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Monodispersed MCM-41 large particles by modified pseudomorphic transformation: Direct diamine functionalization and application in protein bioseparation

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ABSTRACT

Direct diamine functionalization of large spherical MCM-41 (uniform particle diameter of 15 μm) was achieved via the co-condensation of pre-shaped spherical silica gel and [3-(2-aminoethyl aminopropyl)] triethoxysilane (ATMS) using a modified pseudomorphic transformation approach. The functionalized MCM-41 spheres exhibited ordered hexagonal mesostructure, narrow pore size distribution, and high-loadings of diamine group (1.785 mmol/g). The morphology of parent silica gel was well preserved during the simultaneous pseudomorphic transformation and diamine functionalization. Various characterization techniques such as thermogravimetric (TG), ultra violet–visible–near infrared (UV–vis–NIR), and solid state ^{29}Si and ^{13}C MAS NMR, demonstrated that the diamine groups were successfully incorporated in the spherical MCM-41 silica framework. Benzaldehyde adsorption results proved that the distribution of diamine groups was concentrated on the pore wall surface of MCM-41, and they were active and accessible for further post-treatment. The surface density of diamine groups can be easily controlled by changing the ATMS content in the initial synthesis mixture, while the order of mesoporous MCM-41 structure was maintained in appropriate extent of ATMS/SiO_2 molar ratio. This preparation method provides a new synthesis protocol to modify large particle mesoporous materials with different functional groups. Furthermore, it was demonstrated that this diamine functionalized MCM-41 large particle can be used to separate native proteins efficiently.

1. Introduction

Significant efforts have been undertaken to incorporate various organic functionalities within an inorganic framework to achieve the combined properties of both organic and inorganic components [1,2]. Research on organically functionalized mesoporous silicas has attracted considerable attention [1–3]. Modifying the physiochemical properties of mesoporous silicas endowed them many promising applications in diverse fields, such as electric and optical-electronic devices, chemical and biochemical sensors, nanofluidic system, membranes, adsorption, and catalyst [1–5]. Generally, two pathways are available for synthesizing inorganic–organic hybrid mesoporous organosilica materials, (1) post grafting: the subsequent modification of the pore wall surface of a pure siliceous mesoporous material, (2) co-condensation: the simultaneous condensation of inorganic silica and organosilica precursors. However, the argument for post grafting approach is that if the organosilica precursors react preferentially with surface silanol groups at the pore opening, the diffusion of organosilica precursor molecules into the pore is hindered, leading to a non-homogeneous distribution of organic groups. The co-condensation approach offers a better control of resultant materials in terms of a high and uniform surface coverage of organic unit without blocking the mesopores [4,5].

MCM-41 was firstly used as packing materials in size-exclusion chromatography [6]. However, the results showed the materials were not suitable for column packing because of the ultra-fine particle size, irregular morphology and low mechanical strength [7]. Significant progress has been achieved in controlling the morphology and the pore size of mesoporous silica [7–11]. However, the particle size of synthesized mesoporous material is usually small with a typical range between 0.2 and 1 μm [12]. It is difficult to meet the requirement for stationary phases in HPLC applications, which desires a minimum sphere size of 5 μm to facilitate easy packing and reduce hydrodynamic energy cost [7,13]. To overcome these drawbacks, Martin et al. introduced the idea of pseudomorphism to transform pre-shaped amorphous silica sphere (5–130 μm) to MCM-41 and MCM-48 while retaining the spherical morphology [13,14]. Furthermore, the mesoporous silica products have been functionalized with organic groups by post grafting and employed as stationary phases in HPLC [15,16].

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In this study, the advantages of co-condensation and pseudomorphic transformation were combined. A large spherical MCM-41 with high-loading of diamine functional groups concentrated on the pore wall surface was prepared through a one-pot synthesis approach (see Supporting Information Scheme S1). This functionalized MCM-41, with well-defined diamine functionalized silica surface displaying evidence of isolated diamine sites, was also found useful in protein bioseparation. Batch selective adsorption and separation of two model proteins which differ in size and charge (i.e. lysozyme and bovine serum albumin) were investigated as a function of NH_2 density and mobile phase pH conditions. The separation efficiency achieved with functionalized MCM-41 particles was compared with their non-porous counterparts.

2. Experimental

2.1. Materials

The following materials, uniform pre-shaped silica (99.99% SiO_2, product number: S10008B, particle size: 15 μm), cetyltrimethylammonium bromide (CTAB, Sigma–Aldrich), 3-(2-aminoethyl aminopropyl) trimethoxysilane (ATMS, TCI), were used as received without further purification. Hen egg white lysozyme and bovine serum albumin (BSA) were purchased from Sigma–Aldrich. Ethylenediamine tetraacetic acid (EDTA), Trizmabase or (tris[hydroxymethyl]amino methane, urea, dithiothreitol (DTT) and sodium chloride (NaCl) were of reagent grade (99.95% purity), and purchased from Merck.

2.2. Synthesis

In the typical synthesis, 1.82 g CTAB and 0.5 g NaOH were dissolved in 18 g H_2O under stirring at room temperature. After adding pre-shaped silica, the resultant mixture was stirred for 30 min, then ATMS was added to the mixture and stirred for another 30 min. The molar composition of the mixture was $1SiO_2$:0.1CTAB:0.25NaOH: $20H_2O$:*x*ATMS, where *x* varied from 0 to 0.4. The resultant mixture was transferred into autoclaves with Teflon liners and aged at 120 °C under static condition for 6 h. The solid product was recovered by filtration and washed with deionized water, and dried at 80 °C overnight. The surfactant was removed by ethanol extraction.

2.3. Characterization

Powder X-ray diffraction patterns were recorded with a Bruker AXS D8 diffractometer (under ambient conditions) using filtered Cu Kα radiation. Diffraction data were collected from 0.5° to 8° (2θ) with a resolution of 0.02° (2θ). (see Supporting Information Fig. S1). Nitrogen adsorption/desorption isotherms were measured at 77 K with a static volumetric instrument Autosorb-6b (Quanta Chrome). The specific surface area was calculated by the Brunauer–Emmett–Teller (BET) equation [17]. The pore size distributions (PSD) were calculated from desorption isotherm branch using the Barrett–Joyner–Halenda (BJH) method [18,19].

Thermogravimetric (TGA) in combination with differential thermo gravimetric analyses (DTG) was carried out on a SDT Q600 thermogravimetric analyzer with heating rate of 10 °C/min under air flow. Nitrogen elemental analysis (EA) was performed on a Vario EL III CHNS elemental analyzer. The UV–vis–NIR diffuse reflectance spectra were collected on a Varian Cary 5000 UV–vis–NIR spectrophotometer at room temperature with $BaSO_4$ as background. The ^{29}Si NMR and ^{13}C NMR experiments were carried out at frequency of 400 MHz on a Bruker NMR spectrometer. All spectra were measured at room temperature and the magic-angle spinning frequencies were set at 5 kHz. Chemical shifts were externally referenced to tetramethylsilane (TMS).

The surface density of diamine groups in all samples was investigated by the condensation reactions of the diamine group with benzaldehyde (See Supporting Information Scheme S2). Sample (200 mg) was incubated in 6 ml anhydrous toluene with 2 mmol benzaldehyde under constant stirring at room temperature for 18 h. The catalyst was centrifuged and washed with toluene, and the supernatant was analyzed by gas chromatography with (2 mmol) dodecane as an internal standard.

2.4. Protein adsorption studies

Protein concentrations for separation studies were determined by reverse phase high performance liquid chromatography (RP-HPLC) using a C_5 column (5 μm particle size, 300 Å pore size, 150 × 4.6 mm, Phenomenex). The mobile phase comprises two solvents; 0.05% (v/v) aqueous trifluoroacetic acid (TFA) in H_2O (solvent A) and acetonitrile with 0.05% (v/v) TFA (solvent B). The gradient program employed was as follows: equilibration with 25% solvent B for 10 min, gradient increase from 25% solvent B to 65% solvent B for 50 min for elution, column stripping at 65% solvent B for 10 min, and re-equilibration at 25% solvent B for 20 min. The amount of protein was quantitatively determined by peak integration. The denatured protein mixtures were prepared by mixing 1 mg/ml denatured-reduced BSA with 1 mg/ml denatured-reduced lysozyme in 1 ml denaturant (8 M Urea, 50 mM Tris, 1 mM EDTA, 5 mM DTT). The denatured-reduced protein was prepared by dissolving lyophilized BSA and lysozyme in the denaturant, followed by 3 h incubation at room temperature. Three different pH conditions for protein denaturation were studied (i.e. pH 5, 8.5 and 11). Native protein mixtures were prepared by mixing 1 mg lyophilized BSA and 1 mg lysophilized lysozyme in 1 ml 50 mM Tris buffer (pH 8.5). 20 mg functionalized MCM-41 particles were dispersed into 2 ml denatured or native protein mix solution with continuous stirring at 200 rpm at 21 °C for 30 min. After adsorption, unbound protein was recovered by centrifugation at 10,000*g* for 5 min. Amount of protein adsorbed was determined by subtracting the amount of unadsorbed protein present in the supernatant after centrifugation from the initial protein mixture. To elute the adsorbed protein, the mesoporous particles were stirred at 200 rpm for 60 min (at 21 °C) in elution buffer (8 M Urea, 50 mM Tris, 5 mM DTT, 1 mM EDTA, 0.5 M NaCl, pH 8.5). The eluted protein in solution was recovered for HPLC analysis. Separation factor was calculated based on Eq. (1):

$$\text{Separation factor} = \frac{|\text{mass of lysozyme adsorbed (mg)- mass of BSA adsorbed (mg)}|}{\text{total protein load (mg)}} \times 100\%. \quad (1)$$

3. Results and discussion

Nitrogen physisorption isotherms (Fig. 1 left) are of type IV in accordance to the IUPAC classification, where they exhibit sharp step increases at $P/P_0 = 0.3–0.4$ due to the capillary condensation of nitrogen, implying the uniform-size pore structure. The sample exhibits sharper step increase when less ATMS is added. In addition, the lack of coincidence between adsorption and desorption of the isotherm occurs, showing a hysteresis loop with high ATMS content. This hysteresis can be explained by the presence of a secondary meso-porosity, where the bottle neck pores can be created by the stacked spherical particles. Accordingly, desorption of nitrogen occurs in these pores via cavitation [16]. Table 1 lists the cell parameter, BET specific surface area, pore size, pore volume, and