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Preparation of amino-functionalized mesostructured cellular foams and application as hosts for large biomolecules

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Abstract Large mesopores cellular foam (LMCFs) materials were synthesized using microemulsion templating in acidic solutions. The amine functional groups were attached to channels of LMCFs materials via post-synthesis grafting. The structural and chemical properties of these prepared materials were characterized by TEM, XRD, FTIR and nitrogen adsorption. These resulting materials had disordered mesopores with well-defined large mesopore. The bovine serum albumin (BSA) and glucose oxidase (GOx) were used for adsorption experiment. The biomolecule was immobilized by covalently couple to the interior surface of amino-functionalized mesostructured cellular foams (AF-MCFs). The results showed that AF-MCFs had high-capacity bioimmobilization ability.

Introduction

The specific immobilization of biomolecular such as proteins or enzymes onto solid supports is of considerable interest for their potential applications in biotechnological industries, and biomedical fields [1, 2]. Functionalized mesoporous materials with high surface area, controllable pore size, narrow pore size distribution, thermal and mechanical stability, and featuring high functionality, provide high affinity with the protein and enzyme molecules leading to high protein and enzyme loading [3–5].

The preparation of new materials with large mesopore frameworks or complex macroscale foams is a challenging task due to the control of pore size and structure [6]. In biotechnological applications, the use of high-molecular-weight materials requires mesoporous materials with well-defined large mesopores (15–50 nm) or macroporous pores (>50 nm) for the diffusion of reagents and products through pore channel in order to avoid undesired pore blocking phenomena [7–10]. Large mesopores and macroporous materials enhance the mass transfer and overcome the diffusion resistance. However, it is difficult to prepare mesoporous materials with large and ultralarge pores by existing synthetic methods using only surfactants as templates. Several template methods have been developed and improved to prepare large mesopores and macropores materials. These methods use polymer gels [11, 12], emulsion [13, 14], latex spheres [15], even bacteria [16] as templates. However, these studies focused mainly on pure silica materials, which limit their applications in biotechnology. The synthesis of mesostructured cellular silica foams has been studied by several researchers [17–21]. The major advantage of the combination of surfactant templating techniques and emulsion or microemulsion templating is that it provides a simple and effective way to fabricate mesoporous materials with well-defined pore structures from mesoporous to large and ultralarge pores.

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It is possible to chemically modify the surface of mesoporous materials with various functional groups, enabling these materials to hold great promise for use as supports to immobilize proteins and enzymes. The method of post-synthetic grafting can create high-density functional groups on mesoporous materials without diminishing the porous structure, which is essential for high loading of proteins and enzymes. Therefore, functionalized large mesopores materials with a high-density of amino groups and well-defined mesochannels have resulted in a revolution of the use of biomolecule for applications in separation, catalysis and sensors, which typically depends on the successful immobilization of biomolecule onto suitable host [22, 23]. Despite their importance for the biotechnology applications, the preparation of the functionalized large mesopores materials and applications for the immobilization of enzyme or protein of functionalized mesostructured cellular foams with large mesopores by oil/water microemulsion templating have not been reported.

In this paper, we report the synthesis of amino-functionalized mesostructured cellular foams (AF-MCFs) with large mesopores by the post-synthetic grafting of Tetraethoxysilane (TEOS) and 3-Aminopropyl-triethoxysilane (ATES) using the microemulsion templating. The relationships between synthesis conditions and structural features of AF-MCF materials are described and discussed. AF-MCF materials characterized by transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared (FTIR), and nitrogen sorption are presented. Good composition control and pore size distribution of AF-MCF materials have been achieved. Adsorption performances of protein and enzyme on these functional mesoporous materials are presented and discussed.

Experimental

Preparation of the AF-MCF materials

Tetraethoxysilane (TEOS) and EtOH were obtained from Aldrich. 3-Aminopropyl-triethoxysilane (ATES) was obtained from (ACROS). All chemicals were used as received. Water used in all synthetic procedures was deionized to 18 MΩ cm. Large mesopores cellular foam (LMCFs) materials were synthesized using microemulsion templating in acidic solutions. LMCF materials were prepared as follows: P123 (poly (ethylene oxide)-block-poly (propylene oxide)-block-poly (ethylene oxide), EO_{20}–PO_{70}–PO_{20}, M_{av} = 5,800) of 2 g (0.4 mmol) was dissolved in 75 mL 1.5 N HCl solution at room temperature with stirring. 1,3,5-trimethylbenzene (TMB, 8 g 68 mmol) as the organic solvent (oil phase) was then added to the surfactant solution with stirring to form an oil-in-water microemulsion. The amount of TMB used was controlled by the weight ratios of TMB to P_{123} from 0 to 10, a typical ratio was 4:1. The TEOS (TEOS: 4.4 g, 21 mmol) was then added to the surfactant solution and this mixture was covered and stirred at 38–40°C for 24 h. The synthesized materials were aged at 110°C for 24 h in an autoclave. These as-synthesized materials were rinsed with deionized water and dried at air for 2 days. These synthesized productions were calcined at 500°C for 5 h in air to remove surfactant and form LMCF materials. Post-synthesis functionalization of LMCF materials: 0.2 g of calcined LMCF sample was suspended in 20 mL of ethanol. Then, appropriate amount of 3-Aminopropyl-triethoxysilane (ATES) was added to this silica suspended solution, in which the molecule ratio of ATES to TEOS was controlled from 0 to 2.5. This reaction mixture was refluxed at 85°C for 8 h. The white solid was filtered off, washed with deionized water and ethanol and dried at 60°C for 24 h.

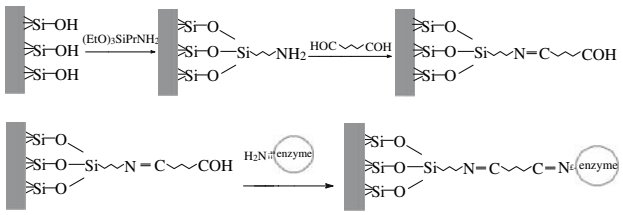
Characterization of the AF-MCF materials

The AF-MCF materials were observed with a JOEL 2000FX transmission electron microscope and an electron diffraction technique with an accelerated electron voltage of 200 kV. Small-angle XRD measurements were performed on an X-ray diffractometer (Rigaku Rotflex D/Max-C) using CuKα radiation (λ = 0.15064 nm). Nitrogen adsorption measurements were carried out with a Tristar 3000 Micromeritics at –196°C. Each sample was degassed for 2 h at 200°C under vacuum of about 10^{-3} Torr before being analyzed. Surface areas were calculated by the Brunauer–Emmett–Teller (BET) method. The pore size was calculated using Barrett–Joyner–Hatenda (BJH) model. All five samples (the molar ratio of ATES to TEOS: 0, 0.25, 0.5, 1.0, 2.0) were examined by Thermo Nicolet (US) Avatar 360 FTIR spectrometer.

Protein adsorption

The bovine serum albumin (BSA) and glucose oxidase were used to investigate adsorption on the amino-functionalized mesostructured cellular foams

(AF-MCFs) materials. In each experiment, the standard protein solution was prepared by dissolving 5 mg protein or glucose oxidase in a 5 mL buffer solution (0.1 M phosphate, pH 6.5). First, AF-MCFs materials react with amyl aldehyde to change amino groups into aldehyde groups, which can covalently couple biomolecule to the interior surface of AF-MCFs materials. The reaction can be represented by the following equation:



Then, the 8 mg of mesoporous solid was suspended in 4 mL of protein solution (protein concentration: 1 mg/mL) and was shaken overnight at room temperature. The amount of protein adsorbed was measured by analyzing the protein content of the supernatant liquid using Bradford method and the amount adsorbed on the mesoporous solid was calculated by the difference [24].

Results and discussion

Characterization results

Figure 1 shows a typical TEM image of amino-functionalized mesostructured cellular foam (AF-MCF) materials at large mesopores. These AF-MFC materials possess a disordered mesoporous structure consistent with the structural features of MCFs reported by previous studies [17–21]. Larger mesopores with diameters ranging from 17 nm to 34 nm were observed from Fig. 1 and the wall thickness of the AF-MCFs was estimated to be from 2.5 nm to 4 nm using TEM. Low angle X-ray diffraction (XRD) was performed for the amino-functionalized MFC materials to analyze the structure of the mesoporous materials. Figure 2 shows the XRD peak in the low angle region after calcinations, which indicates the presence of a mesoporous structure without any long range ordering [22, 23]. The XRD pattern of large meso and macropore materials indicates only information on the intrananoparticle mesopores due to the large dimensions of the motive and the disordered characterization of these materials [10]. The pore size and pore structure of AF-MCF materials depend on the composition of

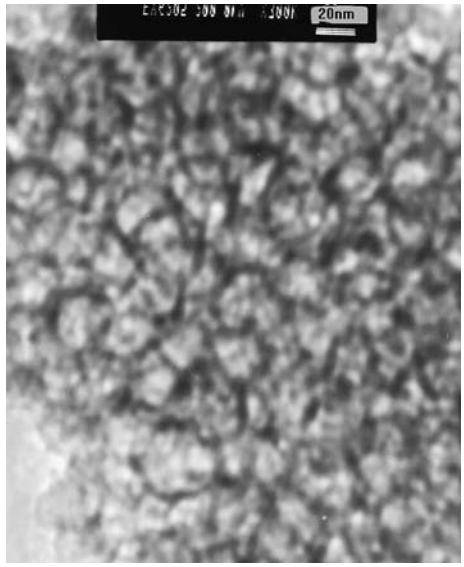


Fig. 1 Transmission electron microscope (TEM) image of the Amino-functionalized mesostructured cellular foam (AF-MCF) materials prepared by microemulsion templating after surfactant removing

precursor and preparation conditions. The ratio of mesitylene to surfactant (P_{123}) has a significant effect on the pore size and size distribution in the AF-MCFs. The average pore size increases with increasing ratio of mesitylene (TMB) to surfactant (P_{123}). The appropriate weight ratio of mesilylene to P_{123} in 4:1 was used to obtain the range of pore size from 17 to 34 in this study. Another factor that influences the pore size and pore structure is the aging time and aging temperature. The average pore size increases and the pore size distribution narrows with increasing aging time at 100–110°C.

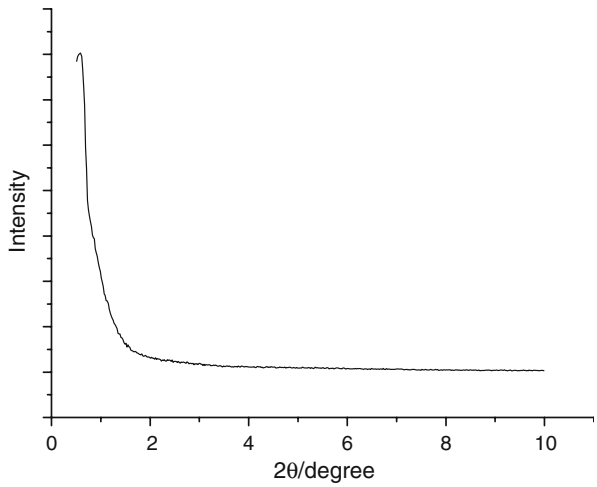


Fig. 2 X-ray diffraction patterns for the Amino-functionalized mesostructured cellular foam (AF-MCF) materials prepared by microemulsion templating after surfactant removing

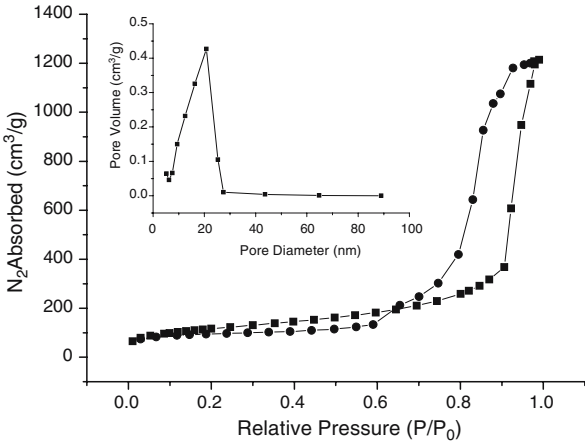


Fig. 3 Nitrogen adsorption isotherms and corresponding pore size analyses for the Amino-functionalized mesostructured cellular foam (AF-MCF) materials prepared by microemulsion templating after surfactant removing

The experimental results indicate that the acid preparation condition allows slow and moderate condensation of the TEOS, and the rates of condensation of TEOS can be controlled by microemulsion templating. A representative nitrogen adsorption–desorption isotherm of the amino-functionalized MFC materials is presented in Fig. 3. The isotherm is a type IV curve of mesoporous materials. A steep hysteretic loop is observed from this curve, which is typical for mesoporous materials that exhibit capillary condensation and evaporation [25, 26]. The sharp rise in the nitrogen adsorption/desorption isotherms at high relative pressures (P/P_0 near 1) indicates the existence of large mesopores and macropores in these materials [21, 27]. Figure 3 (inset) shows the pore size distributions measured by the Barrett–Joyner–Hatenda (BJH) model. The BET surface area is 520.7 m^2/g and the total pore volume is 1.38 cm^3/g. It is known that the large mesopores and macroporous materials have lower surface area and higher pore volume than small mesoporous materials [28, 29].

The presence of the amino-group onto the large mesopores framework after post-synthesis functionalization of LMCF materials has been confirmed by FTIR spectra. After post-synthesis, the C–N stretching vibration at 1,180 cm^{-1} overlapping with Si–O–Si band at 1,054 cm^{-1} and the symmetrical $-NH_3^+$ bending band at 1,505 cm^{-1} can be observed from Fig. 4 and the weak symmetrical $-NH_3^+$ vibration at 1,625 cm^{-1} overlapped with C=C band [9, 30, 31]. These amine function groups do not exhibit by MCFs materials prepared by TEOS alone as shown in Fig. 4a. This is evidence that the amino-functional groups can bond to the interior mesoporous surfaces by this preparation method. The N–H absorption bands overlap with O–H bands at 3,300–3,500 cm^{-1} [5, 9]. The strong Si–O–Si band at 1,054 cm^{-1} and three weak bands at 940, 794, and 444 cm^{-1}, belonged to the silica framework vibration bands, consistent with previous reports [9, 32]. There were a small observable line in C–H vibrations at 2,880 cm^{-1}, but no peaks in the C–O–C vibrations at 1,375 cm^{-1} and 1,456 cm^{-1}. The density of amino-functionalized groups on the pore surfaces of MCFs was controlled by the molar ratio of ATES to TEOS from 0 to 2.5 in the initial precursor of post-synthesis procedure. It can be seen from Fig. 4 that the symmetrical bending band at 1,505 cm^{-1} and 1,625 cm^{-1}

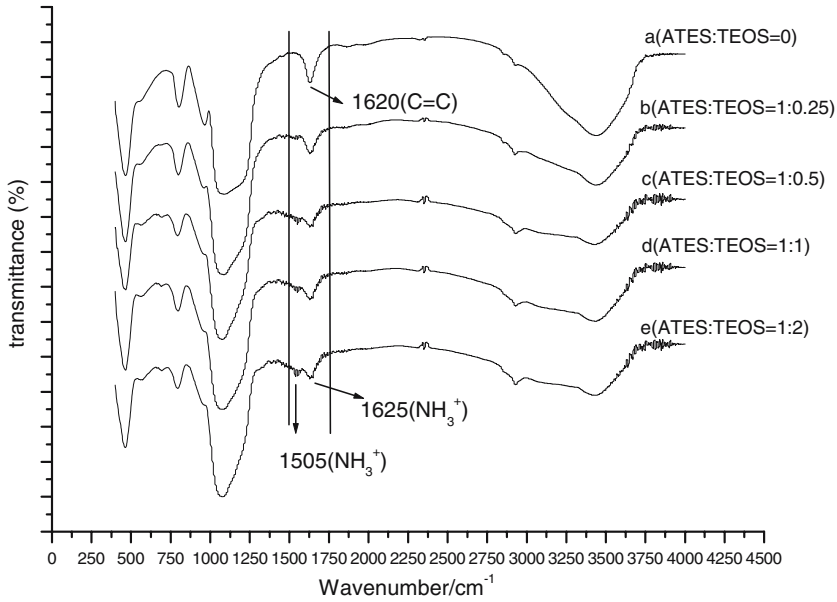


Fig. 4 FTIR spectra of the Amino-functionalized mesostructured cellular foam (AF-MCF) materials prepared by microemulsion templating with different molar ratio of TOES: ATES in the initial precursors. (a) 0, (b) 1:0.25, (c) 1:0.5. (d) 1:1 (e) 1:2

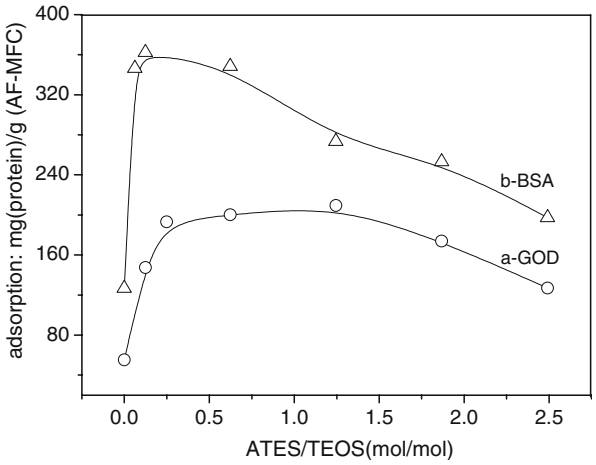


Fig. 5 The amount of protein adsorption onto AF-MCF materials as a function of the density of amino-functionalized groups on the pore surfaces of MCF materials. (a) GOx, (b) BSA

corresponding to the $-NH_3^+$ group increases with the molar ratio of ATES to TEOS in the initial precursor of post-synthesis procedure.

Protein adsorption

The adsorption of glucose oxidase (GOx) and bovine serum albumin (BSA) with molecular weights 33,000 D and 66,000 D onto AF-MCF materials was performed in order to evaluate the adsorption ability of prepared amino-functionalized mesostructured cellular foam (AF-MCF) materials. Figure 5 shows the amount of protein adsorption onto AF-MCF materials as a function of the density of amino-functionalized groups on the pore surfaces of MCFs. The plateau of adsorption was found in the adsorption of glucose oxidase. However, there was a maximum amount in the adsorption of bovine serum albumin (BSA). The adsorption amounts of two proteins on AF-MCF materials increase with the increase of the density of amino-functionalized groups on the pore surfaces of MCFs when the molar ratio of ATES to TEOS was below 0.5. These phenomena can be explained as the increase of the number of amino groups on surface leading to increase amount adsorbed at low molar ratio of ATES to TEOS. This is because the amino groups worked as adsorption site for proteins effectively. When the molar ratio of ATES to TEOS was above 0.5, two proteins showed different adsorption performances, the adsorption amount of bovine serum albumin (BSA) decreases with the increase of the molar ratio of ATES to TEOS after a maximum amount of adsorption (at 0.25), but the adsorption of glucose oxidase remained about equal amounts until 1.25, beyond which the adsorption amount decreases with the increase of the molar ratio of ATES to TEOS. The different adsorption behaviors of two biomolecules onto AF-MCF materials may be attributed to their molecule dimensions. The present of adsorption plateau can be assigned to limited accessibility of the amino groups in the interior of the walls, which depends on pore volume of AF-MCF materials. It is reasonable to assume that high amino concentration will result in a block of mesoporous channel by siloxane groups at post-synthesis procedure and the decrease of pore size has larger effect on bovine serum albumin (BSA) than glucose oxidase due to their different molecule size. Therefore, the decrease of S_{BET} and pore volume on adsorbent causes the decrease of amounts adsorbed for proteins at high amino groups loading. The optimum molar ratio of ATES to TEOS is 1:0.25. The maximum amount adsorbed for GOx and BSA in this study is 210 mg/g AF-MCF and 360 mg/g AF-MCF, respectively, which is higher than that of literature report [33]. These results demonstrate the advantage of AF-MCF materials prepared by this method and their potential for the biotechnological application as supports. Yiu et al. [33] reported that BSA with molecule weight 66,000 D had lower amount adsorbed than Cytochrome *c* with molecule weight 12,400 D onto the thiof-functionalized SBA-15 with a pore diameter of 56.3 Å, in which molecules of BSA can only partially enter the pores and be block at the openings of mesoporous channels. However, according to our experiment results, the protein with large molecule weight (such as BSA) have high amount of protein adsorption onto AF-MCF materials, indicating that the pore diameter of AF-MCF materials is large enough for proteins with large molecules weight to enter. After protein adsorption, the pore volume of AF-MCF materials decreased from 1.38 cm^3/g to 1.32 cm^3/g (BSA) and the decrease value was about the same volume of protein adsorption. These results provided evidence that the protein molecules can enter mesoporous channels but not on the external surface.

Conclusions

Large mesopores cellular foam (LMCFs) materials were synthesized using microemulsion templating in acidic solutions. The amino-functionalized LMCFs materials were prepared by post-synthesis method. The structural and chemical properties of these amino-functionalized porous materials were characterized. These resulting materials showed a high affinity for the

adsorption of protein molecules. It is found that the dimensions of the pores of the AF-MCF materials were large enough for adsorbed large biomolecules (such as BSA and GOx) into the mesoporous pores. These amino-functionalized MCF materials show the advantage as receptors for biomolecules in many interesting biotechnological applications where mass transport is often limited by small pore size.

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