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Adsorption of biomolecules on mesostructured cellular foam silica: Effect of acid concentration and aging time in synthesis

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ABSTRACT

Mesostructured cellular foam (MCF) silica was synthesized using a non-ionic surfactant template-directed method without ammonium fluoride; the acid concentration and aging time were varied to determine the effects of these parameters on the final material. Increasing the acid concentration and aging time resulted in larger window size, which is critical in gating of biomolecule access to the interior of the MCF silica. In particular, when the acid concentration was changed from 1.6 to 3.5 M the window pore dimension approximately doubled, although the pore size distribution was broader. In this study, the optimal synthesis conditions to produce large, narrowly distributed window pores are 3.5 M HCl with an aging time of 20 h. The loadings of L-tryptophan (Trp), lysozyme (LYS) and bovine serum albumin (BSA) on the MCF samples were measured using batch adsorption. Adsorption data followed a Type I isotherm. The monolayer adsorption capacity of Trp on acid-washed MCF was several times higher than that of LYS and BSA, because of the smaller size of Trp. Protein adsorption onto MCF silica showed minimal size exclusion until the window size of the silica was barely larger than the largest protein dimension.

1. Introduction

The adsorption behavior and biocatalytic activity of various immobilized biomolecules, from amino acids to proteins, on several mesoporous silicas have been investigated [1], because these materials have superior physical properties and can accommodate biomolecules into their pore structures. Controlled pore size, high adsorption capacity, and minimal intraparticle mass transfer resistance are desirable for successful applications of these materials as immobilized enzyme supports and as chromatographic stationary phases [2–4]. Intraparticle transport can be facilitated through the use of a 3-dimensional mesoporous structure.

Mesostructured cellular foams (MCF) consist of a 3-dimensional pore structure of large cells interconnected by narrower windows [5]. MCF silicas show enhanced performance as a support for immobilized enzymes and as adsorbents as compared to other mesoporous silicas. For example, the larger pore size and higher pore volume of MCF silica permitted higher chloroperoxidase loading than was observed for MCM and SBA-15 silicas [6]. Similarly, a study comparing the performance of MCF silica with other mesoporous silicas, MCM-41 and SBA-15, for the immobilization of horseradish peroxidase showed that the use of MCF resulted in the highest enzyme capacity, enzyme activity, and immobilized enzyme stability [7]. In high-speed size-exclusion chromatography, MCF outperformed commercial silica in the separation of polystyrenes due to its relatively high porosity and narrow window size distribution [8].

The synthesis of MCF silica is closely related to the synthesis of SBA-15 mesoporous silica except that ammonium fluoride (NH_4F) is typically used as a mineralizing agent; the non-ionic surfactant Pluronic 123 forms the pores, while 1,3,5-trimethylbenzene (TMB) serves as a pore expander [5,9]. The TMB/surfactant ratio determines the template structure: below a ratio of 0.2–0.3 the hexagonal SBA-15 template is formed, but with additional TMB the surfactant solution undergoes a phase transition to form the mesocellular MCF template [10]. Due to the unique structure of MCF silica, the main focus in the synthesis process is to control the size of the windows, which are gates for macromolecule access into the large cells. The window pore size can be tuned by adding small amounts of ammonium fluoride, a mineralizing agent, and altering the hydrothermal treatment [11].

Previous studies have reported the effects of silica source, alcohol and aging process on the synthesis of mesoporous silica materials [12]. Interestingly, minor changes in acid concentration lead to significant changes in particle morphology, window size, and cavity size [8] without the use of ammonium fluoride. The acid concentration also has a significant effect on the hydrolysis rate of the silicon alkoxide that serves as a silica source. High acid concentration results in fast hydrolysis and rapid initiation of the condensation reaction that form the silica network [13]. Also, the acid concentration in synthesis can affect the extent of template

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Nomenclature

A	total area under the fitting of Gaussian function, cc g^{-1} adsorbent Å	n_m	monolayer capacity in Type I isotherm, μmol g^{-1} adsorbent
C	concentration of adsorbate in solution, mM	r^2	Correlation coefficient, dimensionless
C_0	initial concentration of adsorbate in solution, mM	V_P	Adsorbed nitrogen volume, cc g^{-1} adsorbent
d	pore size, Å		
d_c	mean pore size, Å	*Greek letter*	
K	equilibrium constant in Type I isotherm, mM^{-1}	σ	Standard deviation from mean pore size, Å
n	amount adsorbed, μmol g^{-1} adsorbent		

self-assembly, which impacts the pore structure [14]. The aging time also affects mesopore structure [15] and micropore area [16]. However, the effects of simultaneous variation of the acid concentration and aging time on the MCF silica window size have not been investigated.

The study reported here is an investigation of the influences of acid concentration and aging time on the physical properties of MCF silica materials. In addition to physical characterization, the MCF silica materials were probed with three biomolecules selected to provide a broad molecular weight range with easy detection using a UV spectrophotometer. The batch adsorption of L-tryptophan, lysozyme and BSA was studied to understand the effects of physicochemical properties of the synthesized MCF silicas on adsorption of these representative biomolecules.

2. Experimental

2.1. Materials

Pluronic P123 surfactant ($PEO_{20}PPO_{70}PEO_{20}$) was obtained from BASF (Mt. Olive, NJ). 1,3,5-Trimethylbenzene (TMB) was obtained from Alfa Aesar (Ward Hill, MA). Tetraethyl orthosilicate (TEOS, 98%), lysozyme from chicken egg white (LYS, 90%), bovine serum albumin (BSA, 98%) and sodium acetate trihydrate (99%) were obtained from Sigma–Aldrich (St. Louis, MO). L-Tryptophan (Trp) was obtained from Fluka (99%). Hydrochloric acid and acetic acid were obtained from Pharmco-Aaper (Brookfield, CT). Sodium azide (Biotech research grade) was obtained from Fisher Scientific (Fair Lawn, NJ).

2.2. Synthesis of MCF silica

The synthesis of MCF silica followed the previously reported procedure [5] except that the acid concentration, aging temperature, and aging time were varied. In a typical synthesis, 2 g of Pluronic P123 surfactant were dissolved in 75 mL of aqueous HCl; acid concentrations of 1.6, 3.5 and 5.4 M HCl were used. Next, 2 g of TMB were added and the system was held at 35 °C with constant stirring, after which 4.4 g of TEOS were added. The hydrolysis and condensation reactions were carried out for 20 h at 35 °C with constant stirring. The resulting liquid was aged at 100–110 °C for 20–72 h without stirring. The solid MCF was recovered by filtration, washed with deionized water (resistivity of 17.5 MΩ cm) and dried at room temperature. The dried material was then calcined under air at 500 °C for 7 h using a 1 °C min^{-1} heating rate and a 5 °C min^{-1} cooling rate. The synthesized MCF silica was acid-washed using 10% HCl solution for 3 h at 100–110 °C to remove the carbon residues that might be present after calcination, and to maximize the surface silanol concentration. Acid washing increases the surface silanol group density [17,18]; acid washed amorphous silicas typically have 4.9 silanol groups per nm^{-2}.

2.3. Characterization of MCF silica

Nitrogen adsorption–desorption measurements were performed at 77 K using a Micromeritics Tristar 3000 (Norcross, GA) to determine pore size, total pore volume and BET surface area of the functionalized MCF silica materials. All samples were degassed for 2 h at 120 °C before the measurement. The cell size and window size of MCF silica were determined using the BdB (Broekhoff–de Boer)–FHH (Frenkel–Halsey–Hill) method [5,19] from nitrogen adsorption and desorption branches, respectively. A normal probability distribution function [20] was applied to correlate the experimental data for each nitrogen adsorption and desorption branch using the OriginPro 8 software package (OriginLab Corp., Northampton, MA, USA) to investigate the pore size distributions corresponding to different synthetic conditions. In this work, the probability distribution was expressed as:

$$V_p = \frac{A}{\sigma\sqrt{2\pi}} e^{\frac{(d-d_c)^2}{2\sigma^2}} \quad (1)$$

The BET surface area was calculated from the nitrogen adsorption data over the relative pressure range of $P/P^0 = 0.01–0.30$. The total pore volume was determined by the amount of nitrogen adsorbed at $P/P^0 = 0.99$.

The long-range order of the MCF silica was verified with Ultra-Small-Angle X-ray Scattering (USAXS) [21]. Transmission electron microscopy (TEM) was performed using a 1230 Jeol Field Emission microscope at 120 kV. For the TEM imaging, powdered MCF silica was well dispersed in isopropyl alcohol under ultra-sonication and then dried on a copper grid under vacuum.

2.4. Adsorption of biomolecules on MCF silica

L-Tryptophan, lysozyme from chicken egg white and bovine serum albumin, which represent a broad range of molecular weight, were used as adsorbates; the adsorbate concentrations ranged from 2 to 10 mg mL^{-1}. The biomolecules were dissolved in 0.1 M sodium acetate buffer at pH 4 or pH 5.2 with 0.05% sodium azide. In each adsorption measurement, 10 mg of MCF silica was mixed with 1.5 mL of a buffered solution (0.1 M sodium acetate buffer with 0.05% sodium azide) of the adsorbate in a protein low binding centrifuge tube (Eppendorf North America, Hauppauge, NY). The mixture was incubated at room temperature (25 °C) with 150 rpm shaking, after which the tubes were centrifuged for 5 min at 10,000 rpm at room temperature. Initial experiments used incubation times of 20–140 h; it was determined that 20 h was sufficient to achieve equilibrium. The initial and final protein concentrations were determined using a UV spectrophotometer (Spectronic 1001, Milton Roy) at 280 nm; the amount of protein adsorbed was determined by difference. LYS (pI = 10.8) and L-Trp (pI = 5.8) adsorption isotherms were obtained at pH 5.2, the pH at which LYS activity is maximized. Adsorption of BSA (pI = 4.7) was measured at pH 4 and pH 5.2. The selected