Sol-gel preparation of apatite-coated silica macrospheres from water glass and their adsorption of bovine serum albumin and lysozyme

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Silica gel macrospheres were prepared from water glass as the silica source. The water glass solution was mixed with sodium alginate solution and added dropwisely to 1 M CaCl_2 solution to form macrospheres with ~ 4 and 2.6 mm in diameter. Those SiO_2 macrospheres were soaked in 1:1 (volume) mixture of ethanol and 0.1 M Na_2HPO_4 to deposit hydroxyapatite layer (HAp- SiO_2 macrospheres). Bovine serum albumin (BSA) and egg lysozyme (LYZ) were adsorbed those SiO_2 and HAp- SiO_2 macrospheres. The amount of proteins adsorbed was well correlated to the Langmuir-type adsorption equation. The electrostatic interaction was predominant between the macrosphere surface and the protein molecules. Protein molecular entrapment in the mesopores (5–25 nm) was also probable for the SiO_2 macrospheres but not applicable to the HAp- SiO_2 ones without the mesopores. From consideration that protein molecule has a few groups with negative or positive partial charges, a multi-layer adsorption model was proposed.

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1. Introduction

Blood compatible adsorbents find various biomedical applications, such as medical implants,^{1)–3)} biondiagnostics,⁴⁾ targeted drug delivery,^{5),6)} biosensors and devices,^{7)–9)} and blood purification or hemoperfusion.^{10)–14)} Apatite is one of such adsorbents and is employed for chromatographic separation of serum proteins.^{15)–18)} Because of their high tissue compatibility, non-inflammation, and non-immunogenesis,¹⁹⁾ hydroxyapatite [HAp; $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$] and ion-substituted non-stoichiometric apatite particles are also employable to remove pathogenic substances. For example, Takashima et al.²⁰⁾ confirmed good blood compatibility of a few non-stoichiometric hydroxyapatite, and showed better adsorption of β_2 -microglobulin (β_2 -MG) than that of bovine serum albumin (BSA). Fujii et al.²¹⁾ and Hayakawa et al.²²⁾ studied the selective adsorption of β_2 -MG and BSA on zinc-substituted hydroxyapatite as a function of the Zn content and Zn distribution in the apatite particles. Takemoto et al.²³⁾ synthesized hydroxy-carbonate apatite and showed that a larger carbonate ion content was responsible for better selectivity of β_2 -MG.

Adsorbent materials for biological applications have been prepared in various forms such as membranes,^{24),25)} fibers,²⁶⁾ sheets,²⁷⁾ nano- or microparticles,²⁸⁾ and consist of organic, inorganic, or hybrid components: polymers (resins),²⁹⁾ ceramic materials such as titania¹³⁾ and hydroxyapatite,³⁰⁾ metal granules,³¹⁾ amorphous graphitic or fullerene-like carbon nitride,³²⁾ or porous silicates.^{33),34)} For blood purification clinics like hemoperfusion and dialysis including peritoneal dialysis, packed columns of macrospheres or suspension of macrospheres with mm in size would be advantageous because the macrospheres in

that size range are more convenient in handling and easier to control quantity. Moreover, one may avoid least risk that their fragments are carried into blood vessel.

Since the adsorption takes place at the interface between the material and surrounding moiety involving adsorbates, the most important factors for optimizing the adsorbents include the particle size (surface area and curvature), surface charge density, crystallography, and surface morphology, while the pore size and its distribution are also predominant factors if the adsorbates are able to be trapped in the pores. Moreover, the adsorbents in the form of fine particles set in hemoperfusion blood purification columns might introduce any diverse problems when they are in contact with flowing blood or blood plasma and some might be released in the flow. Fixing those active adsorbents on some neutral substrates to form a core-shell architecture can avoid those problems with preserving their adsorbing ability.

When the fabrication cost is taken into account, silica is the best substrate among possible candidates, like polymers, active carbon, chemically stable ceramics, or composites. Flexible fabrication of silica would rely on the sol-gel synthesis procedure with which the particles of any shape and size are produced and various surface modifications are possible. Silica gel has been studied extensively in the past four decades where hydrolysis of alkoxy-silanes and condensation of the resulted silanol groups under acid or basis catalysis are the primary topics. More feasible source of Si(IV) is water glass, or highly hydrolyzed sodium disilicate, because of its commercial availability. After use in hemoperfusion or blood purification, the adsorbent silica particles could be abandoned after calcining or sterilization. Moreover, securing blood or plasma flow under least pressure needs mm-sized spheres packed in about a few hundred ml of column volumes. The fabrication of the silica substrates must be highly cost-oriented.

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Silica gel granules are commercially available since long as a drying agent, and they are good candidates as the substrates for such applications. However, their dry surface must be appropriately hydrolyzed by the treatment with acid or alkaline solutions to fix an active layer. Studies of such hydrolysis and adsorbent coating are under way, and the results will be later presented elsewhere. In the present study, a convenient sol-gel approach was developed to prepare silica microspheres (~2 mm in wet states) on which an apatite layer was deposited via the procedure similar to glass-apatite conversion, provided by Rahman, Day, and co-workers.³⁵⁾ The microstructure of the gel spheres and surface morphology were characterized. Adsorption of model proteins, bovine serum albumin (BSA) and egg lysozyme (LYZ), was analyzed and the mechanism of adsorption was discussed.

2. Experimental

2.1 Preparation of silica microspheres and apatite deposition

2.1.1 Silica gel microspheres

Water glass, purchased from nacalai tesque, Osaka, Japan, involved 55 mass % sodium disilicate, $\text{Na}_2\text{O} \cdot 2\text{SiO}_2$, after a chemical analysis. Distilled water was added to the original solution to obtain 30 mass % water glass solution. Then, gelation time (t_{gel}) of the silicate solutions with varied pH in the range 2–10.5 were examined as described later in §3. With its enough long t_{gel} , the silicate solution of pH 3 was employed in the following experiments.

The silicate solution of pH 3 was mixed with 1.5 mass % sodium alginate solution in the mass ratio 2:3 under stirring. t_{gel} for this mixture was about 85 min. The silicate-alginate mixture of 12 ml was immediately added dropwisely to 50 ml 1 M CaCl_2 solution (pH 4.8) at a rate of 0.3 ml/min, using a syringe feeder through a needle (the inner diameter: 0.8 mm), to yield approximately 800 microspheres. These microspheres were aged for 1 d at 40°C in the CaCl_2 solution. Here, the CaCl_2 solution contained a highly excess amount of calcium ions over the stoichiometric amount necessary for the alginate molecules to form Ca-alginate chelate. All of those ~800 microspheres were subsequently soaked in 40 ml 0.1 M NH_4OH solution (pH 9.7) for 1 d at 25°C. The final pH of the ammonia solution was ~8.5. Those wet Ca-silica microspheres were washed in 100 ml distilled water for 10 min under gentle stirring. This procedure was iterated for 3 times to obtain wet Ca-silica microspheres.

About 2 g of wet microspheres were used to examine silica content: they were washed with distilled water to drive off free sodium and calcium ions, freeze-dried, and then subjected to heating at 800°C for 1 h in air. The silica content in the wet gel was 7.6 mass %, while the freeze-dried microspheres consisted of ~71.5 mass % SiO_2 , 17.4 mass % organic component or alginate, and 10.8 mass % H_2O held in closed pores. The silica content in the calcined body was 99.6 mass % with amount of calcium ions in the calcined was negligibly small CaO content (~0.4 mass %).

2.1.2 HAP-coating on silica microspheres (HAP- SiO_2)

The as-washed Ca-silica gel microspheres were placed in a tightly capped polystyrene bottle (50 ml) together with 40 ml of a 1:1 (volume) mixture of ethanol and 0.1 M Na_2HPO_4 . The volume ratio of the spheres vs. the mixture was set at ~1:7. The bottles were then kept in an oven at 80°C for 1 d. During the incubation, it is expected that the phosphate ions took out the calcium ions from the Ca-alginate chelate molecules in the SiO_2 microspheres to yield apatite via the reactions similar to the glass-apatite conversion.³⁵⁾

To stabilize the free alginate molecules, those microspheres were soaked again in the 1 M CaCl_2 solution for 30 min. Then, those HAP- SiO_2 microspheres were rinsed with distilled water three times as above described, dried in an oven overnight, and stored in a desiccator until use.

2.2 Characterization

2.2.1 Microstructure analyses

X-ray powder diffraction (XRD) was taken for the freeze-dried HAP- SiO_2 microspheres to identify the deposited phase: Cu $\text{K}\alpha_1$; an Ultima IV machine (Rigaku, Tokyo, Japan), operated at 40 kV and 40 mA, $\theta/2\theta$ step-scanned with a 0.02° step in the 2θ range 10–70°, and holding time of 1 s per step. Surface microstructure of the microspheres was observed under a scanning electron microscope (SEM; DS720, TOPCON, Tokyo, Japan: 20 kV acceleration), equipped with an Energy dispersion X-ray (EDX) analyzer with which the presence of component atoms was to be confirmed. Fourier-Transform Infrared (FT-IR) spectra of the microspheres were also measured using the KBr method where 2 mg dry samples were shaped into discs together with 200 mg KBr (Nicolet, Nexus 470TH, Thermo Scientific, Yokohama, Japan). Crush resistance and strain at failure of the silica gel microspheres were measured using a Rheoner RE-33005 machine (Yamaden, Osaka, Japan) with the strain rate of 0.5 mm/s. After dried in vacuo, pore size distribution and specific surface area were derived from the nitrogen adsorption and B.E.T. analysis (Gemini 2360, Micromeritics, Osaka, Japan). Particle sizes were measured under an optical microscope, equipped with an image analyzing software.

2.2.2 Protein adsorption

Human serum contains 30–50 mg/ml (450–750 $\mu\text{mol/L}$) albumin but only a trace amount 4–11 $\mu\text{g/ml}$ (0.27–0.75 $\mu\text{mol/L}$) of LYZ. Albumin and lysozyme are typical acidic and basic proteins, and they are negatively and positively charged, respectively, under physiological conditions. They are then good model proteins for which the adsorbent capability is to be evaluated, because the adsorption is frequently interpreted in terms of the electrostatic interactions between the adsorbents and the relevant proteins. The present study employed bovine serum albumin (BSA) and egg lysozyme (LYZ): molecular weight (Mw) of BSA is about 67200D while that of LYZ is 14600D, and their sizes are about $4 \times 4 \times 16$ nm and $3 \times 3 \times 4.5$ nm, respectively. Both were purchased from Wako Chemical, Osaka, Japan, and used without further purification for the adsorption experiments. They were dissolved into saline to attain solutions of concentration up to 10 mg/ml or 2.0 mM, and their pH was adjusted to 7.4 using tris(hydroxymethyl)aminomethane (TRIS) buffer. The microspheres (~0.2 g in dry weight) and 5 ml BSA or LYZ solutions were placed in tightly capped 5 ml glass bottles, mounted on a thermostatic shaking-bath, and the bottles were shook at 2 Hz at 37°C for 0–300 min. After filtering out the microspheres, the amount of residual proteins in the protein solutions was measured photometrically,^{10),20)} using a UV-Visible spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan), assuming that the optical density of the peaks around 280 and 270 nm was proportional to the amount of BSA and LYZ, respectively. The adsorption data were fitted with Langmuir-type adsorption isotherm.

$$\Gamma = \Gamma_{\text{max}} Kc / (1 + Kc) \quad (2)$$

Fig. 1. Optical photo images of the SiO_2 and HAp- SiO_2 macrospheres. Wet: as-prepared, FD: freeze-dried. Their average size is indicated in each frame.

Here, Γ is the amount of protein adsorbed on the macrospheres in equilibrium with the protein concentration c , $\Gamma_{\max}$ stands for the maximum adsorption where all of the active sites are occupied by the adsorbate molecules. K is the constant related to the rate constants of adsorption and desorption. The original Langmuir equation was derived for gas adsorption under several assumptions. Equation (2) has been sometimes applied to other cases, like the protein adsorption. Rearranging Eq. (2) leads to Eq. (3):

$$1/\Gamma = (1/\Gamma_m) + (1/\Gamma_m \cdot K)(1/c) \quad (3)$$

If the adsorption data are plotted to correlate $1/\Gamma$ to $1/c$ and a linear relation is obtained, the parameters in Eqs. (2) or (3) will be given for discussion.

3. Results

3.1 Macrospheres

Gelation time (t_{gel}) of the silicate solution without alginate was obtained as a function of pH, since t_{gel} was a measure of stability of the present water glass sol/gel system and determined the workability. t_{gel} showed a U-letter shaped dependence on pH. An alkoxide system $\text{Si}(\text{OC}_2\text{H}_5)_4\text{-H}_2\text{O-C}_2\text{H}_5\text{OH-H}_2\text{O}$ [the molar ratio $\text{Si}(\text{OC}_2\text{H}_5)_4/\text{H}_2\text{O} = 4$] showed a similar t_{gel} dependence on pH.³⁶⁾ In the range $\text{pH} < 3$, t_{gel} was substantially long, ~ 1000 min or longer. The silicate solution of pH 3 showed $t_{\text{gel}} \sim 40$ min and was transparent—little opaque when prepared. The opacity increased with the aging and a slight amount of supernatant was detected on the top of the solution body, indicating syneresis due to gelation. In the range pH 3 through 9.5, the system gelled very quickly within < 15 min. When pH exceeded 9.5, the silicate solution looked milky already when prepared, and sedimentation on the container bottom was found when held for 1 d at 25°C . Moreover, mixing alginate elongated t_{gel} for the silicate solution of pH 3 to ~ 85 min, and hence, this solution in the range $\text{pH} = 3$ was concluded optimum for the macrosphere fabrication.

The feeding rate of the silicate solution controlled the size of the silica macrospheres, ranging from ~ 4 to ~ 1 mm in diameter. **Figure 1** shows typical wet silica gel macrospheres of 2.6 mm in diameter. The size decreased a little with the apatite deposition:

from 2.6 to 2.1 mm, while the freeze-drying procedure naturally shrunk the SiO_2 macrospheres by $\sim 40\%$ and the HAp- SiO_2 ones by $\sim 20\%$. The both wet macrospheres bounced when dropped from ~ 5 cm in height, but the dry ones behaved like ceramics, or they were brittle. The force and apparent strain at collapse were measured as a function of the period of aging in the 1 M CaCl_2 solution. The as-prepared macrospheres, or wet SiO_2 in Fig. 1, collapsed at 27 g-f (or 0.26 N), and those aged for ~ 3 h were broken at 38 g-f (0.37 N), whereas those aged for 12 h or longer collapsed at ~ 47 g-f (0.46 N). For the aging within 1 day, the apparent strain (diameter at collapse/diameter before the force being loaded) decreased slightly from ~ 62 to 58%, or remained almost constant. Not only the silica network development due to gelation but also reinforcement with the Ca-alg chelate formation supposedly were effective for strengthening the gel macrospheres, and they were strong enough to be handled with tweezers for removal or transport.

The XRD patterns for the dry HAp- SiO_2 in **Fig. 2** show the diffraction peaks of hydroxyapatite (JCPDS 9-0432), and their intensity increased slightly with aging in the ethanol/ Na_2HPO_4 solution. The XRD profile for the SiO_2 macrosphere at the bottom indicated no detectable peaks assignable to NaCl, CaCl_2 or other possible ingredients. This might indicate the absence of sodium and chloride ions in the system. Yet, one should examine the EDX profiles before giving definite remarks because the possibility remained that their weak diffractions were masked by the XRD signal noise. Note that the peak width at half height maximum was almost independent of the aging period, $\sim 0.38^\circ$ in 2θ , and the aging longer than 3 h indicated no further increase in the diffraction intensity.

Figure 3 shows the FT-IR spectra of the SiO_2 macrospheres after aged under different conditions and those of HAp- SiO_2 ones, where the bands have been assigned according to the literatures.³⁷⁾⁻³⁹⁾ A broader band $\nu_s(\text{O-H})$ of the residual H_2O was detected around 3430 cm^{-1} , while the strong one, denoted as $\nu(\text{Si-O, P-O})$, around 1080 or 1101 cm^{-1} with a shoulder ($\sim 1200\text{ cm}^{-1}$) was the $\nu_3(\text{Si-O})$ band superimposed with the $\nu_3(\text{PO}_4^{3-})$ band. The two bands at 804 and 468 cm^{-1} in the low

energy region were assigned to $\nu_1(\text{SiO}_4)$ and $\delta(\text{Si-O})$. The ν_1 mode of the SiO_4 tetrahedron, belonging to representation A_1 , is only Raman active, but sometimes the $\sim 800\text{ cm}^{-1}$ IR band has been assigned to this mode. The 964 and 563 cm^{-1} bands were assigned to the ν_1 and ν_4 modes of the orthophosphate ions. It is reasonable that those phosphate bands were detected only for the HAp-SiO₂ macrosphere. In addition, the ν_{as} and ν_{s} modes of the carboxyl groups of alginate were found at 1625 and 1423 cm^{-1} for both SiO₂ and HAp-SiO₂, indicating that the alginate molecules remained in the gel spheres.

The SEM images in Fig. 4 compare the surface microstructures of (a) SiO₂ and (c) HAp-SiO₂. The EDX signals from their surface were presented in (b) and (d), respectively. The SiO₂ microspheres had smoother surface, while flaky crystallites covered the HAp-SiO₂ surface. Those crystallites were apatite according to the XRD in Fig. 3. Predominant EDX signals were due to Si and O in both spectra (b) and (d). The Ca peak in (b) was attributed to the presence of Ca-alginate chelate molecules. The peak for P was detected only in (d) in agreement with the apatite deposition, indicated by the SEM image (c) and the XRD

Fig. 2. XRD patterns of the SiO₂ microspheres after HAp-coating by soaking them in the 1:1 (vol/vol) mixture of ethanol/0.1 M Na₂HPO₄ at 80° C for 1 h–7d.

profile in Fig. 2. In addition, the peaks of Na and Cl, though trace, were detected in both (b) and (c), indicating that the sodium ions were not thoroughly washed away from the microspheres.

Pore size distribution profiles in Fig. 5 for SiO₂ microspheres showed a sharp peak at $\sim 3\text{ nm}$, and a broad peak in the mesopore region, extending from 5 to 20 nm . In contrast, the mesopores seemed to disappear due to the HAp-coating, leaving the peak at $\sim 3\text{ nm}$. Specific surface area (SSA) data were also derived from the N₂ adsorption experiment: 220 and $173\text{ m}^2/\text{g}$ for SiO₂ and HAp-SiO₂ microspheres, respectively, as listed in Table 1 together with some other properties characteristic of the protein adsorption.

Fig. 3. The FT-IR spectra of SiO₂ and HAp-SiO₂ microspheres together with the spectrum of sodium alginate as a reference. The aging time in the solutions of pH 3.0 and 9.7 for the SiO₂ microspheres was indicated like SiO₂:24 h-3.0. Some bands characteristic of each unit or group were presented with arrows.

Fig. 4. SEM images (a, c) and EDX point analysis (b, d) for the SiO₂ (a, b) and HAp-SiO₂ (c, d) microspheres. The EDX peaks were assigned to the component atoms.

Fig. 5. The pore size distribution of the SiO_2 and HAp- SiO_2 microspheres.

Table 1. Size, SSA, and some Langmuir isotherm constants for BSA and LYZ adsorption for SiO_2 and HAp- SiO_2 microspheres

	Size ^{a)} (mm)	SSA (m ² /g)	Γ_m ^{b)} ($\mu\text{g}/\text{m}^2$)	K ^{b)} (L/mg)	θ ^{c)} (-)	Γ_m ^{d)} (n mol/m ²)
SiO_2	1.52	220	26. (B) ^{e)} 476. (L)	0.20 ₃ (B) 0.30 ₀ (L)	0.013 (B) 0.206 (L)	0.4 (B) 32.6 (L)
HAp- SiO_2	1.77	173	400. (B) 1667. (L)	0.09 ₁ (B) 0.66 ₇ (L)	0.201 (B) 0.722 (L)	6.0 (B) 114.2 (L)

a) Average diameter of the freeze-dried microspheres.

b) Eq. (3).

c) Eq. (4). Molar weight of BSA and LYZ: 67200D and 14600D; molecular size: BSA $4 \times 14 \text{ nm}$, LYZ $3 \times 3.5 \text{ nm}$ (Ref. 26).

d) Γ_m in molar basis.

e) B: BSA, L: LYZ.

3.2 Protein adsorption

Figure 6 shows that BSA and LYZ adsorption on the SiO_2 and HAp- SiO_2 microspheres was dependent not only on the contact time but also on the protein concentrations of the solutions. Although similar adsorption profiles were obtained also for the SiO_2 microspheres, the data only for the 10 mg/ml BSA and LYZ solution were presented in Fig. 6, for avoiding clutter. Figure 6(a) indicates that BSA adsorption on both microspheres increased rapidly but it reached an equilibrium state for any concentration within 100 min. The equilibrium adsorption increased with the BSA content of the solutions. In Fig. 6(b), LYZ showed more vigorous adsorption since the beginning, and reached their equilibrium adsorption $\sim 80 \text{ min}$ for the HAp- SiO_2 microspheres, but it took longer for the SiO_2 ones. It follows that both microspheres adsorbed several times as much LYZ as BSA: for example, $800 \mu\text{g}/\text{m}^2$ LYZ but only $120 \mu\text{g}/\text{m}^2$ BSA were adsorbed on HAp- SiO_2 .

4. Discussion

4.1 Growth of the Si–O IR band

It is noticeable in the IR spectra in Fig. 3 of SiO_2 : 0 h and SiO_2 : 24 h-3.0 that the Si–O band around 1100 cm^{-1} grew with aging under pH 3.0. A similar increase in the IR band intensity was also exhibited for SiO_2 : 24 h-9.7. The band intensity is a measure of the Si–O bond density in the microspheres, and the increase in the Si–O band superficially agrees with the gelling observations described in §3.1. Yet, the Si–O band intensity relative to the $\nu_{\text{as}}(\text{COO}^-)$ and $\nu_{\text{s}}(\text{COO}^-)$ bands due to the alginate molecules was independent of the aging. Therefore, the 1100 cm^{-1} band growth should not be interpreted by the gelation or increased

Fig. 6. (a) BSA and (b) LYZ adsorption kinetics for the SiO_2 (open circles) and HAp- SiO_2 (filled symbols) microspheres in contact with the solutions of varied protein concentrations. The curves are guides for the eye.

Fig. 7. Langmuir plot [Eq. (2)] of BSA and LYZ adsorption on the SiO_2 (filled symbols) and HAp- SiO_2 microspheres (open symbols).

network density, whereas the increase in the band for the HAp- SiO_2 was definitely attributed to overlap of the $\nu(\text{P-O})$ signal from the apatite deposited on the silica microspheres.

4.2 Protein adsorption

4.2.1 Adsorption simulation from Langmuir model

Figure 7 shows the data presented in Langmuir type plot, according to Eq. (2). All systems, i.e., BSA adsorbed on SiO_2 and HAp- SiO_2 , LYZ adsorbed on SiO_2 and HAp- SiO_2 gave good linear correlations between $1/\Gamma$ and $1/C$, with good reliability factor $R^2 \sim 0.97$. Table 1 lists the parameters Γ_m and K , where Γ_m represents the amount of relevant adsorbate molecules when they occupy all active sites. Assuming such an extreme case, the

surface coverage θ_m (area covered by the proteins/unit surface area) was derived from Eq. (4),

$$\theta_m = \Gamma_m \cdot (N_A/Mw) \cdot S_p \quad (4)$$

Here, N_A is the Avogadro's number and Mw is the molar weight of BSA and LYZ, namely, 67200 and 14600, while S_p is the molecular size: $4 \times 14 \text{ nm}^2$ for BSA and $3 \times 3.5 \text{ nm}^2$ for LYZ.⁴⁰⁾ Then, the results were listed in Table 1. The coverage by BSA was smaller than that by LYZ, in agreement with the amount of adsorption in both mass (Γ_m) and molar (Γ_{mol} : the last column of Table 1) basis. In practical cases, a part of the sites available for adsorption remain unoccupied, controlled by the equilibrium.

4.2.2 Adsorption mechanisms

It is common to interpret protein adsorption in terms of electrostatic interactions between the proteins and the adsorbent. From the respective isoelectric point (iep): 4.7, 11.1 and 1.8–2.2 for BSA, LYZ⁴⁰⁾ and silica,⁴¹⁾ negative charges rest on BSA and silica, and a positive charge is on LYZ under physiological conditions, pH 7.2–7.4. Thus, the electrostatic interaction model can interpret the LYZ adsorption on the SiO_2 microspheres by 20 times as much mass as that of BSA, indicated in Fig. 6 or by Γ_m in Table 1. In molar basis, the LYZ adsorption was ~ 8 times as much as the BSA adsorption.

Some complexity was introduced in the HAp- SiO_2 microspheres because of the apatite crystallites, which have two kinds of crystallographic planes to provide good adsorption sites with varied partial charges. The C-plane and A-plane of the apatite crystal will be positively and negatively charged under pH 7.4, according to the lattice characteristics: the calcium ions are rich on the former, and the phosphate ions are rich on the latter. The HAp- SiO_2 gave polycrystalline diffractions of apatite in Fig. 2, and the random orientation was confirmed in the SEM image in Fig. 4(c). Thus, it is reasonable that each plane should be exposed to the protein molecules in similar probability, or the protein molecules would have an even chance to find the planes of either charge. That is, the apatite deposition gave the silica microspheres active sites suitable for BSA for which the electrostatic interactions worked. Actually, the BSA adsorption on the HAp- SiO_2 microspheres increased by a factor of ~ 15 . Even LYZ adsorption increased by a factor of ~ 3.5 . This LYZ case, however, seems strange because the sites with negative charges appeared due to the apatite deposition. The present study failed to identify the orientation of those apatite lattice planes by transmission electron microscopy, and hence, no definite description is possible on the preferably grown plane. Yet, the results at least have indicated that both microspheres are highly active to adsorb positively charged species, and that the apatite layer on the HAp- SiO_2 microspheres possesses enhanced ability to adsorbing such species with a negative charge.

Another possible mechanism is trapping molecules in such pores that are large enough to accommodate them. This mechanism is applicable to the SiO_2 microspheres that had mesopores ranging 5 to 20 nm in size, but is inappropriate to the HAp- SiO_2 microspheres without such mesopores. Their SSA ($173 \text{ m}^2/\text{g}$), almost comparable to the SiO_2 ones ($220 \text{ m}^2/\text{g}$), could not be attained if those microspheres were solid dense spheres. It probably resulted partly from the spaces among the apatite crystallites on the surface layer. Moreover, it seems unlikely that, even if the microspheres had a larger volume in the mesopore region, the protein molecules would migrate deep into the pores one after another to fill them. The larger values of coverage θ_m in Table 1 have been derived by assuming that such mesopores should be filled with the protein molecules.

In this regard, another mechanism should work for the present protein adsorption.

4.2.3 Validity of Langmuir type adsorption

The protein adsorption on the present silica gel microspheres would not match the assumptions in deriving Langmuir equation, i.e., a single layer adsorption of gas molecules on a part of the active sites on a flat surface. Thus, despite that the coverage θ_m derived from Eq. (4) was smaller than unity in Table 1, or the present microspheres had surface area enough to adsorb the proteins in a single layer, it is probable that the mode of the protein adsorption in reality did not follow the Langmuir assumptions. It is emphasized that a protein molecule has the groups with a negative (C=O , or C-O) or positive partial charge ($-\text{NH}-$) though it may have either charge in total depending on pH of the medium in which they are placed. Such charged groups may find good attractive adsorption sites on the adsorbent surface to settle the molecule. It is reasonable to suppose, thus, that a protein molecule still keeps such charges at those groups even after settled on an adsorption site, and that those groups with active charges then should deserve attracting another protein molecules to be adsorbed. That is, once a protein molecule is attached on an adsorption site, new adsorption sites are provided one after another to attain multi-layer adsorption. In this model, the factor to control the total amount of adsorption is the true surface coverage by the molecules that would first come onto the surface.

5. Conclusions

Silica gel microspheres were prepared from water glass as the silica source. The water glass was diluted with 2 M HCl to adjust pH of the system, with which sodium alginate solution was mixed. The mixture was added dropwisely to 1 M CaCl_2 solution to form microspheres with ~ 3 and 2.6 mm in diameter. After aged in the CaCl_2 solution for 1 d (SiO_2 microspheres), they were soaked in 1:1 (volume) mixture (pH 7.8) of ethanol and 0.1 M Na_2HPO_4 , and hydroxyapatite was deposited on their surface (HAp- SiO_2). According to SEM observation and N_2 gas adsorption, the SiO_2 microspheres had smooth surface with a large pore distribution in the mesopore region (5–20 nm) together with $220 \text{ m}^2/\text{g}$ in specific surface area (SSA). The HAp- SiO_2 microspheres lost those mesopores but had $173 \text{ m}^2/\text{g}$ in SSA. FT-IR spectra of the microspheres exhibited the IR bands characteristic of the component bonds, such as Si–O and P–O. Bovine serum albumin (BSA) and egg lysozyme (LYZ) adsorption on the SiO_2 and HAp- SiO_2 microspheres were measured and analyzed on the basis of Langmuir-type adsorption model. Simple electrostatic interaction between the charged microsphere surface and protein molecules was predominant, and entrapment of the protein molecules in the pores was partially effective. Yet, the latter was not the case for HAp- SiO_2 without mesopores. A multi-layer adsorption model was proposed, from the presence of both negative and positive charges in a protein molecule.

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