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Immobilization and Characterization of Serum Albumin in SBA-15 Mesoporous Material

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Bovine serum albumin was immobilized inside SBA-15 mesoporous silica through physical adsorption method. Chemical analysis, powder X-ray diffraction, Fourier transform infrared spectroscopy, low-temperature N_2 adsorption-desorption at 77 K, scanning electron microscopy, high resolution transmittance microscopy, ultraviolet-visible solid diffusion reflection spectroscopy and luminescence spectroscopy were used to characterize the prepared (SBA-15)-bovine serum albumin hybrid material and luminescent spectrum of the hybrid composite material was studied. The results showed that the guest bovine serum albumin had already been encapsulated in the host molecular sieve and its immobilization amount was 72.99 mg/g (BSA/SBA-15) and bovine serum albumin had partially come into molecular sieve pore channels and the molecular sieve framework in composite material was remained intact. The ultraviolet-visible solid diffusion reflection spectra indicated that absorption peak of the composite material brought an obvious blue shift compared with that of bovine serum albumin, showing that the bovine serum albumin was already assembled in the channels of molecular sieve. The average particle diameter of (SBA-15)-bovine serum albumin sample measured from SEM was 345 ± 10 nm. Blue light of hybrid material (SBA-15)-bovine serum albumin was emitted at 439 nm.

Key Words: Serum albumin, Mesoporous SBA-15, Physical adsorption, Immobilization.

INTRODUCTION

In 1992, the American scientists, Kresge *et al.*[1] of mobil oil company, first successfully developed the ordered mesoporous material-M41S mesoporous molecular sieves series materials, in which MCM-41 (MCM is the name of the mesoporous molecular material synthesized by mobil company, represents the mobil composition of matter) came to front and is most studied. Although the appearance of MCM-41 is ground-breaking milestone in the synthesis of mesoporous molecular sieves, there are some shortcomings of its own, such as its smaller pore size and not high hydrothermal stability, which makes its application subjected to certain limitations. In 1998, Zhao *et al.*[2], for the first time synthesized a hexagonal pore structure mesoporous molecular sieve SBA-15 (the SBA represents Santa Barbara Amorphous), whose pore wall is thickness of 3.1-6.0 nm and nanopore size is adjustable from 4.6 to 30 nm and the specific surface area is up to above 700 cm^2/g. The mesoporous molecular sieve not only has large specific surface area, narrower pore size distribution, tunable pore size, high mechanical strength, good hydrothermal stability and higher than 900 ºC thermal stability, but also has long use life and non-toxic to biology. The appearance of SBA-15 makes mesoporous molecular sieves start moving to the practical direction. SBA-15 molecular sieve was synthesized in acid medium using amphiphilic non-ionic polymer surface active agent poly(ethylene glycol)-block-poly(propylglycol)-block-poly(ethylene glycol) (PEO-PPO -PEO) triblockco-polymer and tetraethyl orthosilicate (TEOS) interaction. It has highly ordered hexagonal phase. Mesoporous molecular sieve SBA-15 displayed a great potential applied value in the biological, separation and catalytic and nano assembly aspects, *etc.*, due to these advantages. Some transition metals have been assembled into the SBA-15 molecular sieve, which has prepared some novel catalysts[3]. Metivier *et al.*[4] incorporated organic fluorescent silicon source into the SBA-15 mesoporous materials and the prepared materials could detect Hg^{2+} in aqueous solution. Xu and Zhai[5] used ion exchange method and hydrothermal synthesis to prepare (SBA-15)-ZnS host-guest nanocomposite materials with good luminescent properties. Yu and Zhai[6] have encapsulated nimodipine into SBA-15 host and studied slow-release effect of the drug in SBA-15 and good slow-release effect was achieved. Mesoporous silica siliceous materials SBA-15, with non-toxic, high specific surface area, high pore volume and highly ordered hexagonal structure, is suitable to support the adsorption and immobilization of proteins and enzymes. On the basis of this, a large pore molecular sieve SBA-15 may have more potential in

immobilization of enzymes than MCM-41. Enzyme, as biological catalyst, has high specificity. And, catalytic effect of enzyme catalyst is high and reaction conditions are mild. Free enzymes, with poor stability, difficult recovery, make their practical application be limited. However, the immobilized enzymes have wide applied prospects, especially, using molecular sieve inorganic carrier to be immobilized, is the advantages of non-toxicity and of that surface properties can be adjusted and of being easy to recycle and be regenerated, has wide applied prospects. Manyar *et al.*[7] immobilized the porcine pepsin in mesoporous SBA-15. Xiao *et al.*[8] studied the lysozyme immobilization in silicon dioxide nanometer material. Liu *et al.*[9] have immobilized hemoglobin on SBA-15 and used for the electrocatalytic reduction study of H_2O_2 and achieved a certain catalytic effect. Serum albumin is the main component of the protein in blood. In the circulatory system, it occupies about 60 % of the plasma protein content, provides 80 % of the osmotic pressure for blood[10], to take charge of the blood pH value by serum albumin. For bovine serum albumin, the molecular size is 5 nm × 7 nm × 7 nm. It is composed of 583 amino acid residues with a molecular weight of 68000 (Da) and isoelectric point (pI) = 4.7[10,11]. The aim of this paper was to use SBA-15 mesoporous material for immobilization of bovine serum albumin and study the optimum immobilization conditions. Chemical analysis, powder X-ray diffraction, Fourier transform infrared spectroscopy, 77 K low temperature N_2 adsorption-desorption, scanning electron microscopy, transmission electron microscopy, UV-visible solid diffuse reflectance spectra and luminescence spectra were used to characterize the products. The luminescence properties of the composite hybrid materials were also observed.

EXPERIMENTAL

Tetraethyl orthosilicate (TEOS, Shanghai Chemical Reagent Corporation of Chinese Medicine Ltd.); triblock copolymer, poly(ethylene glycol)-block-poly(propylglycol)-block-poly(ethylene glycol) ($EG_{20}PG_{40}EG_{20}$, average molecular sieve 5800, Aldrich); Hydrochloric acid (Beijing Chemical Factory, China); Bovine serum albumin (BSA, Huishi Biochemical Reagents Corporation, China). The reagents used in the experiment were of analytical purity and the water was doubly deionized water.

Synthesis of SBA-15 material: In 15 g of deionized water and 60 g of 2 mol L^{-1} HCl mixed solution, 2 g of amphiphilic triblock copolymer was added with continuous stirring. After dissolution, 4.25 g of tetraethyl orthosilicate was slowly added until forming homogeneous solution. At 40 ºC, stirring was lasted for 24 h. The solution was placed in a Teflon-lined autoclave and crystallized at 100 ºC for 2 days. Finally, the crystallized product was filtrated and washed several times with deionized water and dried at room temperature. Then, the product was calcined at 550 ºC for 24 h to prepare SBA-15 mesoporous molecular sieve[2].

Bovine serum albumin protein adsorption: 0.25 g of calcined SBA-15 was taken and to it was added 5 mL of 4 mg/mL bovine serum albumin solution. 5 mL of pH = 4.5 CH_3COOH + CH_3COONa buffer solution was added. The mixture was shaken for adsorption for 38 h at 4 ºC. The mixed system was centrifugated at a speed of 8000 rpm and the supernatant clear solution was retained. The precipitate was washed with distilled water until bovine serum albumin can not be detected in solution. And the supernatant clear solution and precipitate were obtained and the supernatant clear solutions were merged.

By arsenazo-III spectrophotometric method[12] the enzyme protein content in the supernatant clear solution was determine by subtraction to calculate the content of the enzyme protein adsorbed in the molecular sieve.

Adsorbed enzyme protein amount = (Total amount of enzyme protein) - (The amount of enzyme protein in the supernatant clear solution) (1)

The optimum experiments of conditions were respectively made according to the following methods:

The other conditions were fixed and unchanged and the bovine serum albumin was adsorbed and vibrated in SBA-15 for 0, 1, 4, 8, 15, 24, 38, 48 and 52 h, respectively. The amount of bovine serum albumin protein encapsulated in 1 g of SBA-15 molecular sieve was calculated.

The other conditions were fixed and unchanged and the immobilization of bovine serum albumin in different concentrations of bovine serum albumin solution was made. The concentrations of 0, 1, 2, 3, 4, 5 and 6 mg/mL of bovine serum albumin solution were taken. The amount of bovine serum albumin encapsulated in 1 g of SBA-15 molecular sieve was calculated.

The other conditions were fixed and unchanged and the immobilization of BSA at different pH was made. The pH values of 4.0, 4.5, 4.8, 5.0, 5.5 and 6.0 were taken. The amount of BSA encapsulated in 1 g of SBA-15 molecular sieve was calculated.

For the sample prepared under the optimum conditions, the situation of bovine serum albumin assembly was analyzed with different characterization methods.

Chemical analysis: Using arsenazo-III spectrophotometry[12] for the determination of bovine serum albumin content, absorbance measurements was completed on a 722 S type spectrophotometer (Shanghai Lingguang Technology Co. Ltd., China).

Powder X-ray diffraction analysis: Powder XRD experiments were finished on D_8/ADVANCE diffractometer (Bruker Company, Germany) using CuK_α target, the determination wavelength was 0.154 nm, tube voltage was 30 kV and tube current was 20 mA. The determination angle 2θ value of small angle XRD was from 0.4º to10º and the determination step size was 0.02º. The determination angle 2θ value of wide-angle XRD ranged from 10º to 80º with measured step length of 0.2º.

Fourier transform infrared spectroscopy: FT-IR experiments were made on a BRUKER Vertex-70 spectrometer (American Mike Company) by the KBr pellet technique. Range of the determination wavenumber was 4000-400 cm^{-1}.

Low temperature N_2 adsorption and desorption: The experiments were made in liquid nitrogen at 77 K and ASAP 2020 MV 3.01 H adsorption instrument (Micromeritics Company, USA) was used. The sample was vacuumized at room temperature for 2 h. Use of room temperature prevented

protein denaturation. Specific surface area was measured by BET (Brunner-Emmett-Teller) method. BJH (Barrett-Joyner-Halenda) method was used for the analysis of pore structure of the sample.

Scanning electron micrograph and high resolution transmission microscopy photo were respectively shot on a PHILIPS XL30-type field emission scanning electron microscopy and the FEI Tecnai G2 F20-type field emission transmission electron microscope (the working voltage was 200 kV).

UV-visible solid diffuse reflectance spectroscopy: The experiments were carried out on a spectrophotometer U-4100 UV-Visible (Hitachi Electric Company, Japan) at the detection wavelength from 200 to 800 nm. α-Al_2O_3 was taken as matrix.

Luminescence experiments: Fluorescence spectrometry experiments were carried out at room temperature 20 ºC using the dual-grating fluorescence spectrometer SPEX-FL-2T-2 (American SPEX Company).

RESULTS AND DISCUSSION

Effect of adsorption time, bovine serum albumin concentratrion and different pH on bovine serum albumin adsorption: The effects of adsorption time, different BSA concentration and different pH on BSA adsorption amount are respectively shown in Figs. 1-3. The results show that when the adsorption of BSA on SBA-15 was over 0-38 h, adsorption amount increased as the time increased. At 38 h, the adsorption achieved equilibrium. Effect result of BSA concentration on adsorption amount revealed that over the range of 0-4 mg/mL as BSA concentration increased, BSA adsorption amount increased. At 4 mg/mL, the adsorption achieved equilibrium. The experimental results of acidity influence proved that in pH = 4.5 CH_3COOH + CH_3COONa buffer solution the adsorption amount of bovine serum albumin on SBA-15 was the largest, which is in agreement with the isoelectric point pH 4.7 of bovine serum albumin. The biggest adsorption took place in the bovine serum albumin isoelectric point (pH = 4.7) nearby place, pH = 4.5. At this time, the immobilization amount is 72.99 mg (BSA)/g (SBA-15).

At this time the electrostatic repulsive-force between bovine serum protein molecules and the surface of SBA-15 is the smallest. The isoelectric point of various kinds of SiO_2 materials ranges from 0.5 to 3.7[13,14]. The isoelectric point of SBA-15 is 2.7-3.7[15]. As pH increases, results in increase of electrostatic repulsive-force between bovine serum albumin and the surface of BSA-15 and results in lowering of adsorption amount because both bovine serum albumin and SBA-15 take negative electrical charges. Furthermore, repelling force between the adsorbed bovine serum albumin molecules in a higher surface coverage increased. With the pH being higher than the isoelectric point of bovine serum albumin, the adsorption amount of bovine serum albumin declines as a result of the synergy of the two kinds of actions.

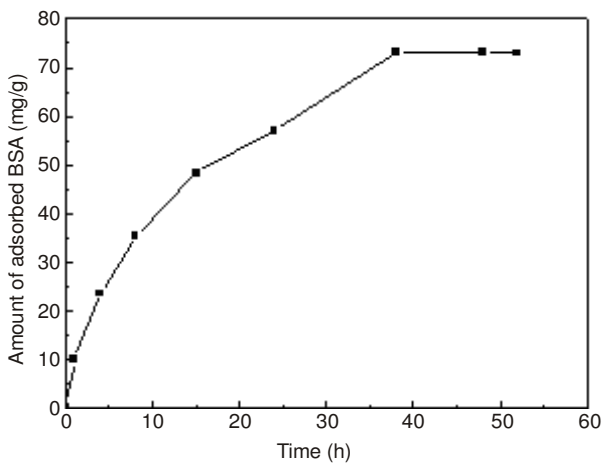


Fig. 1. Effect of adsorptive time on adsorption of bovine serum albumin on SBA-15

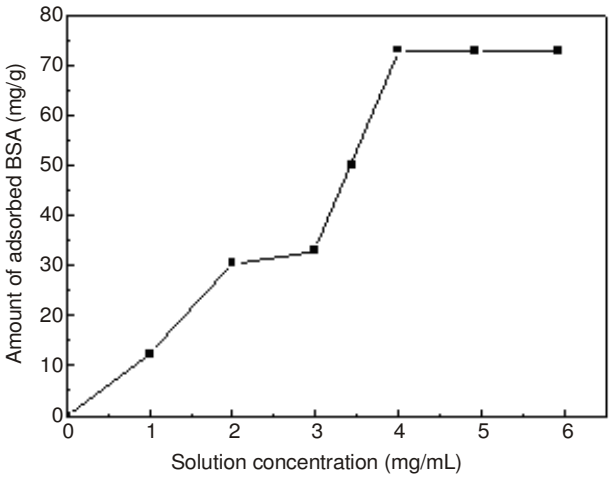


Fig. 2. Adsorption of different concentration bovine serum albumin on SBA-15

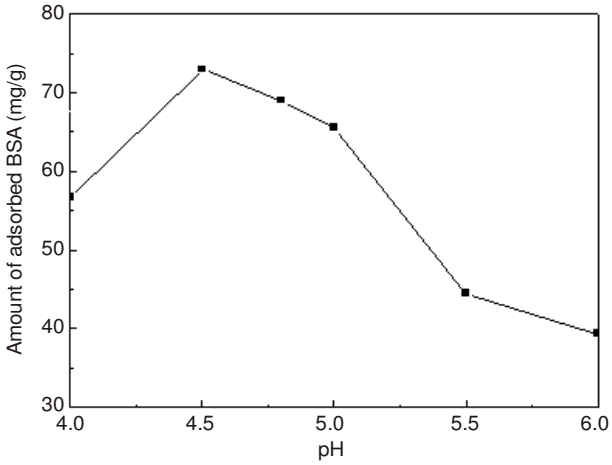


Fig. 3. Effect of different pH on adsorption of bovine serum albumin on SBA-15

Powder X-ray diffraction analysis: Fig. 4 is the small angle XRD diffraction patterns for bovine serum albumin, SBA-15, (SBA-15)-BSA samples. As can be seen from the graph, XRD diffraction curve (b) of SBA-15 sample has a strong diffraction peak d_{100} in the low diffraction area, weaker diffraction peaks d_{110}, d_{200}, also are clearly visible, showing that the quality of SBA-15 prepared was good. The d(100) and unit cell parameters a_0 obtained by calculation are listed in Table-1. Fig. 5 is the wide angle XRD diffraction patterns of bovine serum albumin, SBA-15, (SBA-15)-BSA samples. It can be seen by the graph that the (SBA-15)-BSA sample did not appear other characteristic peak except SBA-15 phase, indicating in the composite material no new phase formed.

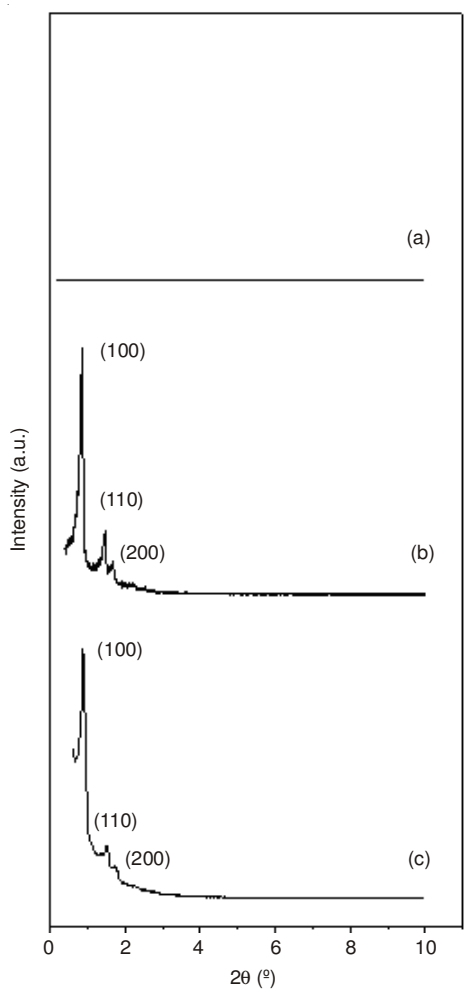


Fig. 4. Small angle XRD diffraction patterns of samples: (a) bovine serum albumin; (b) SBA-15; (c) (SBA-15)-BSA

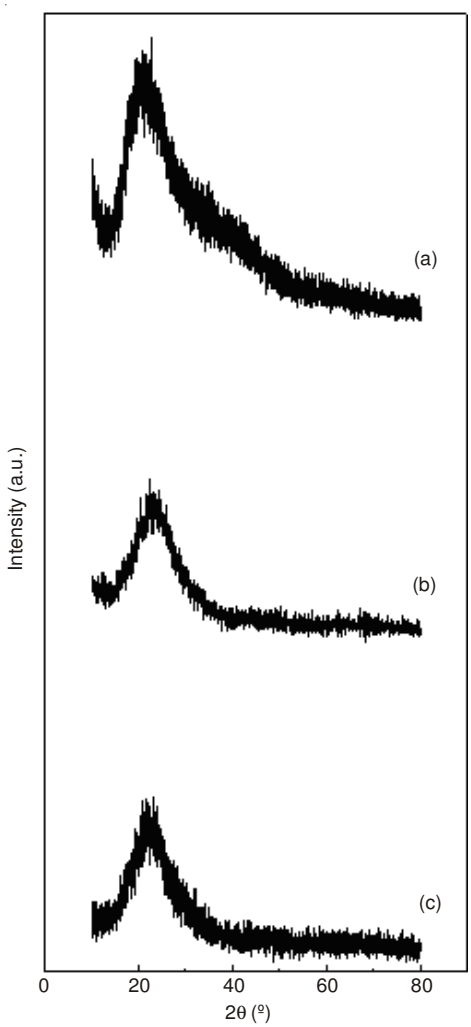


Fig. 5. Wide angle XRD diffraction patterns of samples: (a) bovine serum albumin; (b) SBA-15; (c) (SBA-15)-BSA

Fourier transform infrared spectroscopy: The infrared spectra of each sample from 4000 to 400 cm^{-1}, conducting a comprehensive comparison with FT-IR spectroscopic situation of the bovine serum albumin, the SBA-15 and (SBA-15)-BSA (Fig. 6). Curve b is the infrared spectrum of SBA-15 prior to assembly, curve c for the infrared spectrum of bovine serum albumin assembled into SBA-15. For SBA-15 sample at 461 cm^{-1} the bending vibration of Si-O-Si bond of the molecular sieve framework made the curve show absorption peak. The absorption peaks of 1092 cm^{-1} and 795 cm^{-1} was caused due to the stretching vibration of Si-O-Si bond. Absorption peak at 950 cm^{-1} appeared because of the Si-OH bending vibration. There was an absorption peak at 3433 cm^{-1} due to the adsorbed water. Curve (c) is the IR spectrum of sample (SBA-15)-BSA. Composite material still has characteristic peaks of SBA-15, just the intensity decreased but did not appear characteristic absorption peaks of bovine serum albumin, which can illustrate that bovine serum albumin has spread into the molecular sieve channels and was not adsorbed in molecular sieve outer surface.

Adsorption and desorption of N_2 at low temperature: The adsorption and desorption isotherm of nitrogen gas and pore size distribution of the calcined SBA-15 and composite material (SBA-15)-BSA at 77 K are reported in Fig. 7. It can be known from Fig. 7(A) that the adsorption and desorption isotherm as well as hysteresis loop of the samples SBA-15 and (SBA-15)-BSA at 77 K are similar, which indicates that after the introduction of bovine serum albumin the molecular

TABLE-1
PORE STRUCTURE PARAMETERS OF SAMPLES

Sample	Interplanar spacing, d_{100} (nm)	Unit cell parameter, a_0[a] (nm)	BET Specific surface area (m^2/g)	Pore volume[b] (cm^3/g)	Pore size[c] (nm)	Pore wall Thickness[d] (nm)	Content of BSA (BSA/molecular sieve) (mg/g)
SBA-15	10.91	12.60	662	1.14	6.77	5.83	0
Composite hybrid material (SBA-15)-BSA	10.04	11.59	570	0.83	5.70	2.89	72.99

a– Unit cell parameter, $a_0 = \frac{2}{\sqrt{3}} d_{100}$; b – BJH adsorption cumulative volume of pores; c– Pore size calculated from the adsorption branch; d– Wall thickness calculated by (a_0 - pore size)

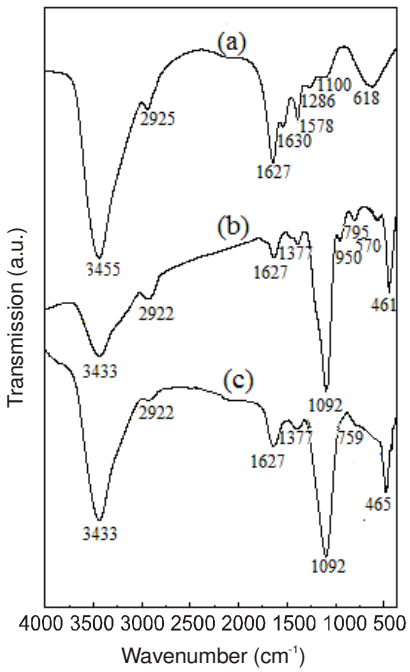


Fig. 6. Infrared spectra of samples: (a) bovine serum albumin; (b) SBA-15; (c) (SBA-15)-BSA

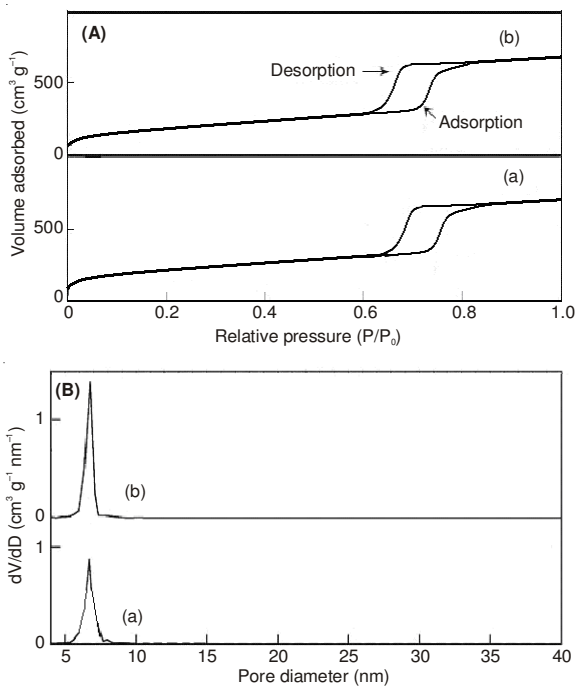


Fig. 7. (A) Low temperature N_2 adsorption-desorption patterns of samples; (B) Pore size distribution patterns: (a) SBA-15; (b) (SBA-15)-(BSA)

sieve porous channels structure of SBA-15 was not destructed. From the curves of the adsorption and desorption of N_2, the samples before and after assembly show three stages, which match with adsorption characteristics of mesoporous materials. (SBA-15)-BSA sample has the smaller relative partial pressure range of sudden jump than the non-assembled SBA-15 sample, because after the encapsulation of sample the guest bovine serum albumin entered into the pores of molecular sieve and occupied a part of the pore volume, making the adsorbed range of relative partial pressure decrease compared with the unassembled SBA-15 sample. As bovine serum albumin molecules partially occupied the pore channels of SBA-15, this made the pore volume of 1.14 cm^3/g reduce to 0.83 cm^3/g, with a reduction of 27.2 %. The BET specific surface area decreased from 662 m^2/g to 570 m^2/g,with a reduce of 13.9 %. It shows that the bovine serum albumin molecules have been assembled into the pores of SBA-15. These reults indicate that bovine serum albumin was confined within the SBA-15 pores, rather than adsorbed on the outer surface of SBA-15. Fig. 7(B) is pore size distribution pattern of SBA-15 and (SBA-15)-BSA samples. It can be seen from the figure that (SBA-15)-BSA sample aperture has been reduced. This shows that the bovine serum albumin was encapsulated within the molecular sieve pore channels and partially occupied the channels of SBA-15 material. The sample pore structure parameters and related physical and chemical parameters are listed in Table-1.

SEM and TEM analysis: Fig. 8 is SEM images of the sample (SBA-15)-BSA. The shape of particles presents fibriform appearance. The average particle diameter is 345 ± 10 nm through measurement. The high resolution transmission electron microscopy (Fig. 9-A) of (SBA-15)-BSA taken with the beam direction perpendicular to the pores. Fig. 9-B is the TEM images taken with the beam direction parallel to the pores. It can be seen that the pore stripes are clear and orderly, showing that the prepared (SBA-15)-BSA composite hybrid material is a typical mesoporous material. Pore size is 7.2 ± 0.1 nm and the introduction of bovine serum albumin did not make the mesoporous channels change.

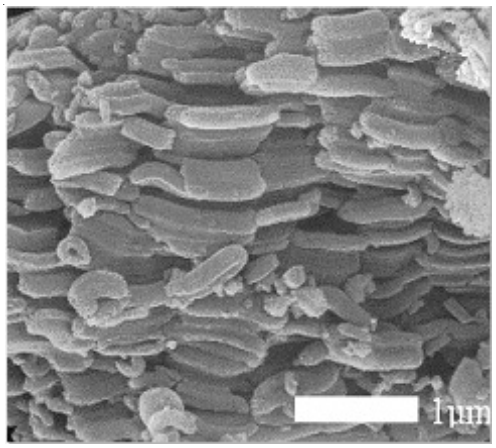
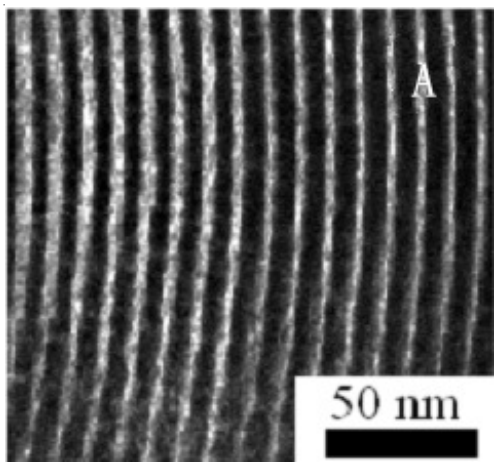


Fig. 8. SEM photograph of (SBA-15)-BSA sample



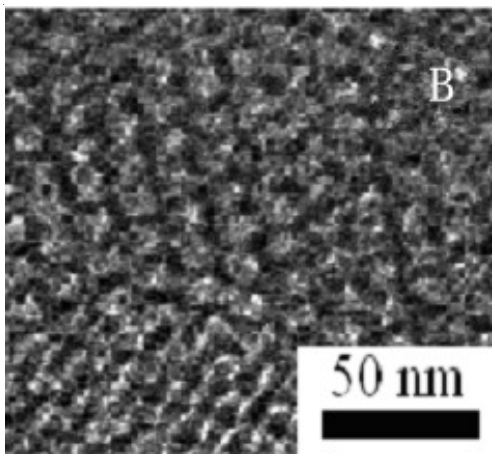


Fig. 9. TEM photograph of (SBA-15)-BSA sample: (a) taken with the beam direction perpendicular to the pores and (b) taken with the beam direction parallel to the pores

UV-visible solid diffuse reflectance spectra: Fig. 10 is UV-visible solid diffuse reflectance spectra of samples. It can be seen from curve (a) that SBA-15 has not any absorption peak in the visible-UV region, while bovine serum albumin has an absorption peak at 287 nm on curve (b). Curve (c) represents absorption curve of sample (SBA-15)-BSA, has an absorption peak at the 280 nm, which resulted from the bovine serum albumin encapsulated in the molecular sieve pore channels. Compared with absorption peak of bovine serum albumin, the peak location be decreased a blue shift of the range of 7 nm, which indicates bovine serum albumin entered into pores of SBA-15.

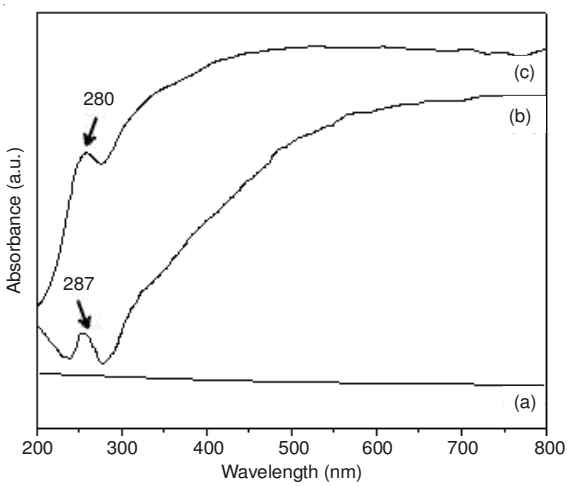


Fig. 10. UV-visible solid diffuse reflction absorption spectra of samples: (a) SBA-15; (b) BSA; (c) (SBA-15)-(BSA)

Luminescence research: Fig. 11 is the excitation and emission spectra of bovine serum albumin and (SBA-15)-BSA composite material. In this study, the emission spectrum peak of (SBA)-15-BSA composite material appears at 439 nm. This shows that after bovine serum albumin is adsorbed, its structure and the conformation have not been damaged. Composite material shows bovine serum albumin luminescent properties due to the unspoiled conformation of bovine serum albumin. SBA-15 itself does not have the luminescence properties, but (SBA)-15-BSA has good luminescence properties, thus the

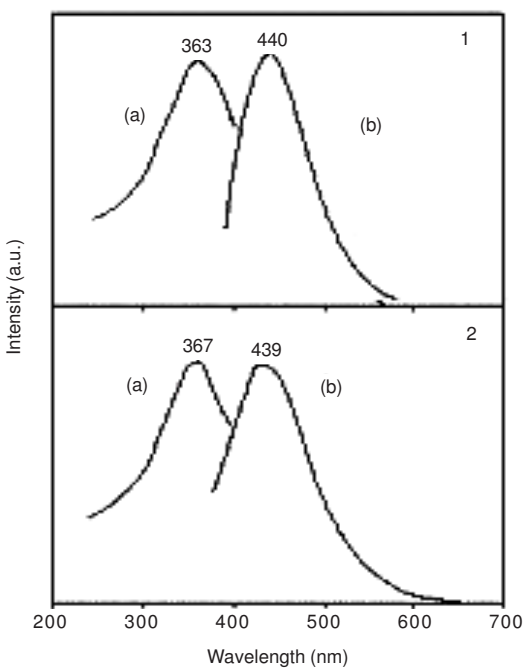


Fig. 11. Luminescent spectra of samples: (1) SBA-15; (2) (SBA-15)-(BSA). (a) Excitation spectrum; (b) Emission spectrum

obtained (SBA)-15-BSA composite material can be used as a luminescent material with potential application prospects. The obtained (SBA)-15-BSA composite material in this experiment has a emission spectrum peak at 439 nm which is in the range of visible light and emits blue light, therefore, it can be applied as a blue light-emitting materials with potential applications. In addition to blue laser device, the hybrid materials prepared in this article can be potentially used as catalysts and chemical sensor material.

Conclusion

The study prepared mesoporous SBA-15 molecular sieve by the hydrothermal method and bovine serum albumin object material was assembled into the above-stated host material by physical adsorption method, successfully preparing host-guest composite material (SBA-15)-BSA. Finally, the composites prepared were characterized by chemical analysis, X-ray powder diffraction, Fourier transform infrared spectroscopy (FT-IR), low temperature N_2 adsorption-desorption, SEM, high resolution transmission microscopy, UV-visible solid diffuse reflectance spectra and luminescence spectra. The results proved that the bovine serum albumin object had been assembled into the host molecular sieve and partially gone into its pores with the immobilization amount of 72.99 mg (BSA)/g (SBA-15) and composite material's molecular sieve skeleton was retained intact. The average diameter of (SBA-15)-BSA sample is 335 ± 10 nm. Composite material (SBA-15)-BSA emits blue light at 439 nm. The composite material prepared has potential applied foreground in blue lasers, catalysts, chemical sensors, *etc*.

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