A mesoporous SiO₂/dense SiO₂/Fe₃O₄ multiply coated hollow microsphere: synthesis and application on papain immobilization

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Abstract

The mesoporous SiO₂/dense SiO₂/Fe₃O₄ multiply coated hollow microspheres were prepared by a multistep coating technique. At first, the dense SiO₂ layers with the thickness of 4.2nm were coated on the Fe₃O₄ nanorods to prevent them from being corroded in acidic system. Then, the mesoporous SiO₂ was coated on the composite microspheres via a sol-gel and hydrothermal synthesis process by using TEOS as silicon source, P123 in combination with co-surfactant CTAB as template in acidic system. The mesoporous SiO₂/dense SiO₂/Fe₃O₄ multiply coated hollow microsphere has a narrow pore size distribution around 3.74nm, a large surface area of 505m²g⁻¹, and a pore volume of 0.47cm³g⁻¹. The composite microspheres had a high papain immobilization (222mg/g) since the hollow cavity in the composite microsphere is a natural warehouse storing papain molecules effectively. The pH stabilities and temperature stabilities of the immobilized papain activity were much higher than that of the free papain activity.

Keywords: dense SiO₂; mesoporous SiO₂; hollow microspheres; Fe₃O₄; immobilized papain

1. Introduction

The magnetic mesoporous SiO_2 material has attracted much attention in the past decades due to its unique properties, such as excellent magnetic responsivity, uniform pore size distribution, high surface area, low cytotoxicity, etc [1-3]. The magnetic mesoporous SiO_2 microspheres are a new-emerging kind of carrier material in some biomedical applications, especially on enzyme immobilizations [4-5], due to pore size of magnetic mesoporous SiO_2 material do match enzyme molecules size. Moreover, enzyme molecules has a broad application, such as medicine, food, cosmetic and wine industry. However, the enzyme molecules is easy to be inactivated due to environmental factors, such as pH and temperature in the catalytic process. Moreover, it is difficult that the enzyme molecules are separated from the mixed reactant, resulting in the difficulty in recycling[6-7]. Therefore, these problems largely restrict the application of the enzyme molecules. However, the magnetic mesoporous SiO_2 material can solve the above problems well.

The synthetic system of mesoporous SiO_2 generally could be divided into two categories: alkalic system [8] and acidic system [9-10]. The synthesis technology of the magnetic mesoporous SiO_2 material has been quite mature in the alkalic system. However, the magnetic mesoporous SiO_2 material prepared in the acidic system has not yet been reported, since the magnetic layer of iron source would be corroded in the acidic system. Zhao and co-workers [11-14] have synthesized pure mesoporous SBA-15 with nearly spherical morphology by using a triblock copolymer P123 and a co-surfactant CTAB as composite surfactant templates in the acidic system. Moreover, the co-surfactant CTAB can adjust the microstructure of mesoporous SiO_2 , such as surface area, pore volume and pore size. It is well known that these microstructure of mesoporous SiO_2 are the key factors to affect the enzyme immobilization. Therefore,

it is significant to synthesize the magnetic mesoporous SiO_2 material using co-surfactant CTAB in the acidic system. Meanwhile, before the coating of mesoporous SiO_2 on magnetic layer, it is essential to prevent the magnetic layer from being corroded in the acidic system by coating prior dense SiO_2 on the magnetic layer of iron source.

In the present work, the mesoporous SiO_2 /dense $\text{SiO}_2/\text{Fe}_3\text{O}_4$ multiply coated hollow microspheres were prepared by a multistep coating technique. The dense SiO_2 layer was coated on the magnetic layer before the mesoporous SiO_2 coated. The mesoporous SiO_2 was coated on the composite microspheres via a sol-gel and hydrothermal synthesis process by using TEOS as silicon source, P123 in combination with co-surfactant CTAB as template. The effect of the dense SiO_2 layer on the magnetic layer, and the effect of the amount of co-surfactant CTAB and hydrochloric acid concentration on microstructure of obtained materials were investigated in detail. Furthermore, the papain immobilization property of the mesoporous SiO_2 /dense $\text{SiO}_2/\text{Fe}_3\text{O}_4$ multiply coated hollow microspheres was discussed.

2.Experimental Section

2.1Chemical

Tetraethyl orthosilicate (TEOS, 99.99%) and Cetyltrimethylammonium bromide (CTAB, 99%) were purchased from Tianjin Fuchen Chemical Co. Ferrous chloride ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 99.70%), ethanol, sodiumhydroxide (NaOH , 97%), hydrofluoricacid (HF , 40Wt%), and ammonia solution ($\text{NH}_3 \cdot \text{H}_2\text{O}$, 25Wt%), were purchased from

Beijing Chemical Co. Poly(ethylene oxide)-block-poly (propylene oxide) -block-poly (ethylene oxide) (Mn=5800, EO₂₀PO₇₀EO₂₀, P123), Papain (powder, 2500 units per mg) was purchased from Sigma Aldrich. Casein (C₄₇H₄₈N₃NaO₇S₂) was purchased from Meryer Chemical technology Co. Deionized water was produced by water purification system (Ulupure, Chengdu, China). All reagents were used as-received without further purification.

2.2 Preparation of mesoporous SiO₂/dense SiO₂/Fe₃O₄ hollow microspheres

2.2.1 Preparation of β -FeOOH hollow microspheres

The monodisperse SiO₂ microspheres were obtained in the ethanol medium by a seeding technique in the presence of TEOS as silicon source and ammonia as catalyst [15]. Hydrofluoric acid was added into ferrous chloride (Fe²⁺) solution (0.1M) to make the ratio of Fe²⁺ to F⁻ 3:1, adjusted the solution pH to 4.50 by dropping an ammonia solution. Then, the monodisperse SiO₂ microspheres were added into the solution, which was stirred at 333K in a water bath for 2h. Removed the SiO₂ cores through the NaOH solution (5wt%) and kept in a water bath at 313K for 2h.

2.2.2 Preparation of the dense SiO₂/ β -FeOOH hollow microspheres

The as-synthesized β -FeOOH hollow microspheres were dispersed in ethanol solution containing ammonia (0.05M) and water (7.00M) [16]. Then, the dense SiO₂ layer was obtained by dropping the TEOS, and stirring for 2h at 308K. The as-prepared samples were denoted as T1, T2, and T3, when the amount of TEOS is 0.63g, 1.26g, 2.52g, respectively. The microspheres were washed with ethanol and distilled water, then separated by centrifugation.

2.2.3 Preparation of the mesoporous SiO₂/ dense SiO₂/Fe₃O₄ hollow microspheres

Firstly, P123 (0.40g), CTAB and KCl (0.50g) was dissolved in mixture of hydrochloric acid (30ml) under magnetic stirring at 40°C for 2h. Afterwards, the dense SiO₂/β-FeOOH hollow microspheres and 15ml hydrochloric acid were added into the above mixture. After ten minutes, the TEOS (1.26g) was dropped into the above mixture and then stirring for 24h at 313K. When the amount of CTAB is 0.05g and hydrochloric acid concentration is 0.5M, 1M, 2M, 3M, the as-prepared samples were denoted as H0, H1, H2 and H3, respectively. When hydrochloric acid concentration is 2M and the amount of CTAB are 0g, 0.05g, 0.10g, 0.15g, the as-prepared samples were denoted as C0, C1, C2, C3, respectively. The final mesoporous SiO₂/ dense SiO₂/Fe₃O₄ hollow microspheres were obtained by calcination at 823K in air and then being reduced in a flowing gas mixture of H₂ (5%) and Ar (95%) at 623K. The detailed formation process of the mesoporous SiO₂/dense SiO₂/Fe₃O₄ hollow microspheres was depicted in Scheme 1.

Scheme 1. Formation process of mesoporous SiO₂/dense SiO₂/Fe₃O₄ hollow microspheres

2.3 Immobilization of papain on the mesoporous SiO₂/dense SiO₂/Fe₃O₄ hollow microspheres and activity stabilities

The mesoporous SiO₂/dense SiO₂/Fe₃O₄ hollow microspheres (10mg) were first

stirred at room temperature for different time (0.5~7h) in 10 ml of sodium phosphate buffer (pH 7.0) containing 12mg papain. The supernatant was separated from solid materials by centrifugation. The amount of papain was monitored by an ultraviolet-visible spectrophotometry (UV-vis).

The activity of free and immobilized papain were determined in a reaction medium containing 2ml casein aqueous solution(1mg/ml) as substrate in phosphate buffer (pH=7.0) at 50°C in the absorbance at 277 nm. The papain was added into the substrate solution and stirred immediately for 5h. The absorbance of the supernatant obtained through centrifuging the reaction solution was determined by a UV-vis spectrophotometer. The free and immobilized papain were put in different buffer solutions (pH=3.0~8.0) at room temperature for 24h to investigate the pH stabilities. The thermal stabilities of free and immobilized papain activity were determined after being kept in the buffer solution (pH=7.0) of different temperature (30~70°C) for 2 h. Here the papain activity was expressed in relative units (%), and the maximal activity value of free and immobilized papain measured at pH=7.0 and 50°C.

2.4 Characterization

Transmission electron microscopy (TEM) images were recorded on a JEOL JEM-2010 electron microscope operated at 200 KV. The magnetization curve was measured at room temperature under a varying magnetic field with a vibrating sample magnetometer (VSM). N₂ adsorption–desorption isotherms were carried out on a Micromeritics ASAP 2020 pore analyzer at 77K under continuous adsorption condition. BET and BJH analyses were used to determine the surface area, the pore

size distribution, and the pore volume. The UV/Vis absorption spectra was measured using an Evolution 600 UVV spectrophotometer of Thermo Electron Corporation. Powder X-ray diffraction (XRD) pattern was recorded on a XRD-7000 diffractometer using a high power Ni-filtered Cu-K α radiation (1.541 Å) source.

3. Results and discussion

3.1 The effect of the dense SiO₂ layer on the magnetic layer

Fig. 1 shows the TEM images of the monodisperse SiO₂ microspheres, β -FeOOH hollow microspheres and the different dense SiO₂ layers (Sample T1, T2, T3). The SiO₂ microspheres (Fig. 1A) have good monodispersity and uniform size with the seeding technique [17, 18], and its average size was about 300nm. As shown in Fig. 1B, the β -FeOOH nanorods have been successfully coated on the SiO₂ core. The β -FeOOH nanorods has the average length of about 40nm and its average diameter of about 15nm. Fig. 1C shows that the SiO₂ cores are totally removed without causing the collapse of the β -FeOOH hollow microspheres. The β -FeOOH hollow microspheres have a novel network-like composite shell structure through the overlap of β -FeOOH nanorods. The size of cavity in the β -FeOOH hollow microspheres is about 400nm depending on the diameter of the SiO₂ core. The papain molecules could easily enter the cavity as a papain storage warehouse through the network-like composite shell structure. From Fig. 1 (D, E, F), it is seen that the dense SiO₂ has successfully coated on the β -FeOOH nanorods. The average thicknesses of the dense SiO₂ layer were 2.7nm, 4.2nm and 7.1nm with the addition amount of TEOS increasing from 0.63g, 1.26g to 2.52g, respectively. The dense SiO₂ layer becomes

thicker with the adding amount of TEOS increasing.

Fig. 1 TEM photographs of (A) Monodisperse SiO₂ Microspheres; (B) SiO₂/β-FeOOH Microspheres; (C) β-FeOOH Hollow Microspheres; (D, E, F) Dense SiO₂/β-FeOOH Hollow Microspheres (D sample T1; E sample T2; F sample T3)

The dense SiO₂ layer is the basis of the subsequent preparation of mesoporous SiO₂ layer in the acidic system since the dense SiO₂ layer could prevent the β-FeOOH nanorods from being corroded in acidic system. If the dense SiO₂ layer is too thin or incomplete, it couldn't prevent magnetic nanorods from being corroded. On the other hand, the too thick dense SiO₂ layer would jam the pore formed by the interconnections of the magnetic nanorods to hinder the enzyme immobilization. Therefore, the thicknesses of the dense SiO₂ layer have great influence on the papain immobilization of the magnetic mesoporous SiO₂ microspheres. The TEM photographs of the dense SiO₂/β-FeOOH hollow microspheres soaked in 2M hydrochloric acid for 24 hours are exhibited in Fig. 2. It is shown that when the thickness of the dense SiO₂ layer is around 2.7nm, the composite microspheres have collapsed. It is because that the dense SiO₂ is too little to be completely coated on the

β -FeOOH nanorods so that the β -FeOOH nanorods would be corroded in acidic system. When the thickness of the dense SiO_2 layer is around 4.2nm, the dense SiO_2 is completely coated on the β -FeOOH nanorods and prevented β -FeOOH nanorods from being corroded in acidic system. The N_2 adsorption-desorption isotherms and the corresponding pore size distributions of the dense SiO_2/β -FeOOH hollow microspheres are shown in Fig.3. The corresponding structural parameters of the samples are summarized in Table 1. In the dense SiO_2/β -FeOOH hollow microspheres, there are a lot of open pores formed by the interconnections of the β -FeOOH nanorods with the pore size distribution ranging from 5 nm to 120nm (Fig. 3). With the thicknesses of the dense SiO_2 layer increasing from 4.2nm to 7.1nm, the average pores of the composite microspheres have been reduced from 16.20nm to 8.70nm. It is because the too thick dense SiO_2 layer jams the pore formed by the interconnections of the magnetic nanorods, which would hinder the follow-up enzyme immobilization. Therefore, the ideal thickness of the dense SiO_2 layer is 4.20nm.

Fig. 2 TEM photographs of the dense SiO_2/β -FeOOH hollow microspheres soaked in 2M hydrochloric acid for 24 hours (A) sample T1; (B) sample T2; (C)sample T3

Fig. 3 N₂ adsorption-desorption isotherms (A) and pore size distributions (B) of the dense SiO₂/β-FeOOH hollow microspheres

Table 1 Structural parameters of the dense SiO₂/β-FeOOH hollow microspheres

Samples	Surface area(m ² /g)	Pore volume(cm ³ /g)	Average pore size(nm)
T2	62.17	0.24	16.20
T3	60.39	0.12	8.70

3.2 The effect of hydrochloric acid concentration on microstructure of mesoporous SiO₂

TEM photographs of the mesoporous SiO₂/dense SiO₂/Fe₃O₄ hollow microspheres prepared with different hydrochloric acid concentration are presented in Fig. 4. From Fig. 4, the mesoporous SiO₂ has been successfully coated on dense SiO₂/Fe₃O₄ hollow microspheres in the acid system. It is seen that the mesoporous SiO₂ layer coated on the composite hollow microspheres becomes thicker with hydrochloric acid concentration increasing from 0.50M to 2M. It is because that the hydrochloric acid as an acidic catalyst can accelerate the hydrolysis of TEOS. It is well known that the thick mesoporous SiO₂ layer is conducive to the follow-up papain immobilization. When the hydrochloric acid concentration increased to 3M, the hydrolysis reaction is further accelerated, leading to an increase of the nucleation rate and the number of nucleus, and thus resulting in the pure SiO₂ microspheres rather than being coated on the composite microspheres (Fig. 4D). Regular mesoporous SiO₂

layer can be obtained at a suitable hydrochloric acid concentration due to the relatively compromising rate of hydrolysis and condensation reaction of TEOS. The selected area electron diffraction (Fig.4E) gives the characteristic diffraction rings of the Fe_3O_4 polycrystal.

The XRD pattern (Fig. 5A) reveals that the mesoporous SiO_2 /dense SiO_2 / β - FeOOH hollow microspheres are transformed into mesoporous SiO_2 /dense SiO_2 / α - Fe_2O_3 hollow microspheres by calcination in air at 823 K for 6 h. The diffraction peaks match well with the standard PDF data (JCPDS Card No.33-0664). After mesoporous SiO_2 /dense SiO_2 / α - Fe_2O_3 hollow microspheres were calcined at 623 K in a reducing atmosphere(5% H_2 / 95% Ar) for 4 h, the diffraction peaks (Fig. 5B) of the resultant sample matches well with the standard PDF data (JCPDS Card No. 19-0629). This demonstrates that mesoporous SiO_2 /dense SiO_2 / Fe_3O_4 multiply coated hollow microspheres possess a pure Fe_3O_4 crystalline phase [19-21].

Fig. 4 TEM photographs of mesoporous SiO_2 /dense SiO_2 / Fe_3O_4 hollow microspheres prepared with the different hydrochloric acid concentration (A) H0; (B) H1; (C) H2; (D) H3; (E) SEAD image of (A)

Fig. 5 XRD patterns of β -FeOOH hollow microspheres (sample H0) after the calcination in air at 823 K (A) and the calcination in reducing atmosphere at 623 K (B), the standard PDF data of Fe_2O_3 No.33-0664 (C) and Fe_3O_4 No. 19-0629 (D)

The N_2 adsorption-desorption isotherms and the corresponding pore size distributions of mesoporous SiO_2 /dense $\text{SiO}_2/\text{Fe}_3\text{O}_4$ hollow microspheres prepared with the different hydrochloric acid concentration are shown in Fig. 6. The structural parameters of the samples are summarized in Table 2. It can be seen that all samples exhibit a typical type IV isotherm with a capillary condensation of nitrogen at the relative pressure ranging from 0.40 to 0.60 due to the presence of mesopores. Furthermore, their hysteresis loops are obvious, indicating that the mesopores of these samples are open and cylindrical. In addition, all samples have a secondary condensation step at high relative pressure region, which should be attributed to the interparticle space or porosity formed among β -FeOOH nanorods. As shown in Table 2, the surface area increases from 351 to 505 m^2/g , the total pore volume from 0.30 to 0.47 cm^3/g and the peak pore size increases from 3.26 to 3.74 nm, as the hydrochloric acid concentration increases from 0.5M to 2M. It might be owed to the following

reasons. The primary structure-directing agent P123 is composed of hydrophobic PO block inner core and hydrophilic EO block outer shell [22, 23]. When the hydrochloric acid concentration was increased, the cross-linking polycondensation of TEOS and P123 was intensified, leading to the decrease of the micropores and further resulting in the increase of mesopore size.

Fig. 6 N₂ adsorption-desorption isotherms (A) and pore size distributions (B) of mesoporous SiO₂/dense SiO₂/Fe₃O₄ hollow microspheres prepared with the different hydrochloric acid concentration

Table 2 Structural parameters of mesoporous SiO₂/dense SiO₂/Fe₃O₄ hollow microspheres prepared with the different hydrochloric acid concentration

Samples	Surface area(m ² /g)	Pore volume(cm ³ /g)	Peak pore size(nm)
H0	351	0.30	3.26
H1	339	0.30	3.42
H2	505	0.47	3.74

3.3 The effect of the co-surfactant CTAB on microstructure of mesoporous SiO₂

Fig. 7 shows the TEM images of mesoporous SiO₂/dense SiO₂/Fe₃O₄ hollow microspheres prepared with the different amount of CTAB. It is indicated that the concentration of CTAB has a great effect on the morphology of mesoporous SiO₂/dense SiO₂/Fe₃O₄ hollow microspheres. From Fig. 7(A, B), the sample prepared with the co-surfactant CTAB exhibits a more perfectly spherical morphology. From

Fig. 7(B, C, D), it is also seen that the morphology gradually becomes irregular with the increase of CTAB amount. It may be ascribed to an emulsion templating mechanism, where P123 and CTAB act as total structure agents controlling the hydrophilic-hydrophobic balance in the coupled micelles. Then the forces between the composite surfactant micelles and the microspheres are gradually enhanced with the increase of CTAB amount, leading to the difficulty of core-shell structure formation and thus resulting in an irregular morphology of mesoporous SiO_2 /dense $\text{SiO}_2/\text{Fe}_3\text{O}_4$ hollow microspheres [24].

Fig. 7 TEM photographs of mesoporous SiO_2 /dense $\text{SiO}_2/\text{Fe}_3\text{O}_4$ hollow microspheres with the different amount of CTAB (A) C0; (B) C1; (C) C2; (D)C3

The N_2 adsorption-desorption isotherms and the corresponding pore size distributions of mesoporous SiO_2 /dense $\text{SiO}_2/\text{Fe}_3\text{O}_4$ hollow microspheres with different co-surfactant CTAB are shown in Fig. 8. It shows that all the samples exhibit type IV adsorption-desorption isotherms and sharp capillary condensation steps covering narrow relative pressure regions, reflecting that uniform mesopores are present in the mesoporous composite microspheres. The structural parameters of the

samples are summarized in Table 3. It shows that the surface area and the total pore volume increase, and the peak pore size decreases with the addition of co-surfactant CTAB increasing. It is mainly because that the P123 and co-surfactant CTAB have the process of the cross-linking polycondensation due to the charge effect between the hydrophilic and hydrophobic agents [25]. The total surfactant volume gradually increases with the addition of co-surfactant CTAB, causing the surface area and the pore volume increasing. Simultaneously, due to the size of co-surfactant CTAB is smaller than that of P123, peak pore size would decrease with the addition of co-surfactant CTAB. It is indicated that the size of the mesopore in the composite microspheres could be controlled by changing the addition of co-surfactant CTAB.

Fig. 8 N₂ adsorption-desorption isotherms (A) and pore size distributions (B) of mesoporous

SiO₂/dense SiO₂/Fe₃O₄ hollow microspheres with different co-surfactant CTAB

Table 3 Structural parameters of mesoporous SiO₂/dense SiO₂/Fe₃O₄ hollow microspheres with

different co-surfactant CTAB

Samples	Surface area(m ² /g)	Pore volume(cm ³ /g)	Peak pore size(nm)
C0	376	0.36	4.30
C1	505	0.47	3.74
C2	562	0.51	3.21
C3	640	0.56	3.19

3.4 Papain immobilization and activity stability

Papain has been widely used in medicine, food, cosmetic and wine industry. Papain was selected as a model enzyme to investigate enzyme immobilization performance of the composite microspheres. Because papain molecules size closely match pore size of the mesoporous SiO_2 /dense $\text{SiO}_2/\text{Fe}_3\text{O}_4$ multiply coated hollow microsphere. The external size of the papain molecules is $3.7 \times 3.7 \times 5.0$ nm. Meanwhile, the composite microspheres can provide a microenvironment to protect enzyme molecules. Fig.9 shows the amount of papain immobilized on mesoporous SiO_2 /dense $\text{SiO}_2/\text{Fe}_3\text{O}_4$ hollow microspheres at different time. The maximum amount of papain immobilized on different samples is summarized in Table 4. From Fig 9, it can be seen that papain was fast absorbed the composite microspheres in the first hour and reached its adsorption saturation after 5 hours. From Table 4, it is seen that the papain immobilization of the sample C1 was highest. It might be because that the mesopore size of the sample C1 with average 3.74 nm is just suited to the size of papain molecules. However, when the mesopore size is larger, the papain molecules will be separated from the mesopore channel of the composite microspheres (sample C0) due to the solution liquidity. The too small pore size will restrict the enzyme molecules to enter the mesopore channel of the composite microspheres (sample C3).

The stability of the papain activity is one of the most important criteria with respect to its further industrial applications. From Fig. 10A, it could be seen that both free and immobilized papain exhibit high activity when the solution pH is about 7.0. Compared immobilized papain and free papain, it is also seen that the maximum

residual activity of immobilized papain is above 80%, much higher than that of free papain (less than 40%). That is because the hollow cavity of mesoporous SiO_2 /dense $\text{SiO}_2/\text{Fe}_3\text{O}_4$ hollow microspheres provides a “natural warehouse” and microenvironment, avoiding the papain molecules inactivation. Furthermore, the surface of mesoporous SiO_2 materials has lots of silanols, which plays a buffer role for the solution in mesoporous channel [26, 27]. Therefore, the pH change in mesoporous channel is smaller than that outside.

Fig. 10B shows the residual activity of the free and immobilized papain after being kept at different temperatures ($30\sim 70^\circ\text{C}$) for 2h. It indicates that the thermal stability of immobilized papain is better than that of free papain, while the activity of free papain is only less than 40%. It could be explained that the pore walls of mesoporous SiO_2 /dense $\text{SiO}_2/\text{Fe}_3\text{O}_4$ hollow microspheres protect the immobilized papain against being damaged at high temperature due to multipoint bond formation between the support and the papain molecule [28, 29].

Fig. 9 Papain absorption curves at different time

Table 4 The maximum amount of papain immobilized on different samples

Samples	C0	C1	C3
Maximum amount(mg/g)	198	222	203

Fig. 10 pH stabilities (A) and the temperature stabilities (B) of free and immobilized papain activity

3. Conclusion

In conclusion, the mesoporous SiO₂/dense SiO₂/Fe₃O₄ hollow microspheres were prepared by a multistep coating technique. Then the mesoporous SiO₂ was coated on the composite microspheres via a sol-gel and hydrothermal synthesis process by using TEOS as silicon source, P123 in combination with CTAB as template in acidic system. The thicknesses of the dense SiO₂ layer can be easily controlled by changing the amount of TEOS, achieving the desired thickness of the dense SiO₂ layer about 4.2nm with a TEOS amount of 1.26g. The mesoporous SiO₂ layer was coated on the composite microsphere with a hydrochloric acid concentration of 2M and a CTAB amount of 0.05g exhibited a narrow pore size distribution at 3.74nm, a large surface area of 505m²g⁻¹, and a pore volume of 0.47cm³g⁻¹. The papain immobilization tests prove that mesoporous SiO₂/dense SiO₂/Fe₃O₄ hollow microspheres possess a high immobilization capacity due to the novel network like shell hollow structure. The pH stabilities and temperature stabilities of the immobilized papain activity were much higher than that of free papain activity. It is expected that the mesoporous hollow composite microspheres could be a large potential application in the enzyme

immobilization.

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References

- [1] S. Huang, Y. Fan, Z. Cheng, et al., Magnetic mesoporous silica spheres for drug targeting and controlled release, *J. Phys. Chem. C* 113 (2009) 1775-1784.
- [2] M. Liong, S. Angelos, E. Choi, et al., Mesostructured multifunctional nanoparticles for imaging and drug deliver, *J. Mater. Chem.* 19 (2009) 6251-6257.
- [3] I. Ursachi, A. Vasile, H. Chiriac, et al., Magnetic properties of magnetite nanoparticles coated with mesoporous silica by sonochemical method, *Mater. Res. Bull.* 46 (2011) 2468-2473.
- [4] H. S. Park, C. W. Kim, H. J. Lee, et al., A mesoporous silica nanoparticle with charge-convertible pore walls for efficient intracellular protein delivery, *Nanotechnology* 21 (2010) 225101.
- [5] I. I. Slowing, B. G. Trewyn, V. S. Y. Lin, Mesoporous silica nanoparticles for intracellular delivery of membrane-impermeable proteins, *J. Am. Chem. Soc.* 129 (2007) 8845-8849.
- [6] T. Sen, A. Sebastianelli, I. J. Bruce, Mesoporous silica-magnetite nanocomposite: fabrication and applications in magnetic bioseparations, *J. Amer. Chem. Soc.* 128 (2006) 7130-7131.
- [7] I. A. Barbosa, P. C. de Sousa Filho, D. L. da Silva, et al., Metalloporphyrins immobilized in $\text{Fe}_3\text{O}_4@\text{SiO}_2$ mesoporous submicrospheres: reusable biomimetic catalysts for hydrocarbon oxidation, *J. Colloid Interface Sci.* 469 (2016) 296-309.
- [8] T. Xia, M. Kovichich, M. Liong, et al., Polyethyleneimine coating enhances the cellular uptake of mesoporous silica nanoparticles and allows safe delivery of

- siRNA and DNA constructs, ACS Nano 3 (2009) 3273–3286.
- [9] M. Thomas, I. Slipper, A. Walunj, et al., Inclusion of poorly soluble drugs in highly ordered mesoporous silica nanoparticles, Int. J. Pharm. 387 (2010) 272-277.
- [10] H. F. Liu, S. F. Ji, H. Yang, et al., Ultrasonic-assisted ultra-rapid synthesis of monodisperse meso-SiO₂@Fe₃O₄ microspheres with enhanced mesoporous structure, Ultrason. Sonochem. 21 (2014) 505-512.
- [11] D. Y. Zhao, J. Y. Sun, Q. Z. Li, et al., Morphological control of highly ordered mesoporous silica SBA-15, Chem. Mater. 12(2000) 275-279.
- [12] J. W. Zhao, F. Gao, Y. L. Fu, et al., Biomolecule separation using large pore mesoporous SBA-15 as a substrate in high performance liquid chromatography, Chem. Commun. 7 (2002) 752-753.
- [13] G. Feng, J. W. Zhao, S. Zhang, et al., Application of C₁₈-modified mesoporous SBA-15 as the substrate for high performance liquid chromatography, Chem J Chinese U 23 (2002) 1494-1497.
- [14] G. D. Stucky, B. F. Chmelka, D. Y. Zhao, et al., Triblock-copolymer-directed syntheses of large-pore mesoporous silica fibers, Chem. Mater. 10 (1998) 2033-2036.
- [15] S. L. Chen, P. Dong, G. H. Yang, The size dependence of growth rate of monodisperse silica particles from tetraalkoxysilane, J. Colloid Interface Sci. 189 (1997) 268-272.
- [16] Q. Y. Li, P. Dong, R. X. Liu, et al., Preparation of monodisperse SiO₂/TiO₂/SiO₂

- multiply coated submicrospheres, *J. Inorg. Mater.* 16 (2001) 897-902.
- [17] Q. Y. Li, Q. Q. Zhu, Q. Wei, et al., Pore controllable regulation and laccase immobilization of hollow mesoporous $\text{SiO}_2/\text{Fe}_3\text{O}_4$ microspheres, *Adv. Mater.* 833 (2014) 93-98.
- [18] M. M. Wang, Q. Y. Li, Q. Wei, et al., Monodisperse mesoporous $\text{SiO}_2/\text{Fe}_3\text{O}_4/\text{SiO}_2$ microspheres: preparation and laccase immobilization, *Chemical Reaction Engineering and Technology* 28 (2012) 123-128.
- [19] X. H. Li, Q. Y. Li, Q. Q. Zhu, et al., Study on pore size regulation and laccase immobilization of mesoporous $\text{SiO}_2/\text{Fe}_3\text{O}_4$ hollow microspheres, *J Synth Cryst* 43 (2014) 2958-2965.
- [20] M. M. Wang, Q. Y. Li, Q. Wei, et al., Studies on laccase immobilization of mesoporous $\text{SiO}_2/\text{Fe}_3\text{O}_4$ hollow microspheres, *Chem J Chinese U* 2 (2013) 299-305.
- [21] Q. Y. Li, P. P. Wang, Y. L. Zhou, et al., A magnetic mesoporous $\text{SiO}_2/\text{Fe}_3\text{O}_4$ hollow microsphere with a novel network-like composite shell: synthesis and application on laccase immobilization, *J. Sol-Gel Sci. Technol.* 78 (2016) 523-530.
- [22] D. Y. Zhao, Q. S. Huo, J. L. Feng, et al., Nonionic triblock and star diblock copolymer and oligomeric surfactant syntheses of highly ordered, hydrothermally stable, mesoporous silica structures, *J. Am. Chem. Soc.* 120 (1998) 6024-6036.
- [23] C. Y. Lin, Y. Qiu, L. Q. Jiang, Micellar structure of pluronic F127 and P123 triblock copolymers, *Journal of Fuzhou University (Natural Science)* 28 (2000)

77-81.

- [24] W. H. Zhang, L. Zhang, J. H. Xiu, et al., Pore size design of ordered mesoporous silicas by controlling micellar properties of triblock copolymer EO20PO70EO20, *Microporous Mesoporous Mater.* 89 (2006) 179-185.
- [25] D. D. Jiang, Q. Wei, S. P. Cui, et al., Controllable morphology and pore structure of micron-sized organic-inorganic hybrid silica spheres derived from silsesquioxane, *J. Sol-Gel Sci. Technol.* 78 (2016) 40-49.
- [26] B. Zhao, B. Shi, R. Ma, Immobilization of papain on the mesoporous molecular sieve MCM-48, *Eng Life Sci* 5 (2005) 436-441
- [27] J. Z. Tian, T. Jing, X. M. Huang, et al., Cage-shape macroporous/mesoporous 3DOM SiO₂ immobilized papain, *Adv. Mater.* 236-238(2011)954-957.
- [28] Y. Zhu, S. Kaskel, J. Shi, et al., Immobilization of trametes versicolor laccase on magnetically separable mesoporous silica spheres, *Chem. Mater.* 19 (2007) 6408-6413.
- [29] Y. Deng, C. Deng, D. Qi, et al., Synthesis of core/shell colloidal magnetic zeolite microspheres for the immobilization of trypsin, *Adv. Mater.* 21 (2009) 1377-1382.