Graphical contents entry

Magnetically separable and reusable Fe₃O₄-polydopamine hybrid hollow microspheres based on template-induced covalent assembly method exhibit highly efficient peroxidase mimetic catalytic activity and stability.

**Magnetically separable and recyclable Fe₃O₄-polydopamine hybrid hollow
microsphere for highly efficient peroxidase mimetic catalysts**

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Abstract: Magnetic Fe_3O_4 -polydopamine (PDA) hybrid hollow microspheres, in which Fe_3O_4 nanoparticles were firmly incorporated in the cross-linked PDA shell, have been prepared through the formation of core/shell PS/ Fe_3O_4 -PDA composites based on template-induced covalent assembly method, followed by core removal in a tetrahydrofuran solution. The morphology, composition, thermal property and magnetic property of the magnetic hybrid hollow microspheres were characterized by SEM, TEM, FT-IR, XRD, TGA, and vibrating sample magnetometer, respectively. Results revealed that the magnetic hybrid hollow microspheres had about 380 nm of inner diameter and about 30 nm of shell thickness, and 13.6 emu g^{-1} of magnetization saturation. More importantly, the Fe_3O_4 -PDA hybrid hollow microspheres exhibited intrinsic peroxidase-like activity, as they could quickly catalyze the oxidation of typical substrates 3,3',5,5'-tetramethylbenzidine (TMB) in the presence of hydrogen peroxide. Compared with PDA/ Fe_3O_4 composites where Fe_3O_4 nanoparticles were loaded on the surface of PDA microspheres, the stability of Fe_3O_4 -PDA hybrid hollow microspheres was greatly improved. As-prepared magnetic hollow microspheres might open up a new application field in biodetection, biocatalysis, and environmental monitoring.

Keywords: polydopamine; Fe_3O_4 ; incorporated; hollow microspheres; peroxidase-like; catalyst; stability

1. Introduction

Currently, there is growing interest in the use of nanoparticles for a wide range of biomedical and technological applications. Among well-known nanoparticles, magnetic Fe_3O_4 nanoparticles are of special interest because of their potential in biological imaging or separation techniques. For instance, Fe_3O_4 nanoparticles have been widely used experimentally for numerous applications such as separation of biomolecules [1-3], drug and gene targeting [4,5], tissue engineering [6], magnetic resonance imaging [7-9], magnetic biosensors [10], biocatalysts [11], wastewater treatment [12-17] and as mediators of heat for cancer therapy [18,19]. More recently, Fe_3O_4 nanoparticles have been found surprisingly to possess intrinsic peroxidase-like activity [20-26]. Peroxidase catalysis owns a wide range of practical applications. For instance, the ability to catalyze the oxidation of organic substrates to produce a color change or reduce their toxicity is frequently employed as a detection tool or in wastewater treatment. However, naked nanoparticles generally incline to aggregate to form larger particles because of Van der Waals forces, resulting in a significant deterioration of their original activity [27-29]. To solve this problem, many support materials for anchoring Fe_3O_4 nanoparticles have been developed. In general, it is a good method to immobilize magnetic Fe_3O_4 nanoparticles onto the outer surface of the supports [22,23,30-33]. However, the immobilized nanoparticles on the outer surface are often dropped from the substrates in the application process, resulting generally in loss of magnetism and catalytic activity. Therefore, finding a suitable

support and a proper strategy to stabilize Fe_3O_4 nanoparticles should be an attractive target.

Recently, polydopamine (PDA) materials have attracted considerable research activities due to their good coating capability and unique structural feature. First, PDA can exhibit a strong adhesive property to various materials including noble metals, metal oxides, semiconductors, polymers, and so on through spontaneous oxidative self-polymerization [34-38]. Second, the modification of PDA is easy, convenient, substrate-independent and chemistry reactive [39-41]. Third, as-formed adherent PDA coatings have an active surface containing ample catechol and amine groups, which endow as-prepared materials with high dispersibility in solution [42]. Fourth, a large number of hydrophilic groups endow the cross-linked PDA coating with porous hydrogel-like structure, which empower free access of water-soluble reactants to nanocatalyst surfaces [43]. Of equal importance is that PDA coating can be bound to the nanocatalyst surface through weak non-covalent interactions, minimizing passivation of the nanocatalyst.

In this study, based on a template-induced assembly mechanism, we prepared a kind of magnetic Fe_3O_4 -polydopamine hybrid hollow microsphere in which Fe_3O_4 nanoparticles were firmly incorporated in the cross-linked PDA shell. Due to the coverage and isolation of the PDA chain, Fe_3O_4 nanoparticles could hardly escape from the capsule shell or come into contact with each other, thus avoiding falling off or aggregation. Compared with PDA/ Fe_3O_4 composites, where Fe_3O_4 nanoparticles were loaded on the surface of PDA microspheres, the stability of Fe_3O_4 -PDA hybrid

hollow microspheres was greatly enhanced. The method can offer at least three merits: (1) the fabrication of magnetic hybrid hollow microspheres with the highlights of high performance, non-toxicity, and environmental friendliness can be easily carried out at room temperature; (2) as-prepared magnetic hybrid hollow microspheres possess novel hollow structure, where Fe_3O_4 nanoparticles were firmly incorporated in the polymer shell with highly cross-linked chemical structure; and (3) the magnetic hybrid hollow microspheres exhibit highly intrinsic peroxidase-like activity and good stability for the reactions including the oxidation of organic substrates such as 3,3',5,5'-tetramethylbenzidine to reduce their toxicity, whose product color change has been generally used in wastewater treatment detection.

2. Materials and Methods

2.1 Materials

Polyvinylpyrrolidone (PVP, $\text{MW} = 40\ 000\ \text{g mol}^{-1}$), 3-hydroxytyramine hydrochloride (dopamine hydrochloride) and Tris (hydroxymethyl) aminomethane (Tris base, $\geq 99.8\%$), 3,3',5,5'-tetramethylbenzidine dihydrochloride (TMB) were purchased from Sigma-Aldrich and used as received. 2,2'-Azobisisobutyronitrile (AIBN) was purchased from Shanghai Chemical Reagent Co. (China) and was purified by recrystallization in ethanol. Styrene (Shanghai, China) was purified using an inhibitor removal column and stored $-5\ ^\circ\text{C}$ prior to use. All other materials from Shanghai Chemical Regents were of analytical grade and used without any further purification, including $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, ammonium hydroxide, tetrahydrofuran (THF) and absolute ethanol.

2.2 Preparation of magnetic Fe₃O₄ nanoparticles

Magnetic Fe₃O₄ nanoparticles were synthesized by the common co-precipitation method. In brief, FeCl₃·6H₂O (2.365 g, 8.75 mmol) and FeCl₂·4H₂O (0.995 g, 5 mmol) were dissolved in 27.5 mL deoxygenated water at 57 °C under N₂ protection and vigorous mechanical stirring to form a homogeneous solution. Accordingly, 2.5 mL of ammonium hydroxide was quickly injected into above solution, and the black precipitate was observed immediately. After 15 min, another 1 mL of ammonium hydroxide was added and the reaction continued for another 2 h before cooled down to room temperature naturally. Finally, the resulting Fe₃O₄ nanoparticles were separated using a magnet and washed with ethanol and water.

2.3 Preparation of PS microspheres

Monodisperse polystyrene spheres (PS) were prepared by dispersion polymerization according to the previous work [44]. Briefly, all of styrene (5.0 g), PVP (1.5 g), AIBN (0.1 g) were dissolved in 37.5 g ethanol and 12.5 g water under magnetic stirring to form a homogeneous solution. The solution was deoxygenated by bubbling pure nitrogen gas at room temperature for about 1 h. Then the reaction was heated to 70 °C and kept for 24 h under constant stirring with a rate of 100 rpm. Finally, the obtained dispersion was centrifuged and washed with ethanol for several times, and dried in a vacuum oven at room temperature for 24 h.

2.4 Preparation of PDA microspheres

In brief, the Tris-buffer solution (50 mL, 10 mM L⁻¹, based on deionized water) was first mixed with 10 mL of alcohol under mild magnetic stirring. Then, 75 mg of

dopamine hydrochloride was added to the mixed solution. After stirring for more than 3 days, PDA spheres were obtained.

2.5 Fabrication of PDA/Fe₃O₄ composite

In a typical process, 15 mg PDA spheres were dispersed in 55 mL deoxygenated water to form a homogeneous suspension. Then, 54 mg FeCl₃·6H₂O and 23 mg FeCl₂·4H₂O were added into above suspension in a four-necked flask at 57 °C. After mechanical agitation for 1 h, 5 mL ammonium hydroxide was quickly injected into the suspension followed by refluxing for another 15 min. Afterwards, another 1 mL of ammonium hydroxide was added to the above reaction system to proceed for 2 h. Finally, the product was cooled to room temperature and then washed with ethanol repeatedly followed by drying at 50 °C in a vacuum oven.

2.6 Fabrication of magnetic Fe₃O₄-PDA hybrid hollow microspheres

To fabricate PS/Fe₃O₄-PDA composites, first, the PS microspheres (11.25 mg) and as-prepared Fe₃O₄ (4 mg) were mixed with 150 mL Tris-HCl solution (pH = 8.5) under mechanical agitation for 2 h. Subsequently, 45 mg dopamine was added into the system under vigorous stirring. Then, the reaction continued for another 48 h. Finally, the solution was centrifugated and washed with ethanol several times. After drying overnight at 50 °C under vacuum, core/shell PS/Fe₃O₄-PDA composites were obtained.

To obtain Fe₃O₄-PDA hybrid hollow microspheres, the as-prepared PS/Fe₃O₄-PDA composites were suspended into 40 mL THF in a 100 mL round-bottomed flask. After

mechanically stirring for 3 h, the resulting products were separated using a magnet and washed with ethanol for several times, then dried in a vacuum oven at 50 °C.

2.7 Peroxidase mimetic catalyst

60 μL of 30% H_2O_2 and 2 mL of 0.15 mM TMB-HCl (freshly prepared) were mixed in a cuvette. Then, a certain amount of the hollow Fe_3O_4 -PDA suspension was added to the above mixture. The oxidation reaction progress with 0.15 mg catalysts for 18 min was monitored at 1 min intervals at 25 °C by recording the absorption spectra in a scanning kinetics mode. In the recycling study, the catalysts (0.12 mg) were separated from the solution by a magnetic when the reduction reaction was completely finished. After washing with water twice, they were reused in the next reaction run. The procedure was repeated 6 times. The absorbance intensity of the reaction solution was measured and the rate constant k of the reaction was calculated. For a comparison, the peroxidase mimetic experiment was also conducted by PDA/ Fe_3O_4 catalysts on the same condition, and the rate constant k of the reaction was studied.

2.8 Characterization

The microstructure of PS microspheres, PS/ Fe_3O_4 -PDA composites, magnetic Fe_3O_4 -PDA hybrid hollow microspheres and PDA/ Fe_3O_4 composites were characterized by field emission scanning electron microscopy (JEOL JSM-7401F) and transmission electron microscope (JOEL JEM-100CX) with the accelerating voltage of 100 kV. Fourier transform infrared (FT-IR) spectra were measured using a Perkin-Elmer Paragon 1000 FT-IR spectrophotometer in the wave number range from

4000 to 500 cm^{-1} . X-ray diffraction (XRD) pattern was recorded at a Bruker D8 Advance instrument. A Perkin-Elmer TGA 7/DX thermogravimetric analyzer was used to get the precise loading amount of Fe_3O_4 nanoparticles in the magnetic Fe_3O_4 -PDA hybrid hollow microspheres at a heating rate of 10 $^\circ\text{C min}^{-1}$ under a flowing oxygen atmosphere. The magnetization curve was measured at 300 K under a varying magnetic field with physical properties measurement system (MPMS SQUID, Quantum Design, USA). UV-vis spectroscopy was performed on Shimadzu UV-240 spectrometer ranging from 500 to 800 nm.

3. Result and discussion

Fig. 1 shows the preparation scheme of magnetic Fe_3O_4 -PDA hybrid hollow microspheres. First, polystyrene microspheres with an average diameter of 400 nm were fabricated through dispersion polymerization, and Fe_3O_4 nanoparticles with an average particle size of 15–20 nm were prepared through the common co-precipitation method. Accordingly, Fe_3O_4 nanoparticles were embedded by active PDA generated through self-polymerization of dopamine in a weakly alkaline aqueous environment to form the Fe_3O_4 -PDA hybrid particles. Then, the particles could self-assemble onto the surface of PS microspheres to form PS/ Fe_3O_4 -PDA (core/shell) composites. As soon as the core was removed in a THF solution, magnetic Fe_3O_4 -PDA hybrid hollow microspheres were obtained. As the PDA shell has highly cross-linked structure to incorporate Fe_3O_4 nanoparticles, magnetic Fe_3O_4 -PDA hybrid hollow microspheres exhibit good stability. Furthermore, the hybrids are proven to exhibit ultra high peroxidase mimetic activity, as described in Fig. 2. The

intermediate HO_2^- was generated through the oxidation and reduction process, and finally oxidized TMB to the non-toxic product. Fe_3O_4 NPs which were embedded in the PDA shell could show the peroxidase mimetic activity for the reactions.

As observed in Fig. 3a, the homogeneous Fe_3O_4 nanoparticles with an average diameter of 15-20 nm were synthesized through the co-precipitation method successfully. The SEM images in Fig. 3b confirm that the original PS spheres prepared by dispersion polymerization are uniform at a large scale with an average diameter of 400 nm. Moreover, it is obvious that the surfaces of the PS microspheres are very smooth. In comparison with the original PS spheres, both the average size and surface roughness of PS/ Fe_3O_4 -PDA composite spheres (see Fig. 3c and d) are increased. After coating, the average size of the obtained PS/ Fe_3O_4 -PDA composite spheres is increased from 400 to 460 nm, which suggested the successful coating of Fe_3O_4 -PDA shell around the PS spheres surface, and the shell thickness was calculated to be 30 nm. To obtain Fe_3O_4 -PDA hybrid hollow microspheres, THF solution was selected to etch the PS core in PS/ Fe_3O_4 -PDA composites. The inner hollow structure is clearly observed from the ruptured capsules as pointed out by the arrow in Fig. 3e. TEM characterization results from Fig. 3f further confirmed the above conclusion, as a large void appears owing to the removal of the PS core as reported other hollow spheres [45]. The inner diameter of the magnetic hybrid hollow microspheres is estimated to be about 380 nm (less than the diameter of PS templates), which due to the significant shrinkage of hybrid hollow microspheres during the removal of colloidal templates. The Fe_3O_4 -PDA shell in which Fe_3O_4 nanoparticles

(pointed out by the red arrow in Fig. 3f) were embedded is measured to be 30 nm in thickness, which is in good agreement with the value calculated from the SEM image. In the high-resolution TEM image (inset of Fig. 3f), the crystalline lattice of Fe_3O_4 nanoparticles can be well observed. The distance between two adjacent lattice planes is approximately 2.971 Å, which corresponds to (220) lattices of Fe_3O_4 nanoparticles [46]. For comparison, PDA/ Fe_3O_4 composites with Fe_3O_4 nanoparticles loaded on the PDA surface were also prepared. As seen in Fig. 4a and b, PDA microspheres with the average diameter of 500 nm exhibit a smooth surface. However, when PDA microspheres were coated by Fe_3O_4 nanoparticles, the PDA surface became rough (Fig. 4c). TEM characterization resulting from Fig. 4d further proved that Fe_3O_4 nanoparticles were successfully loaded on the PDA surface. The great difference of the Fe_3O_4 locations between Fig. 3d and Fig. 4d indicated that the template-induced assembly method is good route to prepare magnetic Fe_3O_4 -PDA hybrid hollow microspheres.

Fig. 5 shows the FT-IR spectra of PDA spheres, Fe_3O_4 nanoparticles, PS microspheres, PS/ Fe_3O_4 -PDA composites and Fe_3O_4 -PDA hybrid hollow microspheres. In Fig. 5a, the PDA main peak is located at 1650 cm^{-1} , which is attributed to the stretching mode of amine in polydopamine. The characteristic peaks of PS spheres appear at 3024 , 1492 , 1452 and 698 cm^{-1} , as seen in the spectrum (Fig. 5c). In the spectrum of PS/ Fe_3O_4 -PDA composites (Fig. 5d), the characteristic peaks of PS and PDA spheres are observed, and the characteristic peak of Fe_3O_4 nanoparticles (Fig. 5b) at 583 cm^{-1} could be also found in it, which indicated that the

spheres were composed of PS, Fe₃O₄ and PDA materials. After removing the PS cores by THF washing, the characteristic peaks of PS spheres disappear accordingly, while the peaks of Fe₃O₄ nanoparticles and PDA spheres are observed only (Fig. 5e), which indicated that the magnetic Fe₃O₄-PDA hybrid hollow microspheres were successfully formed. Also, for comparison, the FT-IR spectrum of the PDA/Fe₃O₄ composites was also provided (Fig. 5f). Obviously, the FT-IR spectra of the Fe₃O₄-PDA hybrid hollow microspheres and the PDA/Fe₃O₄ composites show an almost identical shape, indicating that the two materials should own same components. In addition, the stability of Fe₃O₄-PDA hybrid hollow microspheres was tested initially by FT-IR spectrum. Fig. 5g shows the FT-IR spectrum of Fe₃O₄-PDA hybrid hollow microspheres after being cycled for 6 times. It can be seen that there is no significant peak position change compared with the original catalysts, indicating that the Fe₃O₄-PDA hybrid hollow microspheres own good stability.

XRD analysis shown in Fig. 6 further confirmed the formation of magnetic Fe₃O₄-PDA hybrid hollow spheres. Obviously, in Fig. 6a, the XRD pattern of PDA only shows a very broad diffraction peak at about 20°, which is usually the characterization peak of amorphous polymer material. Fig. 6b and 6d show the XRD patterns of Fe₃O₄-PDA hybrid hollow microspheres and PDA/Fe₃O₄ composites, respectively. In the two XRD patterns, besides the characterization peak of PDA, other five diffraction peaks are in good accordance with those of magnetite Fe₃O₄ (JCPDS no. 19-0629). The peaks at $2\theta = 30.6, 35.8, 43.4, 53.6, 57.5$ and 63.2° can be indexed to the (220), (311), (400), (422), (511) and (440) crystal planes, respectively

[47]. Additionally, no other signals were detected, proving that the magnetic Fe_3O_4 -PDA hybrids and PDA/ Fe_3O_4 composites were successfully fabricated. To further demonstrate the stability of magnetic Fe_3O_4 -PDA hybrids, the XRD test of Fe_3O_4 -PDA hybrid hollow microspheres after being cycled for 6 times was also conducted. As seen in Fig. 6c, there is no significant change of peak position or intensity after being cycled compared with Fig. 6b, indicating the good stability of the as-prepared Fe_3O_4 -PDA catalyst.

One of the most key requirements for magnetic materials is their good magnetic sensitivity and magnetic manipulation when employed in catalysis. Herein, the magnetic properties of the magnetic Fe_3O_4 -PDA hybrid hollow microspheres were analyzed by MPMS at room temperature (Fig. 7a). The saturation magnetization was found to be 13.6 emu g^{-1} for Fe_3O_4 -PDA hybrid hollow microspheres, which is less than that for the pure Fe_3O_4 nanoparticles (71.8 emu g^{-1}), which is reasonable considering of the lower magnetic content in the Fe_3O_4 -PDA hybrids. The magnetic manipulation of the Fe_3O_4 -PDA hybrids was examined by placing a magnet near the side of glass bottle. As seen in Fig. 7b, the Fe_3O_4 -PDA hybrid hollow microspheres could be separated easily from the solution within 3 min in a magnetic field. Moreover, when the external magnetic field was removed, the magnetic hybrid hollow microspheres could be re-dispersed by gentle shaking. As normal polymer materials can almost decompose completely under high temperature, the thermogravimetric analysis (TGA) of the Fe_3O_4 -PDA hybrid hollow microspheres was performed under a flowing oxygen atmosphere at the temperature ranging from 30 to 800°C to get the

precise loading amount of Fe_3O_4 nanoparticles. For a comparison, the pure PDA hollow microspheres were utilized under the same condition (Fig. 7c). According to the TGA data, the residual ratios of both Fe_3O_4 -PDA hybrid hollow spheres and hollow PDA microspheres are about 22.59% and 2.59% respectively. Hence, the loading amount of Fe_3O_4 nanoparticles embedded in Fe_3O_4 -PDA hybrid hollow spheres was about 19.8 wt%. Moreover, the calculated M_s value (13.6 emu g^{-1}) was a little smaller than theoretical M_s value (14.2 emu g^{-1}), indicating that Fe_3O_4 nanoparticles were embedded successfully in the PDA layer which could lead to weaken the magnetic moment.

Also, we have analyzed the peroxidase-mimetic activity of Fe_3O_4 -PDA hybrid hollow microspheres at room temperature through the measurement of the oxidation extent for a peroxidase substrate TMB by H_2O_2 to the oxidized product, where it changes to blue color as demonstrated in Fig. 8a. With regards to TMB oxidation, the oxidation product shows a typical absorbance at 650 nm and was immediately measured using the UV-Vis spectrum. In the experiment, the absorbance at 650 nm increased dramatically with time after the addition of Fe_3O_4 -PDA hybrid hollow microspheres (0.15 mg) within 18 min (Fig. 8b). As the concentration of H_2O_2 was much larger than that of TMB, the reduction reaction could be considered as a pseudo-first-order reaction with regard to TMB only [48]. The rate constant k was determined by a linear plot of $\ln (C_t/C_0)$ and reaction time t (the ratio of C_t to C_0 , where C_t and C_0 are the TMB concentrations at time t and 0 respectively, was measured from the relative intensity of the respective absorbance A_t to A_0 . Fig. 8c

shows the linear relationship between $\ln (C_t/C_0)$ and reaction time t in reduction reactions catalyzed by Fe_3O_4 -PDA hybrid hollow microspheres. As all these plots matched the pseudo-first-order reaction kinetics very well, the rate constant k could be calculated from the rate equation and the value was about 0.183 min^{-1} . Compared with other rate constant, this should be a very considerable value (see Table 1). In addition to catalytic activity, stability was another important property for catalysts as it determines their service life. The magnetic Fe_3O_4 -PDA hybrid hollow microspheres could be easily collected from the reaction solution by using a magnet and used for the next reaction due to their magnetic properties (Fig. 7b). Herein, we repeat the separation–reuse cycles 6 times. After reaction with 0.12 mg catalysts in each cycle, the absorbance intensity of the reaction solution was immediately measured using the UV-Vis spectrum and the rate constant k of each cycle was calculated. As shown in Fig. 8d, even after six cycles, the rate constant k almost unchanged. In contrast, the rate constant k decreased by 55.4% after six cycles using PDA/ Fe_3O_4 composites as the catalyst. Thus, compared with Fe_3O_4 nanoparticles anchored on the substrate surface, Fe_3O_4 nanoparticles which were embedded in the PDA shell would possess better stability. Such phenomenon could result from that once the Fe_3O_4 nanoparticles are anchored on the support surface, two major problems they face are falling off and deactivation due to erosion by solution. Therefore, the rate constant k in each cycle gradually reduced [46]. Embedding Fe_3O_4 nanoparticles in the hollow capsule shells could well resolve these problems at the same time. Comparing Fe_3O_4 -PDA hybrid hollow microspheres with PDA/ Fe_3O_4 composites, the catalytic activity of the former

was a little lower owing to the poor reactant contacting, however, the stability of the former was much higher. To investigate the iron leaching effect, we added 1 mg of catalysts into 1 mL deionized water and then made it well dispersed for 5 h under ultrasonic irradiation. Then the centrifugation was carried out to separate the hybrids from the suspension. The photographs of the solution before and after centrifugation were shown in Fig. 9. It is obvious that the solution after centrifugation is nearly clear, indicating no iron leaching from the catalysts.

4. Conclusion

In conclusion, we have demonstrated a template-induced assembly route to prepare magnetic Fe₃O₄-PDA hybrid hollow microspheres. During the process, PS spheres as induced template, and the Fe₃O₄-PDA composites as assembly unit to construct core-shell structured PS/Fe₃O₄-PDA composites. After being soaked in THF solution, PS cores were removed and the resulting Fe₃O₄-PDA hybrid hollow microspheres were obtained. For comparison, PDA/Fe₃O₄ composites in which Fe₃O₄ nanoparticles were loaded on the surface of PDA spheres were also synthesized. The typical hollow microspheres had about 380 nm of inner diameter, 30 nm of shell thickness, and 13.6 emu g⁻¹ of magnetization saturation, exhibiting highly magnetic sensitivity, favorable dispersion in aqueous and good stability. Also, the peroxidase-like activity of the Fe₃O₄-PDA hybrid hollow microspheres was verified by the typical color change of the reaction, using 3,3',5,5'-tetramethylbenzidine (TMB) as substrates in the presence of hydrogen peroxide. The rate constant k was about 0.183 min⁻¹. Furthermore, compared with Fe₃O₄ nanoparticles anchored on the PDA surface, there is no

significant decrease detected in the catalytic activity of Fe_3O_4 -PDA hollow microspheres even after the catalytic experiments were repeated 6 times. Since the Fe_3O_4 -PDA hollow microspheres show highly magnetic sensitivity, excellent catalytic activity, good reusability and stability, we think they might have potential applications as good peroxidase-like catalysts in the treatment of industrial waste and environmental monitoring.

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Captions to Figures and Tables

Fig. 1. Schematic illustration of the preparation procedure of magnetic Fe₃O₄-PDA hybrid hollow microspheres.

Fig. 2. Schematics of (a) oxidation and (b) reduction processes on Fe₃O₄ nanoparticles in the presence of H₂O₂, and (c) the peroxidase mimetic reaction of the TMB substrate by Fe₃O₄-PDA hybrid hollow microsphere catalyst. (Fe₃O₄: black sphere, Fe: light purple sphere, O: red sphere).

Fig. 3. SEM images of (a) Fe₃O₄ nanoparticles, (b) PS spheres, and (c) PS/Fe₃O₄-PDA composites; (d) TEM image of PS/Fe₃O₄-PDA composites; (e) SEM image of Fe₃O₄-PDA hybrid hollow microspheres; (f) TEM image of Fe₃O₄-PDA hybrid hollow microspheres and the high-resolution TEM image (inset).

Fig. 4. (a) SEM and (b) TEM images of the PDA microspheres; (c) SEM and (d) TEM images of the PDA/Fe₃O₄ composites.

Fig. 5. FT-IR spectra of (a) PDA spheres, (b) Fe₃O₄ nanoparticles, (c) PS spheres, (d) PS/Fe₃O₄-PDA composites, (e) Fe₃O₄-PDA hybrid hollow microspheres, (f) PDA/Fe₃O₄ composites, and (g) Fe₃O₄-PDA hybrid hollow microspheres after being cycled for 6 times.

Fig. 6. XRD pattern of (a) PDA microspheres, (b) Fe₃O₄-PDA hybrid hollow microspheres, (c) Fe₃O₄-PDA hybrid hollow microspheres after being cycled for 6 times, and (d) PDA/Fe₃O₄ composites.

Fig. 7. (a) Magnetization hysteresis loops at room temperature for as-prepared materials; (b) Photographs of magnetic Fe₃O₄-PDA hybrid hollow microspheres in

water driven by an external magnet; (c) TGA curves under a flowing oxygen atmosphere for hollow PDA microspheres and magnetic Fe₃O₄-PDA hybrid hollow microspheres.

Fig. 8. (a) Photograph of the color changes attributed to the peroxidase mimetic activity tests under magnetic Fe₃O₄-PDA catalysts; (b) UV-Vis spectra of the TMB and H₂O₂ mixture in the presence of 0.15 mg Fe₃O₄-PDA catalysts at different time; (c) The rate constant k estimated by the slopes of straight lines of $\ln (A_t/A_0)$ vs. reduction time; (d) The rate constants k of each cycle catalyzed by 0.12 mg of Fe₃O₄-PDA hybrid hollow microspheres (blue) and 0.12 mg of PDA/Fe₃O₄ composites (red), respectively.

Fig. 9. The photographs of the Fe₃O₄-PDA hybrid hollow microsphere solution before and after centrifugation.

Table 1. Comparison of rate constant k of the Fe₃O₄-PDA hybrids with other catalysts.

Figures

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Table 1.

Catalysts	M (mg)	k (min ⁻¹)	Ref.
Au@PPy@Fe ₃ O ₄	2	0.266	[46]
Au-POMA core-shell hybrids	0.3	0.045	[49]
Au-POMA yolk-shell hybrids	0.3	0.088	[49]
Fe ₃ O ₄ -PDA hybrids	0.12	0.183	This study