⁵⁴ The BET surface area, pore volume and pore size of RChS and MRChS were calculated, and the data are summarized in Table 1. The results reveal that inside the microspheres there are nanosized pores, and the most probable values of the pore size range from 10 to 50 nm (Fig. 3b), indicating that MRChS maintained its initial nanoporous structure after the immobilization of the Ag-Fe₃O₄ nanoparticles. The decrease in surface

Table 1	Surfaces	nronerties	of RChS	and MRChS
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	Structural parameters				
Sample	Surface area $(m^2 g^{-1})$	Pore volume (cm ³ g ⁻¹)	Pore size (nm)		
RChS	34.6	0.252	22.3		
M15	33.1	0.124	14.1		
M30	32.5	0.157	15.3		
M45	18.8	0.084	26.2		

area and pore volume of MRChS compared with RChS indicated that the Ag- Fe_3O_4 nanoparticles occupied the pores of the chitin microspheres.

TEM and high resolution TEM (HRTEM) were used to study the microstructure of the magnetic chitin microspheres in more detail. Fig. 4 shows the TEM and HRTEM images of the magnetic microspheres of M10 (a), M20 (b) and M30 (c). The Ag-Fe₃O₄ nanocomposites with spherical shapes and mean diameters of 10-40 nm were dispersed uniformly in the chitin matrix. The size of the Ag-Fe₃O₄ nanoparticles in these microspheres changed slightly with an increase in the Ag-Fe₃O₄ content (see Fig. 4a-c, and Fig. S6[†]). This could be explained as the acetyl amino groups on the RChS surface captured a certain amount of Fe²⁺, which could terminate the reduction action between Fe(OH)2 and Ag2O, and the micro- and nanosized pores of the chitin matrix acted as micro-chambers to limit the growth of the Ag-Fe₃O₄ nanoparticles. In our findings, the preparative chitin microspheres could be used as an excellent metallic nanoparticle support. From the HRTEM image and the corresponding energy-dispersive spectrum (EDS) in Fig. 4d and e, clear crystalline lattices were attributed to the (111) (d = 0.235 nm) and (200) (d = 0.203 nm) phases of Ag, and (311) (d = 0.251 nm) and (511) (d = 0.162 nm) phases of Fe₃O₄. The results confirmed the successful synthesis of Ag-Fe₃O₄ nanoparticles. Interestingly, there were some rod-like crystals of Fe₃O₄ around the spherical Ag-Fe₃O₄ nanoparticles. This could be explained from the EDS curve for the rod-like crystal (see Fig. S7b[†]), where there were only C (sputtered carbon), O and Fe elements in this crystal, so these nanocrystals were made of Fe₃O₄, which result from the oxidation of Fe(OH)₂.⁵⁵

More detailed information regarding the chemical and bonding environment of the chitin microspheres and Ag- Fe_3O_4 nanoparticles was ascertained using X-ray photoelectron spectroscopy (XPS). Fig. 5a shows the fully scanned spectra in the range of 0–800 eV. These results from the overview spectra demonstrate that C, O, N, Ag and Fe exist in the magnetic microsphere of M30, while only C, O and N appear in RChS. Fig. 5b shows the XPS spectrum in the Ag 3d region of M30. Two peaks at 373.8 eV and 367.8 eV corresponded with Ag3d_{5/2}



Fig. 4 TEM images of the magnetic microspheres of M15 (a), M30 (b) and M45 (c). HRTEM image of an individual particle with the lattice fringes marked (d). Typical EDS spectrum of a particle from the HRTEM (e).



Fig. 5 XPS fully scanned spectra of RChS and M30 (a); XPS spectra of Ag 3d (b) and Fe 2p (c) for M30; XPS spectra of N1s (d), C1s (e) and O1s (f) for RChS and M30, respectively.

and Ag3d_{3/2} binding energies, respectively, consistent with the reported data of Ag^{0,7} Further, the binding energies at 724.6 and 711.1 eV in Fig. 5c were attributed to $2p_{1/2}$ and $2p_{3/2}$ of Fe, which is the typical core level spectrum of Fe₃O₄ with broad peaks, confirming the formation of Fe₃O₄.^{56,57} Interestingly, all peaks of Ag and Fe were shifted to lower binding energies compared with the reported characteristic metallic Ag⁰ peaks at 374.1 and 368.1 eV and Fe₃O₄ peaks at 725.0 and 711.5 eV.^{55,58} However, the peak of N in M30 was obviously shifted to a higher binding energy (398.8 eV) compared with RChS (398.3 eV) (Fig. 5d). These results indicate that the N in the acetyl amino groups acted as the anchor and stabilization for the Ag-Fe₃O₄ nanoparticles, leading to their immobilization on MRChS. This might be explained since the transformation of the electronic coordination from N to Ag-Fe₃O₄ nanoparticles resulted in a decrease of the binding energy for Fe and Ag, but an increase for N. The high resolution XPS spectra for the C1s region around 285 eV is shown in Fig. 5e. The three types of peak at 286.8 eV, 285.4 eV, and 283.7 eV were assigned to the carbon atoms of the C=O group, the C-O or C-N group and C-C or C-H in chitin, respectively.⁵⁹ The similar three peaks of the C1s spectra for RChS and M30 suggested that the Ag-Fe₃O₄ nanoparticles did not attach to the C atoms of chitin. Fig. 5f shows the spectra of O1s for the bare RChS and the Ag-Fe₃O₄(a)chitin composite microspheres (M30). The shape of the wide and asymmetric peak in the O1s spectrum for both RChS and M30 indicated that there was more than one chemical state according to the binding energy. Three peaks of the RChS O1s spectrum were identified: at 532.2 eV, assigned to O-C-O; at 531.3 eV, assigned to C-O-H and C-O-C; and at 530.2 eV assigned to N-C=O, respectively.⁶⁰ For M30, the O1s peak can be generally regarded as a joint contribution of the RChS and Ag-Fe₃O₄ nanocomposites, indicating a clear difference to that of RChS in its lower tails. The peak at 530.4 eV was ascribed to the anionic oxygen in Fe₃O₄ (Fe-O or Fe=O).⁶¹ Interestingly, the N-C=O peak at 529.4 eV of M30 obviously shifted to a lower binding energy compared with that of RChS (530.2 eV), suggesting that the O atom of the N-C=O group served as anchoring sites, together with the N atom, for Ag-Fe₃O₄ nanoparticles. Namely, the acetyl amino the anchoring sites for the Ag-Fe₃O₄ groups were nanoparticles.

Formation mechanism of Ag-Fe₃O₄@chitin microspheres

On the basis of the above experimental results and theoretic analysis, a possible mechanism for the construction of Ag-Fe₃O₄@chitin microspheres is proposed in Scheme 1. There are many micro- and nano-pores in the chitin microspheres, as shown in Fig. 2 and 3 and large amounts of acetyl amino groups exist in the pores of the chitin microspheres (Scheme 1a), supported by the results in Fig. 5 and S3.† Thus, Fe²⁺ was absorbed and stabilized quickly into the pores through binding with the acetyl amino groups. Subsequently, Fe(OH)₂ particles were generated with the addition of alkalinity (Scheme 1b). When AgNO₃ was added into the alkaline suspension, the redox reaction between Ag₂O and Fe(OH)₂



Scheme 1 Schematic depiction for the *in situ* synthesis of Ag-Fe₃O₄ nanocomposites on chitin microspheres: "living" chitin microspheres with surface-bound acetyl amine groups in nano-sized pores (a); Fe(OH)₂ (b) and Ag-Fe₃O₄ (c) nanoparticles anchored with the acetyl amine groups of the chitin microspheres.

took place rapidly, leading to the *in situ* one-pot synthesis of the Ag–Fe₃O₄ nanoparticles in the micro- and nano-sized pores (Scheme 1c), supported by the results in Fig. 3–5 and S4.†

The Ag–Fe₃O₄@chitin nanocomposites were fabricated through the redox reaction between $Fe(OH)_2$ and Ag_2O as follows.⁵⁵

$$Fe^{2+} + 2OH^- \rightarrow Fe(OH)_2$$
 (2)

$$2Ag^{+} + 2OH^{-} \rightarrow Ag_2O + H_2O \tag{3}$$

$$2Ag_2O + 6Fe(OH)_2 \rightarrow 2Fe_3O_4 + 4Ag + 6H_2O$$
(4)

In our findings, the pores in the chitin microspheres served as microreactors for the synthesis and growth of Ag–Fe₃O₄, leading to the formation of the Ag–Fe₃O₄ nanoparticles with a narrow size distribution. The acetyl amino groups as anchoring sites captured the Ag–Fe₃O₄ nanoparticles (Scheme 1c), supported by the results in Fig. 5. All of these results give the insight that (1) the heterostructures were composed of Ag–Fe₃O₄ nanocomposites and chitin; (2) the acetyl amino groups on the chitin microspheres acted as the anchoring sites for the Ag–Fe₃O₄ nanoparticles; (3) the porous microspheres were used as micro-reactors to supply not only cavities for the construction of the nanoparticles, but also a shell to protect the nano-structure and catalytic activity.

Magnetic properties

The magnetic properties of these materials are critical to ensure their application. Fig. 6a shows the magnetization of M15, M30 and M45 as a function of the magnetic field at 298 K. The magnetization of these MRChS microspheres increased considerably with an increase of the Ag–Fe₃O₄ nanocomposites. Though the magnetization of the composite microspheres in each sample was weak and showed a lack of saturation, they exhibited a small hysteresis loop and low coercivity, suggesting superparamagnetic behavior. The saturation magnetization of M15, M30 and M45 obtained from the hysteresis loop was 2.41, 2.81 and 3.42 emu g⁻¹, respectively, depending on the effective mass of the Fe₃O₄ nanoparticles. To further investigate the magnetic response of MRChS, a



Fig. 6 Magnetic hysteresis loops and enlarged view (inset) of the central portion of M15, M30 and M45 (a), and photographs of the MRChS dispersion in water (b), and the attraction behaviors induced by the magnet immersed in the water for 5 s (c) and 13 s (d).

magnet was used to attract the microspheres in water. Fig. 6b–d show photographs of the MRChS dispersion in water and the attraction behaviors induced by the magnet. As shown in Fig. 6b, MRChS were drawn rapidly to attach on the surface of the magnet within 5 s, and were completely collected from the water in the jar within 13 s (Fig. 6c and Video S1†). Obviously, a magnet can be employed efficiently to recycle MRChS. Moreover, the magnetism of MRChS was not only strong enough to separate them out from the action system with the help of an external magnetic field, but also beneficial for easy redispersion for reuse (see Video S1†). Therefore, MRChS could be fast removed from water under the magnetic field, leading to easy recycling and reuse. The convenient recycling of the magnetic microspheres conformed to the call of green chemistry.

Catalytic activity

It is well-known that metallic silver nanostructures are excellent catalysts with high activity and selectivity. Thus, silver immobilized on a porous microsphere support could serve as a practical recyclable catalyst and chromatograph packing material for many reactions. In our findings, the reduction of 4-nitrophenolate (4-NP) to 4-aminophenolate (4-AP) in the presence of NaBH₄ was chosen as a model reaction to evaluate the catalytic activity of the as-prepared Ag-Fe₃O₄@chitin catalyst. Usually, a 4-NP aqueous solution has a maximum absorption at 317 nm in the UV-vis spectrum which is remarkably red-shifted to 400 nm when it has been treated with an aqueous solution of NaBH₄, corresponding to a color change from light yellow to bright yellow.⁶² Absorption at 400 nm reflects the formation of 4-nitrophenolate ions, owing to an increase in solution alkalinity, caused by the addition of NaBH₄.⁶³ The reaction conversion can be calculated from C/C_0 , which is measured from the relative intensity of UV-vis absorbance (A/A_0) at 400 nm.²⁸ Herein, C is the concentration of 4-NP during the reaction and C_0 is the initial concentration. Fig. 7a shows the catalytic efficiency of the reduction reaction of MRChS on 4-NP as a function of time. After the addition of Ag-Fe₃O₄(a)chitin into the system, the absorption intensity of 4-NP at 400 nm decreased rapidly with time, and a new peak at 295 nm appeared, indicating the formation of 4-AP,⁶⁴ which was accompanied with its perceptible color change. For

comparison, a control experiment was conducted with a mixture of 4-NP, the NaBH₄ reducing agent and chitin microspheres without Ag–Fe₃O₄@chitin (Fig. 7b). The solution remained bright yellow with a constant absorption peak at 400 nm for the control experiment, indicating that the catalytic reduction of 4-NP did not occur. However, when Ag–Fe₃O₄@chitin microspheres were added into the reaction mixture, such as M15, M30 and M45, the C/C_0 values or the peak intensity at 400 nm of 4-NP rapidly decreased with increasing reaction time. In this experiment, NaBH₄ was used in large excess to 4-NP, and can be considered as constant during the reaction period. So pseudo-first-order kinetics can be applied to evaluate the reaction rate constants. The reaction kinetics can be described as³⁴

$$\ln(C/C_0) = -kt,\tag{5}$$

where *k* is the apparent first-order rate constant (min⁻¹) and *t* is reaction time. Fig. 7c shows a linear relationship for $\ln(C/C_0)$ against *t* in the reduction catalyzed by the heterostructures following pseudo-first-order kinetics. The reaction rate constants were calculated to be $k \sim 0.3139 \text{ min}^{-1}$ for M45, $k \sim 0.2621 \text{ min}^{-1}$ for M30 and $k \sim 0.2236 \text{ min}^{-1}$ for M15. This indicates that the catalytic efficiency was enhanced with increasing content of silver nanoparticles in the Ag–Fe₃O₄@ chitin microspheres (see Fig. S9†).

The stability and recyclability of catalysts are of great importance for practical application. Fig. 7d shows the catalytic activity of M30 (50 mg L⁻¹) for the reduction of 4-NP with 10 successive times of cycling use. The results for the different cycles were very similar and the catalytic activity hardly changed, indicating MRChS possessed a high turnover frequency (at least 10 times) without any loss of catalytic activity. The recyclability of the catalyst may result from the highly efficient stabilization of the Ag–Fe₃O₄ nanoparticles immobilized in the chitin microspheres. Interestingly, considering the high recycling frequency, favorable size (150–400 μ m) and porous structure of the magnetic microspheres, this would greatly promote the application of MRChS in the catalysis field.

A photograph for MRChS acting as a chromatographic packing material in an injector for rapid catalysis is shown in Fig. 8. When the yellow solution (4-NP and NaBH₄) was

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Fig. 7 Catalytic performance of MRChS on 4-NP: UV-vis absorption spectra during the catalytic reduction of 4-NP over M30 (50 mg L^{-1}) (a), the inserts are the reaction scheme associated color change from bright yellow 4-NP to colorless 4-AP; Plots of absorbance at 400 nm for $ln(C/C_0)$ (b) and C/C_0 (c) versus the reduction reaction time for 4-NP, with M15, M30 and M45 at a concentration of 50 mg L^{-1} and the control experiment (50 mg L^{-1} chitin microspheres); Catalytic activity of the sample M30 (50 mg L^{-1}) for 4-NP with 10 cycle uses as a function of time (d).



Fig. 8 Optical photograph of the preparative chromatography column packed with MRChS for the catalytic reaction from 4-nitrophenol to 4-aminophenol.

syringed through the MRChS packing, the Ag immobilized on the MRChS efficiently catalyzed the reduction of 4-NP to 4-AP, and immediately, the colorless product of 4-AP appeared in the collector (also seen in Video S2†). The highly efficient catalytic activity and the facile application of MRChS are important in the catalysis field. Moreover, all samples had adequate thermal stability because decomposition did not occur below 200 °C (see Fig. S9†). This is important for the huge potential application of nanocomposite catalysts because thermal stability of catalysts is necessary for many catalytic reactions. Therefore, this work supplies a broad pathway to immobilize noble metallic nanocatalysts for further industrial application. It is worth noting that chitin can be biodegraded in soil, and could serve as a plant growth regulator to promote plant growth even after it has been discarded. ⁶⁵

Conclusion

Chitin microspheres having acetyl amino groups were prepared successfully in an aqueous system by a sol–gel transition method *via* a green pathway. The Ag–Fe₃O₄ nanoparticles with mean sizes of 10–40 nm were *in situ* one-pot synthesized in the micro- and nano-pores of the microspheres through strong interactions between Ag–Fe₃O₄ and chitin. The porous microspheres were used as micro-reactors to supply not only cavities for the formation of the nanoparticles, but also a shell to protect the nano-structure and catalytic activity. Moreover, the acetyl amino groups of chitin served as an anchoring and stabilizing agent to trap the Ag–Fe₃O₄ nanoparticles and immobilize them in the chitin microspheres. The magnetic composite chitin microspheres were proved to be not only highly efficient for the catalytic reaction of 4-NP to 4-AP, but also an easily recyclable and reusable catalyst. The chitin microspheres containing Ag–Fe₃O₄ nanoparticles could have great potential in industry as a broad platform for metallic nanoparticle immobilization and for rapid catalysis. Both the fabrication and use of the magnetic composite microspheres were very simple and convenient. Therefore, this is an environmentally friendly material and process, which is in accord with sustainability.

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