Journal of Colloid and Interface Science 331 (2009) 40–46

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Biomolecular adsorption behavior on spherical carbon aerogels with various mesopore sizes

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ARTICLE INFO

Article history:
Received 5 August 2008
Accepted 14 November 2008
Available online 20 November 2008

Keywords:
Spherical carbon aerogel
Sol–gel
Mesopore
Biomolecule
Adsorption
Adsorption rate
Adsorption isotherm

ABSTRACT

Spherical carbon aerogels (SCAs) with controlled particle size and mesopore size were synthesized by an emulsified sol–gel polymerization of phenol, melamine and formaldehyde. The adsorption rate and capacity of biomolecules with different molecular dimensions, including L-phenylalanine (Phe), vitamin B_{12} (VB), α-chymotrypsin (Chy) and bovine serum albumin (BSA) onto SCAs were investigated. The mesopore size can be easily tuned in the range from 5 to 10 nm by simply adjusting catalyst concentration in the initial solution and the spherical particle size can be controlled in 50–500 μm by changing stirring speed. The as-prepared SCAs have high specific surface area (>600 m^2/g) and large pore volume (>1 cm^3/g). The hardness of SCAs is ca. 10 times as large as that of commercial spherical activated carbon particles. The adsorption rate of VB is strongly depended on the mesopore size and particle size, and show an increasing tread with the increase of mesopore size and the decrease of particle size. For small molecule Phe, the specific surface area is key factor to determine the adsorption capacity, but the adsorption capacity of large molecules (VB, Chy and BSA) is dependent on the pore size of SCAs, which should be suitably larger than the molecule size of biomolecules.

1. Introduction

The adsorption of biologically relevant molecules from solution onto solid surfaces has attracted much attention due to its scientific importance and potential applications in many frontiers of modern materials science including biocatalysis, biosensing, drug release and separation of biomolecules [1–7]. Activated carbons, known for over 3000 years, still remain the most powerful adsorbents as host for biomolecules, mainly due to their superior physical and chemical properties, such as highly developed porous structure, biocompatibility and chemical stability as well as their low cost and accessibility [8–10]. From the viewpoint of adsorption and separation, the adsorption of a given biomolecule should be related to the pore size distribution of the host and the size of the biomolecule [11,12]. The commonly used activated carbon is microporous, which shows a low efficiency for giant biomolecules and slow mass transport because of space confinement imposed by small pore sizes. For adsorption of enzymes and proteins, which possess a relative high molecular weight and large molecular diameter, mesoporous carbon with the diameter in the range from 2 to 50 nm, may be a potential candidate to accommodate these biomolecules. However, there is only limited research on the adsorption of large biomolecules by mesoporous carbons.

Significant advances have been achieved in synthesizing mesoporous carbons in the past decade [13]. Generally, there are three main methods to introduce mesopore, including a high degree of catalytic activation or combined physical/chemical activation methods [14,15], carbonization of polymer gel issued from sol–gel processing [16,17] and replica synthesis with templates [18–20]. The pore structure, such as channel connectivity and pore size, of the mesoporous carbons is one of the most important physical parameters of these materials for practical applications, and must be designed according to their uses. Activation method produces mesoporous carbon with broad pore size distribution and considerable microporousity, which makes it less attractive. The mesoporous structure of carbons produced by template methods, using silica or metal oxide nanoparticles, anodic alumina membranes and mesoporous silica materials as molds are predetermined by the structure of templates. This method appears to be effective to obtained well-defined porosity structure. However, the furry and complicated steps are required to prepare and remove the templates. Apart from the cost issues, the resulting carbons generally demonstrate poor mechanical integrity, which limit their industrial applications. Mesoporous carbon aerogel produced by sol–gel process is a good candidate as the host for biomolecules because of its continuous porosity, high surface area and large mesoporous volume. It can be produced in different forms, such as monoliths,

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0021-9797/$ – see front matter
doi:10.1016/j.jcis.2008.11.026

spherical particles and films [21,22]. Moreover, the microstructure can be tailored by controlling the sol–gel reaction parameters [23–25] so that the selective adsorption and separation of biomolecules with specific molecular size are feasible.

For industrial applications, particle size and morphology of the host are also important. Small particles (<100 nm) would offer a large external surface area, but they cannot be used in most biomedical applications due to difficulties of flittering them from the biofluids. It has been recognized that a spherical particle is very beneficial to handle in closed packed or slurry bed, due to flow resistance, low fluid resistance and low particle release characters [26]. In addition, an absorbent with spherical shape is more resistant to abrasion and with high mechanical strength, which is significantly important in medical application.

In this paper, spherical carbon aerogels (SCAs) were prepared by combining sol–gel technology with water-in-oil emulsions. The particle size of spheres can be determined by emulsion conditions, whereas the microstructure is entirely controlled by sol–gel solution chemistry. The adsorption properties of SCAs were evaluated with four typical biomolecules with different molecular sizes, L-phenylalanine (Phe), vitamin B_{12} (VB), α-chymotrypsin (Chy) and bovine serum albumin (BSA). The effect of pore size on adsorption capacity was investigated. Commercially spherical activated carbons (SAC) with particle size about 150 μm from Shanghai Carbosino Materials Co. were used as microporous carbon hosts in comparative investigations.

2. Materials and methods

2.1. Preparation of SCAs

The phenol–melamine–formaldehyde (PMF) sol was synthesized by addition and condensation of phenol, melamine and formaldehyde in NaOH solution, according to a previous paper given elsewhere [27]. After pre-polymerization at 85 °C for 3 h, PMF sol of 20 ml was poured into a glass reactor containing a mixture of 500 ml paraffin oil and 5 ml sorbitan monooleate (Span 80). Then the mixture was stirred with a speed of 200 rpm at 85 °C for 8 h and aged at 85 °C for 2 days. The spherical PMF hydrogels were separated from the solvent by filtration and washed with acetone for at least three times. Then, the spheres were transferred into an autoclave to carry out supercritical petroleum ether (boiling range 30–60 °C) drying at 240 °C and 7.0 MPa. The resulting spherical organic aerogels were carbonized at 800 °C for 3 h with a heating rate of 5 °C/min under nitrogen to obtain SCAs. In this work, the mole ratio of phenol:melamine:formaldehyde:water is fixed at 1:0.4:2.8:20, while the mole ratios of NaOH/phenol were changed from 0.2 to 0.05 to obtain SCAs with different mesopore sizes. The synthesized samples were designated as SCA-1, SCA-2 and SCA-3 according to the mole ratio of NaOH/phenol = 0.2, 0.1 and 0.05, respectively. In addition, SCA-4, SCA-5 were prepared at different stirring speeds of 100, 400 rpm, respectively, with a mole ratio of NaOH/phenol at 0.1 under otherwise identical conditions mentioned above.

2.2. Characterization

Hardness of the SCAs was obtained under KQ-3 particle compressive strength instrument by applying 100 N mass. The morphologies of SCAs were characterized by scanning electron microscopy (SEM, JEM S-250). The particle size distributions were measured with a dynamic light scattering analyzer (DLS, Malvern Mastersizer 2000). The microstructures were observed under transmission electron microscopy (TEM, JEOL 2100) operated at 200 kV. Nitrogen adsorption/desorption isotherms were measured at 77 K with a Micromeritcs ASAP2020 analyzer. Before the measurements, the samples were degassed in vacuum at 473 K for at least 12 h. The Brunauer–Emmett–Teller (BET) method was utilized to calculate the specific surface areas. The total pore volume was estimated from the adsorbed amount at a relative pressure of $P/P_0 = 0.985$. The micropore volume was calculated by the t-plot method. The mesopore volume and pore size distributions were derived from desorption branch by using the Barrett–Joyner–Halenda (BJH) model.

2.3. Adsorption measurements

L-phenylalanine (Phe) and vitamin B_{12} (VB) were purchased from Sinopharm Chemical Reagent Co. and α-chymotrypsin (Chy) and bovine serum albumin (BSA) were purchased from Merck Company. The molecular dimensions and molecular weights for Phe, VB, Chy and BSA are about $0.9 \times 0.5 \times 0.5$, $1.4 \times 1.8 \times 1.1$, $2.9 \times 2 \times 4.0$ and $5.0 \times 7.0 \times 7.0$ nm^3 and 165, 1355, 25,000 and 66,000, respectively. The isoelectric points (pI) of Chy and BSA are 8.3 and 4.8, respectively.

Ultrapure water (18 MΩ station Millipore Milli-Q Plus) were used for all adsorption experiments. To determine the adsorption rate, test samples (300 mg) were suspended in 1000 ml of 148 μmol/L VB12 aqueous solution at the initial pH value 7.2 (natural solution pH value of VB). The mixture was continuously shaken in a shaking bath at 150 shake/min and at 25 °C. Three milliliter samples were taken from the reactor at pre-determined time intervals, including equilibrium time.

For the Phe and VB adsorption isotherm determination, 100 mg of different samples was suspended in 100 ml of solutions with concentrations ranging from 150 to 2400 μmol/L for Phe and from 18 to 443 μmol/L for VB. The resulting mixture was continuously shaken in a shaking bath at 25 °C until equilibrium was reached (typically 24 h). The pH values were measured in the range from 5.1 to 5.4 for Phe and from 7.2 to 7.6 for VB with different concentrations. For Chy and BSA, the adsorption isotherms were measured at 25 °C for 24 h by using the 0.2 M phosphate buffer (pH 7.02). In addition, batch pH studies were conducted by shaking 100 ml of each Chy or BSA solution with 0.1 g of the carbons for 24 h at a range of pH values from 2.35 to 10. The pH value of the solutions was changed by using different buffer solution (potassium hydrogen phthalate/HCl, pH 2.35; sodium acetate/acetic acid, pH 5.05; phosphate buffer, pH 7.02; boric acid/sodium tetraborate buffer solution, pH 8.5 and sodium tetraborate/sodium hydroxide buffer, pH 10.0). The amounts of adsorbed biomolecules were calculated as the difference between the amounts of biomolecules in the initial solution and the amount remaining in solution at equilibrium by UV–vis spectrometer (UV-3150). Calibration experiments were done separately before each set of measurements. The wavelengths of spectrometer for Phe, VB, Chy and BSA are 257, 361, 280 and 280 nm, respectively.

Langmuir equations were used to describe experimental adsorption isotherm data [28]. The Langmuir equation can be written as $q_e = q_0 k C_e / 1 + k C_e$, where k is the Langmuir constant, q_0 is the monolayer capacity (μmol/g), q_e is the equilibrium adsorption capacity (μmol/g) and C_e is the equilibrium concentration (μmol/L).

3. Results and discussion

3.1. Characterization of SCAs

SCAs were synthesized by sol–gel process, combined with inverse emulsion polymerization. The sol solution was dispersed into a continuous hot oil phase as primary emulsion droplets by the combined action with steric stabilizer and agitation. The dispersed droplets may be considered as micro-reactors, with the sol–gel