Adsorption and Release Properties of Proteins on Spherical SBA-15 Nanoparticles Functionalized with Aminosilanes

Pham Thi Tra, Jae Wook Lee, Sun Il Kim*
Department of Chemical and Biochemical Engineering,
(sibkim@chosun.ac.kr*)

INTRODUCTION

SBA-15 material is one of mesoporous materials, which has been attracting researcher's attention due to its prominent property. The most common morphology of SBA-15 are fiber-like several tens of micrometers in length, composed of "basic" rod-like particles, or individual well-defined rod-like particles [1]. Other morphologies with doughnut, rope, egg, sausage, shere, gyroid, discoid, and hyperbranched shapes of SBA-15 materials have been obtained, using organic reagents, strong electrolytes, or metal ions as additives. Among various morphologies, spheres are required as column packing materials and easy-to-handle forms in certain applications such as chromatographic separations and controlled drug delivery [2]. In some previous studies, multi-amine-functionalized mesoporous silicas have been prepared and used to adsorb heavy metal ions and carbon dioxide. There are two ways, including post-synthesis (grafting) and direct synthesis (co-condensation) methods to incorporate functional group into the mesoporous matrix. In this study, mesoporous silica spheres have been obtained via a two-step synthesis process by using a triblock copolymer as template in combination with a co-surfactant and co-solvent. Amine-grafted mesoporous silicas SBA-15 were prepared by attaching 3-aminopropyl triethoxysilane, N-2(-aminoethyl)-3-aminopropyltrimethoxysilane and (3-trimethoxy silylpropyl)diethylenetriamine via post-synthesis method. The protein adsorption properties such as equilibrium and kinetics were investigated. The release study also was carried out to assess application for drug delivery system. These proteins were Bovine Serum Albumin (BSA), Lysozyme (LYS) and Myoglobin (MYO).

EXPERIMENTAL

SBA-15 were synthesized by using tetraethyl orthosilicate (TEOS) as the silica source, Pluronic P123 triblock copolymer ($PEO_{20}PPO_{70}PEO_{20}$, Aldrich) as a structure-directing agent, cetyltrimethylammonium bromide (CTAB) as the co-surfactant, and ethanol as the cosolvent. In a typical synthesis, 3 g of P123 was dissolved in 60 mL HCl (1.5 M). The desired amount of CTAB (0.6 g) and TMB were first mixed with 25 mL deionized (DI) water separately, and then the two solutions were mixed thoroughly as 20 mL of ethanol (100%) was added. 10 mL of TEOS was added drop by drop to the surfactant solution, and the mixture was vigorously stirred (~500 rpm) for 45 min at 35 °C. After stirring, the mixture was transferred to a stoppered PTFE bottle and stored under static condition at 75 °C and then aged at 125 °C. The white precipitate was recovered by filtration, dried at 90 °C for 24 h, and calcined in air at from room temperature to 550 °C, with a heating rate of 1 °C/min. The temperature was held constant at 550 °C for 6 h, the cooling rate was 5 °C/min. Modified mesoporous materials with aminopropyl groups have alternatively been prepared by refluxing freshly activated mesoporous silica in toluene solution containing aminosilane. 5.0 g of calcined SBA-15, which was previously dried

at 398 K for 6 hours in air, was refluxed in the toluene solution of aminosilane (1,7%, 250 mL) at 383 K for 24 hours under an Ar flow. The amine-functionalized SBA-15 was collected by filtration, washed with dry toluene, and dried at 333 K overnight. These materials are designated as APTES-SBA-15, AEAPS-SBA-15 and TA-SBA-15, where APTES, AEAPS and TA are 3-aminopropyltriethoxysilane, *N*-2(-aminoethyl)-3-aminopropyltrimethoxysilane, and (3-trimethoxysilylpropyl) diethylenetriamine, respectively. These SBA-15 samples were characterized by XRD, nitrogen adsorption and desorption analysis, SEM, TEM and FT-IR.

Batch adsorption experiments were carried out by contacting 50 mg of SBA-15 with 10 mL of different protein concentrations in buffer solution at 298K. The adsorbent and solution were sealed and kept in a shaker at 250 rpm for 24 h. The supernatant was diluted in a buffer and then filtered through a 0.2 μm HT Tuffryn low protein binding membrane filters. The protein concentration in the supernatant was analyzed on a UV spectrophotometer with wavelength of 280 nm. A mass balance was applied to calculate the protein adsorbed on the SBA-15. The kinetic experiments were performed in a batch at 298K. The concentrations of protein at different time intervals were analyzed using UV-spectrophotometer. For release experiments, 50 mg of treated SBA-15 was soaked in 10 mL of buffer solutions under stirring with a speed of 250 rpm at 310K for 3 days. The cumulative release amount was determined by a mass balance equation similar to the previous section. For all experiments concerning adsorption, kinetic and release, pH values of buffer solutions were 4.8 for BSA, 7.0 for LYS and MYO.

RESULS AND DISCUSSION

The synthesized spherical SBA-15 samples S1, S2 and S3 were characterized by XRD. Fig. 1(a) shows the XRD patterns of these samples, which shows that spherical SBA-15 exhibited single diffraction peaks, characteristic of mesoporous materials with a pore structure lacking long-range order. CTAB was used as a co-surfactant while ethanol was used as co-solvent to synthesize spherical SBA-15 particles. SEM image shows the morphology of SBA spheres Fig. 1(b). A typical high-resolution TEM image of the spherical SBA-15 is presented in Fig. 1(c) which shows irregularly aligned mesopores with relative uniform pore sizes.

Nitrogen adsorption-desorption isotherms of all samples give a typical irreversible type IV isotherm with a H_1 hysteresis loop as defined by IUPAC. The nitrogen adsorption at low relative pressures (P/P_o<0.1) is accounted for by monolayer adsorption of nitrogen on the pore walls, and does not necessarily imply the presence of micropores. The sharp inflection in the P/P_o range from 0.6 to 0.9 of the isotherm is characteristic of capillary condensation within uniform mesopores, the position of which is clearly related to a diameter in the mesopore range. The pore size distribution of samples was determined by the BJH method. Among three samples, S2 has the largest pore size and the most clear spherical morphology, thus it had been chosen for incorporating amine groups onto the surface. The nitrogen adsorption-desorption isotherms and the corresponding BJH pore size distribution curves of the original SBA-15 sphere and functionalized samples are shown in Fig. 2(a) and (b). The uniform pore size distribution was retained for aminosilane-modified SBA-15. The mean pore diameter of amine functionalized SBA-15 were lower than those of the SBA-15 support (S2), indicating that aminosilanes were anchored on pore

walls of SBA-15. The mean pore diameter of APTES-, AEAPS- and TA-modified SBA-15 decreased in the following order: APTES > AEAPS > TA. This order was in accordance with the order molecular sizes of respective aminosilanes.

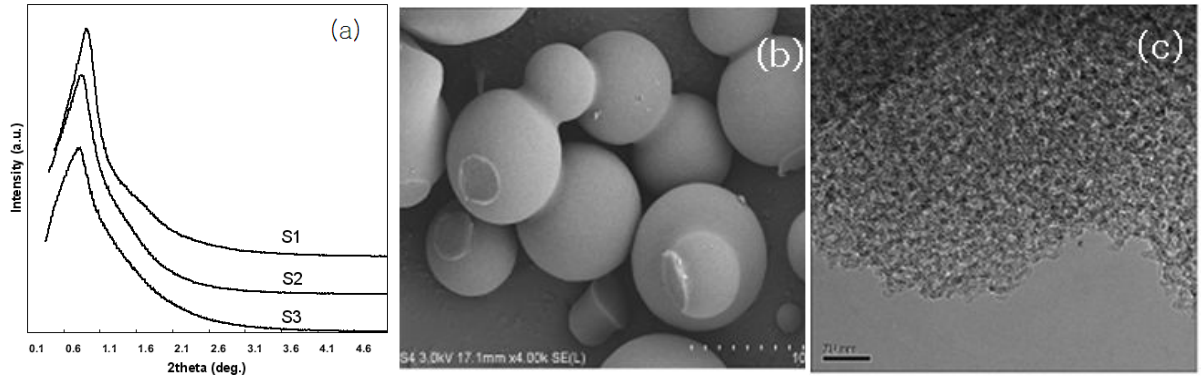

Fig.1 (a). XRD patterns; (b) SEM image; (c) TEM image of spherical SBA-15

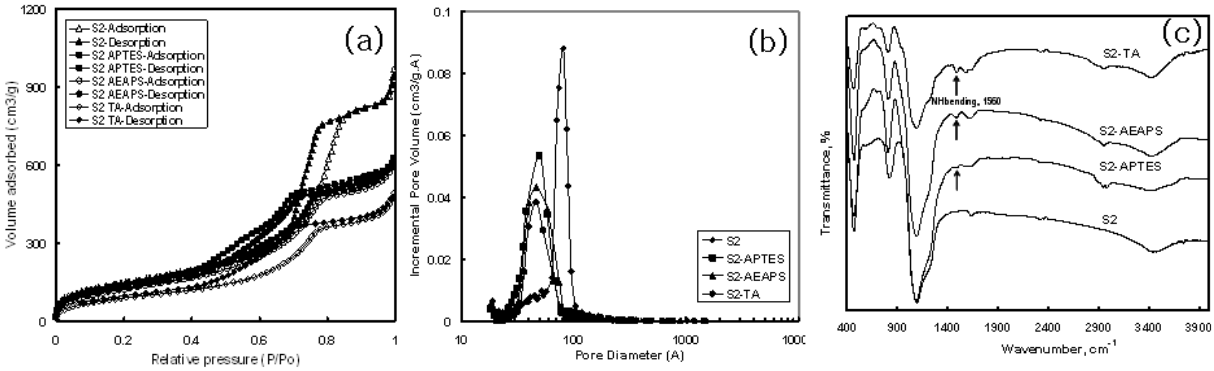

Fig. 2 (a) Nitrogen adsorption-desorption isotherms (b) BJH pore size distribution curves from the desorption branch; (c) IR spectra of original and amine functionalized spherical samples.

Fig. 2(c) shows the infrared spectra of SBA-15 spheres before (S2) and after modification (S2-APTES, S2-AEAPS and S2-TA). There are several bands appearing in the amino-functionalized SBA-15. In fact, it is difficult to determine the functional group NH_2 attached on the surface mesoporous material by observing peak around 3460 cm^{-1} because the band of NH_2 overlaps with that of O-H stretching vibration [3]. The appearance of peaks at 1560 cm^{-1} corresponds to the N-H (primary amine) bending vibration, this confirms the presence of $Si(CH_2)_3NH_2$ functional groups on the wall surface.

In order to investigate the properties of protein adsorption on SBA-15, the isotherm model Langmuir was applied. There are some differences in adsorption capacity of SBA-15 samples between proteins. The sample which has the highest adsorption capacity for all proteins is the original sample S2 due to its largest pore size and internal surface area. After attaching functional groups onto surface, the adsorption amounts of S2-APTES, S2-AEAPS and S2-TA were decreased due to a significant decrease in pore size and surface area. Besides, all modified samples have not adsorption capacity for BSA and S2-TA also has not adsorption capacity for LYS. Only original SBA-15 sample (S2) has adsorption capacity for BSA, but the amount of adsorption is not as high as for LYS and MYO. The interactions between proteins and original SBA-15 sample S2 are both chemical bond and physical bond

due to the presence of hydroxyl groups on the surface of SBA-15 and the functional groups of proteins. After modification via post synthesis, the hydroxyl groups on surface of silica are partially lost because of high temperature hydrothermal and calcinations treatment. Moreover, the attachment of nonpolar and hydrophobic methyl groups in grafted chains made the surface of SBA-15 more hydrophobic and a decrease in pore size. Hence, the amount of proteins adsorbed on the amine-modified sample is reduced in comparison with to that before modification. The results of adsorption kinetics show that original S2 sample has the most rapid uptake for all proteins and can reach saturation level for 10 hours (Fig. 3). The release study was performed for LYS and MYO. Although the adsorption capacity of S2 was highest among all samples, the amount released by functionalized samples was higher than that of S2 for LYS and TA (Fig. 4). Especially, S2-AEAPS has highest release amount, so it is very good candidate to apply in controlled drug delivery system.

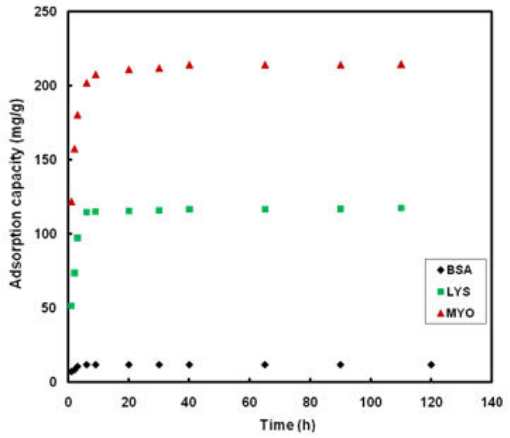

Fig. 3. Adsorption kinetics of S2

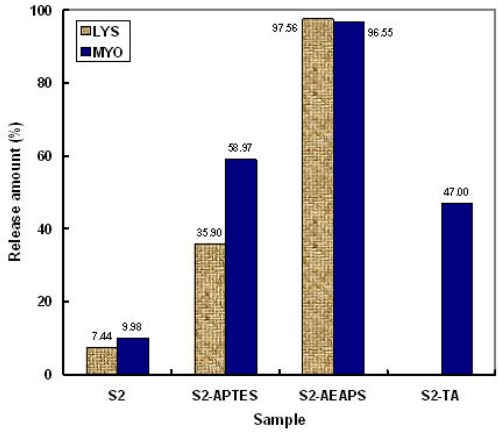

Fig. 4. Release amount of samples

CONCLUSION

In this study, mesoporous silica spheres have been obtained via a two-step synthesis process by using a triblock copolymer as template in combination with a co-surfactant and co-solvent. In addition, the surface characteristics of SBA-15 sphere had been modified by incorporating amine functional groups via post synthesis method. The original SBA-15 has the highest adsorption capacity for all proteins due to its largest pore size and internal surface area. The isotherm data was fitted very well with Langmuir equation. It was also found that the adsorbed proteins can be readily desorbed on amine-modified samples. Especially, the diamine-modified sample (S2-AEAPS) has the highest release amount for two proteins as LYS and MYO. Thus, the modified samples can be applied for controlled drug delivery system.

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