



Preparation, characterization and lysozyme immobilization studies on siliceous mesocellular foams: Effect of precursor chemistry on pore size, wall thickness and interpore spacing



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ABSTRACT

Three homologous series of siliceous meso-cellular foams (MCFs) were synthesized using microemulsion templating by incremental variation of ratio of organic pore expander, trimethyl benzene (TMB), and silica precursor (TEOS) concentrations using Pluronic™ block co-polymer (P123) as the surfactant. The calcined materials were tested for lysozyme immobilization to relate the adsorption capacities to meso-structure. Small angle X-ray scattering (SAXS) shows a short range ordering of pores. The pore size and wall thickness were determined from Porod analysis of the SAXS data combined with gas adsorption data. The pore size and lysozyme loading both increase systematically with TMB/P123 ratio, and decrease with increasing TEOS/P123 ratio. By comparing gas adsorption and SAXS data, we show that the BET method overestimates the mesopore specific surface area and therefore leads to an underestimate of the pore size. The effective pore size determined by the BdB-FHH method on the other hand is consistent with that determined by SAXS surface area analysis. The wall thickness (4.4 ± 0.5 nm), interpore distance (23.9 ± 1.5 nm) and meso-pore volume fraction (0.82 ± 0.04) are independent of synthesis conditions. The mean pore size (33 ± 8 nm, spherical equivalent) is generally larger than the interpore distance, which forces the system to adopt a more cylindrical pore morphology at large pore and window sizes, which is confirmed by SAXS and TEM. TEM shows worm-like pores intermixed along with the familiar cellular foam phase.

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1. Introduction

Siliceous meso-cellular foams (MCFs) were discovered by Stucky and coworkers in 1999 [1]. Their unique three-dimensional (3D) meso-structure with ultra-large, tunable cavities (24–42 nm) and hydrothermally robust framework are favorable for a range of applications in catalysis, separations and bio-sensors [1–5]. By harnessing the flexibility of the synthesis and the high density of silanol groups to engineer surface functionality, one can custom design MCFs to suit the needs of specific applications.

Due to their narrowly distributed pore sizes, large pore volumes (up to $3 \text{ cm}^3 \text{ g}^{-1}$) and tailorable surface chemistry MCFs are potential sorbents for protein immobilization [2,6]. Although proteins and monoclonal antibodies (Mabs) are of great importance in the biopharmaceutical industry, commercial production of these

compounds as therapeutic drugs requires down-stream purification. In the medical and food industries, it is essential to remove adsorbed proteins since even a small amount of deposited protein can give rise to the subsequent adsorption of fibrous proteins, leading to adverse biological consequences [7–9].

MCFs should offer higher adsorption capacities than the mesoporous SBA-15 due to the larger pore volumes and pore sizes of MCFs. Katiyar et al. demonstrated the effect of pH and pore size of SBA-15 on lysozyme adsorption [10]. The highest adsorption capacity occurs at the isoelectric point, ($\text{pI} = 11$ in the case of lysozyme [11]) where the electrostatic repulsion between the adsorbed molecules is at a minimum.

Much research has focused on understanding the formation mechanism, meso-structure and resulting hydrothermal stability of MCFs [12,13]. Literature reports document the effect of type and amount of pore expander on the pore sizes [14,15]. Here we use tetraethylorthosilicate (TEOS) as silica source, tri-methyl-benzene (TMB) as pore expander, and Pluronic™ block co-polymer (P123)

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as surfactant. Schmidt-Winkel et al. found a linear relationship between the diameter of the spherical cavity and the cube root of the amount of TMB, consistent with the proposed micro-emulsion templating mechanism [1]. Although there has been speculation about the effect of the amount of silica precursor (TEOS) on the structure of meso-cellular foam, the relationship of the TEOS/P123 mass ratio to the textural properties of the resulting MCFs has not been established.

In this study, we investigate the effect of TEOS/P123 ratio on the meso-cellular foam structure and the corresponding lysozyme immobilization characteristics by varying the TMB/P123 and TEOS/P123 ratios in the micro-emulsion. Three series of siliceous MCFs with diverse meso-structures were synthesized by systematic variation of the TMB and TEOS concentrations in the micro-emulsion templates. Characterization of these MCFs provides insight on the tailorability of these meso-structures by manipulating the synthesis parameters. By variation of the TMB/P123 (R_1) and TEOS/P123 (R_2) mass ratios, worm-like or ultra-large polyhedral nano foam-like structures were observed to exist along with regular spherical foam texture. Lysozyme adsorption on these MCFs was correlated with the trend in MCF pore characteristics. Isoelectric point was chosen in order to minimize the intermolecular repulsion between the proteins.

2. Experimental

2.1. Synthesis of siliceous meso-cellular foam (MCF)

Three families of MCF samples were synthesized following a modified version of the procedure reported in literature [14]. The mass ratio of the silica precursor to surfactant (TEOS/P123= R_2) differentiates the three series; while the ratio of the mass ratio of organic pore expander to the surfactant (TMB/P123= R_1) was varied to generate members within the series. All the chemicals are used without further purification.

Three series of MCFs are designated as MCF@ R_1 - R_2 2.2, MCF@ R_1 - R_2 3.0 and MCF@ R_1 - R_2 4.4 where R_1 was varied within the range 0.5–2.5 with the interval 0.5 to create five members within each series. In the MCF@ R_1 1.0- R_2 2.2, for example, 4.0 g of Pluronic P123 (Mw = 5800, PEO₂₀PP0₇₀-PEO₂₀) from Sigma Aldrich was dissolved in 130 mL of 1.6 M HCl at room temperature. 4.0 g TMB (Sigma Aldrich) was added under vigorous stirring as the solution temperature was raised to 40 °C. After 2 h, 8.8 g of TEOS (98%, Sigma Aldrich) was added and the resulting solution was maintained at 40 °C under continuous stirring. The TEOS condensation was carried out at 40 ± 2 °C for 20 h under constant stirring. During this stage TEOS hydrolyzes at the surface of the P123-coated TMB droplets and polymerizes to give a composite material with interconnected cells and windows at the regions where the adjacent droplets come into contact. Agitation fractionates the emulsion droplets, leading to better distribution of silica onto the composite [13]. Because of the non-equilibrium nature of the agitated emulsion, however, there is considerable scatter in the observed properties.

After 24 h, 0.046 g of NH₄F (Aldrich, ≥99.99% trace metals basis) was added, the temperature was raised to 120 °C and the solution mixture was aged for another 24 h. Addition of the inorganic mineralizing agent, NH₄F, enlarges the windows between the cells [1].

The as-prepared sample was filtered and washed with water and ethanol repeatedly. The sample was then calcined at 550 °C under 30 mL min⁻¹ of airflow. The samples were ramped at 1 °C min⁻¹, held for 5 h at 550 °C and then cooled. We find no evidence of secondary microporosity arising during the calcination step due to the removal of the polyethylene oxide groups of the P123 template embedded in the inorganic silica framework [16].

2.2. Lysozyme adsorption studies

Immobilization studies were carried out to relate the pore characteristics to lysozyme adsorption. 0.1 M dibasic sodium phosphate buffer was used to maintain a pH of 11 throughout each adsorption experiment. The buffer solution contained 0.05% sodium azide to prevent microbial growth. The starting solutions contained 6 mg mL⁻¹ lysozyme (derived from chicken egg white, 90%, Sigma Aldrich). 1.0 mL of this solution was added to 10 mg of MCFs. The suspended samples, contained in protein, low-binding centrifuge tubes (Eppendorf North America, Hauppauge, NY, USA), were immobilized for 72 h in a mechanical shaker set at 210 rpm and 25 °C. Centrifugation at 10,000 rpm for 10 min was followed by decantation of the supernatant solution. A UV-Vis spectrophotometer (Cary, 50, Varian, Palo Alto, CA, USA) set at 280 nm was used to determine the initial and final concentrations of lysozyme. The amount of protein adsorbed was calculated by a mass balance in conjunction with the Beer-Lambert law. MCFs contacted with lysozyme-free buffer solutions were used as controls. Four replicates of each sample are used to determine the reproducibility (5%) of the adsorption data.

2.3. Textural characterization

2.3.1. BET surface area and pore size distribution measurement

All samples were degassed under vacuum at 180 °C for a minimum of 8 h before analysis. The BET (Brunauer-Emmett-Teller) surface area and pore size distribution (PSD) analyses were conducted by N₂ physisorption at -196 °C using a Micromeritics ASAP 2010 apparatus. Specific surface area (S) was calculated using BET method from N₂ adsorption isotherm recorded in a relative partial pressure (p/p_0) range of 0.05–0.25 [17]. In cases where there was sufficient data in the high relative pressure range (usually only on the desorption branch), the mesoporosity and external surface area were determined by t -plot method. Adsorption isotherm data for the LiChrospher Si-1000 silica reported by Jaroniec et al. was used in the t -plot analysis [18]. The total pore volume was calculated using a single-point adsorption value at the relative pressure of about 0.995. The pore size distributions were calculated using the simplified BdB-FHH method [19].

2.3.2. TEM

Selected samples were characterized with a Philips CM 20 electron microscope. The samples were sonically dispersed in ethyl alcohol and transferred into holey carbon-Cu grid. After the complete evaporation of the ethyl alcohol the particles attached on the walls of holes in the carbon film were examined. The applied accelerating voltage was 200 keV, with a LaB₆ emission current and a point-to-point resolution of 0.27 nm.

2.3.3. SEM

SEM of the samples was performed using a Hitachi S-4000 scanning electron microscope operating at 15 kV. The samples were mounted on a 13-mm aluminum stubs taped with a double-sided adhesive carbon. The samples were sputter-coated with gold/palladium alloy for 120 s to make them conductive.

2.3.4. Ultra-small angle and small angle X-ray scattering (USAXS and SAXS)

USAXS ($\sim 10^{-4} \leq q \leq 0.3 \text{ \AA}^{-1}$) and SAXS ($\sim 10^{-2} \leq q \leq 0.8 \text{ \AA}^{-1}$) were performed at the 15 1D-B and 12 ID-C beam lines, respectively at the Argonne National Laboratories (Argonne, Illinois, USA). In the USAXS measurements, a beam of 12-keV (1.0332 Å) x-radiation strikes a sample and is elastically scattered. A detector records the scattered flux while scattering angle θ is varied. USAXS data were subject to an air background subtraction followed by slit

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