Effect of pH on the Adsorption and Desorption of Human Serum Albumin at Titanium-Water Interfaces

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Adsorption and desorption behaviors of human serum albumin (HSA) on titanium particles were examined as a function of pH. The HSA showed a high affinity-type isotherm with well-defined saturation values, even when HSA had a net charge that was the same type as that of titanium. The saturated amount of HSA adsorbed (Γ_{sat}) depended on pH. The maximum value of Γ_{sat} was obtained at pH around the apparent point of zero charge of HSA, where the net charge ($Z_{\text{H}^+ \text{app}}$) of HSA was zero and where HSA has the minimum intrinsic viscosity ($[\eta]$), reflecting the molecular volume. Results showed that the $Z_{\text{H}^+ \text{app}}$ and $[\eta]$ of HSA are the most important factors determining the Γ_{sat} value. Curves for the surface charge density (σ_{app}) of HSA-adsorbed titanium prepared at initial pH 7.4 showed that carboxyl groups on HSA contributed to interactions even with the negatively charged titanium surfaces. The possible interactions between ionic functional groups on HSA and titanium were discussed based on measurements of variation in pH of solutions, i.e., uptake or release of proton (ΔpH) as a result of adsorption. The removal of HSA from titanium surfaces was more rapid when using alkaline solutions, particularly at pH greater than 11. Results showed that HSA was adsorbed onto titanium surfaces, even under unfavorable electrostatic conditions, through various interactions between ionic groups on HSA and surface OH groups on titanium, the magnitude of those interactions was diminished by the OH⁻ cleaning action.

Keywords : Human Serum Albumin, Adsorption Interactions, Net Proton Charge, Intrinsic Viscosity, Surface Charge Density

1. Introduction

The adsorption behavior of proteins at solid-liquid interfaces is a subject of great importance, particularly in reference to fouling, in biochemical fields. In the medical area, when medical device surface comes in contact with blood, its surface is spontaneously covered with components of blood, including proteins. Human serum albumin (HSA) is recognized as a major protein in blood (4 % in blood plasma) and it tends to be spontaneously adsorbed on medical device and implant surface, thereby constructing the conditioning film ^{1)~3)}. The conditioning film alters the physico-chemical properties of the solid surfaces, and also plays a mediating role in bacterial adhesion. A bacterial infection presents a serious problem especially in the medical area ⁴⁾. To control and minimize protein adsorption and the formation of the conditioning film, it is necessary to elucidate the mode of initial adsorption of HSA onto the blood-contact surfaces.

Metallic titanium has been widely used for the medical device and the dental instrument because of excellent corrosion resistance, mechanical properties, and biocompatibility^{5),6)}. It is known that surface of metallic titanium are covered with stable oxide films, e.g., TiO₂, on which hydroxyl groups (OH groups) are formed through chemisorption of water molecules^{7)~9)}. These OH groups were distinguished between the acidic OH groups (-Ti₂OH) and basic OH groups (-TiOH) depending on the number of coordination to titanium ion. Titanium surface shows an amphoteric nature and its surface charge density and polarity vary as a function of pH. The surface OH groups on titanium play a role in adsorption sites for organic and inorganic materials ⁹⁾. HSA also has both positively (e.g., -NH₃⁺) and negatively charged groups (e.g., -COO⁻), and its net charge and

molecular volume in solution varies widely depending on the solution pH. It is thought that the type and magnitude of the predominant interaction between HSA and titanium are also strongly affected by the solution pH. Although many researchers have studied the adsorption behavior of HSA at solid-liquid interfaces from the viewpoint of electrostatic interaction^{1),10)~12)}, our fundamental understanding of the mode of initial adsorption of HSA onto titanium surface is still lacking.

The purpose of this study is two fold: (i) to elucidate the factors determining the amount of HSA adsorbed on titanium, forming a first monolayer, and (ii) to discuss the type of interaction between HSA and titanium. The adsorption behavior of HSA at water-titanium interfaces was studied as a function of pH. The electrostatic properties of HSA, titanium, and HSA-adsorbed titanium were measured by the potentiometric titration. We show here that the amount of HSA adsorbed on titanium is largely determined by the net charge of HSA, and that ionic functional groups on HSA molecule involved in the irreversible adsorption through electrostatic interaction, hydrogen binding, or ligand exchange with surface OH groups on titanium are proposed as a function of pH.

2. Experimental

2. 1 Materials and chemicals

Nonporous titanium particles (Lot 054370) were obtained from Kojundo Chemical Laboratory Co. Ltd (Saitama). The specific surface area and mean diameter of titanium particles were 0.9 m²/g and 10 μm, respectively. Prior to the use, titanium particles were washed with acetone and ethanol with sonication for 15 min, and then dried at 40 °C. Human serum albumin (HSA) (Fraction V, A-1653, Lot 072K7601) was purchased from Sigma-Aldrich Co. (St. Louis, Mo) and used as received. All other chemicals were of analytical grade.

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2. 2 Measurements of charges of HSA and titanium particles

The apparent values of the net proton charge of HSA (Z_{H^+app}) and the surface charge density of titanium particles (σ_{app}) were measured in the presence of KNO_3 , which is an indifferent electrolyte, using a potentiometric titrator (GT-07, Mitsubishi Chemical Ind. Ltd., Tokyo)^{8),13)}. In this study, 0.1 dm³ of 10⁻¹M, 10⁻²M, and 10⁻³M KNO_3 solutions (reference solution, pH 3.5) were titrated with 0.1 M KOH to pH 9 under N₂ (99.999 %, vol/vol) ambience. Then, a 0.1 g of HSA or a 2.0-g aliquot of titanium particles was dissolved or suspended in 0.1 dm³ of the KNO_3 solution (pH 3.5), and the solution or suspension was titrated as described above. The difference between the amounts of KOH necessary to produce the same pH value in the reference solution and the solution or suspension was attributed to the relative adsorption of H⁺ and OH⁻ ($[\Gamma_{H^+} - \Gamma_{OH^-}]$) onto HSA or titanium surfaces. The Z_{H^+app} and σ_{app} were calculated from $[\Gamma_{H^+} - \Gamma_{OH^-}]$ as a function of pH^{8,13)}. If the intersection of curves for Z_{H^+app} or σ_{app} at three different KNO_3 concentrations is obtained at a single pH value, this pH is regarded as the common intersection point¹⁴⁾, where $[\Gamma_{H^+} - \Gamma_{OH^-}]$ is zero. Accordingly, this pH is regarded as the apparent point of zero charge (pzc_{app}).

2. 3 Intrinsic viscosity of HSA

Specific viscosities ($[\eta_{sp}]$) of HSA at various concentrations (c) of 4 to 30 g per dm³ of 10⁻³M KNO_3 solution were measured at 40 °C in the pH range of 3.6 to 7.4 by using an Ostwald viscometer¹⁵⁾. The intrinsic viscosity ($[\eta]$) was obtained extrapolating the graph of η_{sp}/c vs. c to zero concentration.

2. 4 Adsorption and desorption of HSA

HSA was dissolved in 10⁻³M KNO_3 solution at concentrations of 0.05 to 2 g/dm³. The initial pH (pH_{ini}) of the HSA solution was adjusted in the range of 2.7 to 9.2 with 0.1 M HNO₃ and 0.1 M KOH. A 5-ml aliquot of HSA solutions was introduced into 25-dm³ glass vials containing 0.5 g of titanium particles. Each vial was then sealed with a butyl rubber stopper, laid on its side in a water bath at 40 °C, and reciprocally shaken (140 oscillations per min) for 2 h⁸⁾. After being shaken, the vial was centrifuged at 2,300 × *g* for 10 min. The supernatant containing nonadsorbed HSA was carefully discarded using a micropipette. The HSA-adsorbed titanium particles were washed twice with 5 mL of 10⁻³ M KNO_3 solution by centrifugation (2300 × *g* for 10 min). After being washed twice, the particles were dried at 40 °C for 16 h. The dried particles were transferred into another glass vial and the amount of adsorbed HSA (Γ) was determined as the sum of the amount of HSA removed and that of HSA still remaining on the particles (S_r) after batchwise treatment with 0.1 M NaOH for 2 h with shaking (140 oscillations per min) as described previously¹⁶⁾. HSA in solution or on titanium particles was measured by Lowry-Folin method¹⁷⁾.

To determine the effect of the pH of cleaning solution on the removal of HSA from titanium particle, cleaning solutions of pH_{ini} 1 to 13, which was adjusted with 0.1 M HNO₃ and KOH solution, were used. A 0.5-g portion of HSA-adsorbed titanium particles (2.2 mg/m²), prepared at pH_{ini} 7.4, and 0.005 dm³ of cleaning solutions were put into 25-dm³ glass vials, and then vials were reciprocally shaken (140 oscillations per min) at 40 °C for 2 h as described above. The removal efficiency was expressed as the ratio of the amount of HSA desorbed to the initial amount of HSA adsorbed.

3. Results and discussions

3. 1 Characterization of charging behavior of HSA and titanium

Figure 1 shows the curves for the Z_{H^+app} at different KNO_3 concentrations and the $[\eta]$ in 10⁻³M KNO_3 solution as a function of pH. The Z_{H^+app} and the $[\eta]$ reflect the relative degree of ionization of functional groups and molecular volume of HSA in an aqueous solution, respectively. The pzc_{app} was found to be 5.4. The absolute value of the Z_{H^+app} tended to increase with increasing KNO_3 concentrations.

The $[\eta]$ also depended on the solution pH (Fig. 1B). The minimum $[\eta]$ value of 2.6×10^{-3} dm³/g was obtained at pH 5.3, i.e., around the pzc_{app} of HSA. With increasing difference in pH values from the pzc_{app}, the $[\eta]$ value increased significantly. The $[\eta]$ values obtained in the pH range of 6.6 to 7.4, 3.6×10^{-3} to 5.0×10^{-3} dm³/g, were in good agreement with the value (4.62×10^{-3} dm³/g at pH 7.0) reported by Monkos¹⁸⁾. It was clear that the $[\eta]$ value was correlated with the Z_{H^+app} value. A small Z_{H^+app} results in contraction of the molecule, whereas a large Z_{H^+app} brings about intramolecular electrostatic repulsion, thereby leading to expansion of the molecule. The reversible expansion of globular proteins is general feature^{11),15),19)}. The $[\eta]$ value also gives information related to the area occupied by HSA in the adsorbed state.

Figure 2 shows the curves for the σ_{app} of titanium particles as a function of pH. The σ_{app} of titanium surfaces showed acidic

Fig. 1 Curves for the Z_{H^+app} (A) and the $[\eta]$ (B) of HSA at different KNO_3 concentrations, as a function of pH. Symbols (KNO_3): $\circ$, 10⁻¹M; $\blacktriangle$, 10⁻²M; $\square$, 10⁻³M.

nature that was characterized by small positive σ_{app} values and large negative σ_{app} values. The pzc_{app} was found to be 4.4, which was in agreement with the values (4.4 to 6.2) reported for Ti and TiO_2 particles⁸⁾.

3. 2 Effect of pH on HSA adsorption

Figure 3 shows the typical isotherm for HSA adsorption on titanium particles at pH_{ini} 7.4 which is equal to blood pH. The Γ increased greatly at low equilibrium concentrations of HSA (c_{HSA}) and reached saturation value (Γ_{sat}) of 2.2 mg/m^2 at c_{HSA} more than 0.2 g/dm^3 . When the Γ value was less than 1.8 mg/m^2 , c_{HSA} values were almost zero. This indicates that HSA molecules in solution were almost completely adsorbed on titanium. Such high affinity-type isotherms with well-defined saturation values were obtained in the pH range of 2.7 to 7.5 (data not shown). When all the HSA-adsorbed titanium particles obtained at examined pHs were washed with 10^{-3} M KNO_3 solution at 40 °C for 2 h, no desorption of HSA occurred, indicating that the adsorption of HSA on titanium was irreversible.

Figure 4A shows the Γ_{sat} values as a function of pH_{ini} . The Γ_{sat} depended on the pH_{ini} . The maximum value of Γ_{sat} was 3.8 mg/m^2 and obtained at pH around the pzc_{app} of HSA (5.4) and little pH change followed by the HSA adsorption was occurred at this pH. The obtained Γ_{sat} corresponded to the values (3.0 to 3.6 mg/m^2) reported for monolayer adsorption of HSA^{10),11)}. The occurrence

Fig. 2 Curves for the σ_{app} of titanium particles at different KNO_3 concentrations, as a function of pH. The arrow indicates pzc_{app} . Symbols (KNO_3): $\circ$, 10^{-1}M ; $\blacktriangle$, 10^{-2}M ; $\square$, 10^{-3}M .

Fig. 3 Isotherm for HSA adsorption on titanium particles at 40 °C and at pH_{ini} 7.4. The adsorption experiment was conducted in the presence of 10^{-3} M KNO_3 with reciprocal shaking for 2 h (140 oscillations per min).

of an adsorption maximum of albumin protein in their pzc region is also commonly found on various solid surfaces^{8),10),15),20),21)}. Even when HSA had the same type of net charge as that of titanium, the adsorption of HSA took place spontaneously. Since HSA molecule behaves as soft particles with ion-penetrable layers^{11),22)}, the electrostatic repulsion between HSA and titanium is thought to be extremely low or negligible upon adsorption. Thus, although global electrostatic forces undoubtedly affect adsorption behavior, they do not dominate the Γ_{sat} . In the case of protein adsorption, the contribution of electrostatic repulsive forces might become important for the interaction between HSA molecules. The magnitude of $Z_{\text{H}^+ \text{app}}$ determines molecular structure and strength of electrostatic repulsive forces. A small $Z_{\text{H}^+ \text{app}}$, and hence a small $[\eta]$ enables HSA molecules to form a closely-packed adsorbed layer on titanium surfaces because lateral electrostatic repulsions between adjacent HSA molecules are minimized. As a result, a large Γ_{sat} is obtained at pH around the pzc_{app} of HSA. At pHs far from the pzc_{app} of HSA, a large $Z_{\text{H}^+ \text{app}}$ causes molecular expansion of HSA and intermolecular electrostatic repulsion between HSA molecules, thereby resulting in a sparse arrangement of HSA on titanium and a small Γ_{sat} . It is indicated that the $Z_{\text{H}^+ \text{app}}$ and $[\eta]$ of HSA are the most important factors determining the Γ_{sat} value, aside from the magnitude of interaction forces between HSA and titanium surfaces.

The HSA-adsorbed titanium particles at Γ_{sat} obtained in **Fig. 4A** were washed with 0.1 M NaOH solution and the amounts of HSA remaining on titanium (S_r) were compared as a function of the adsorption pH (**Fig. 4B**). Although there were significant differences in the Γ_{sat} depending on the adsorption pH, the S_r values were almost equal to each other ($0.34 \pm 0.03 \text{ mg}/\text{m}^2$). These data indicate that a certain amount of HSA molecules, that are not able to be removed by cleaning with 0.1 M NaOH, are present irrespective of pH_{ini} and the Γ_{sat} values.

3. 3 σ_{app} of HSA-adsorbed titanium

Experiments were designed to investigate the type of ionic groups on HSA molecule interacting with titanium surfaces. The HSA-adsorbed titanium particles were prepared at pH 7.4 ($\Gamma = 2.2 \text{ mg}/\text{m}^2$), where HSA and titanium have net negative charges,

Fig. 4 Effect of adsorption initial pH on the Γ_{sat} values over the pH range of 2.8 to 9.2. For experimental conditions, see the legend for **Fig. 3**.

and the dried particles were titrated in the presence of 10^{-1} M KNO_3 . **Figure 5** shows curve for the σ_{app} of the HSA-adsorbed titanium particles as a function of pH. The dotted line was the curve obtained by summing the σ_{app} of titanium particles per m^2 and the $Z_{\text{H}^+ \text{ app}}$ per 2.2 mg at 10^{-1} M KNO_3 . If there was no electrostatic interaction between adsorbed HSA and titanium surface, the curve for σ_{app} of HSA-adsorbed titanium particles coincides with the dotted line. However, the curve for σ_{app} shifted to the positive side, giving the more alkaline pzc_{app} (5.9) compared with the calculated pzc_{app} (5.0) of HSA-adsorbed titanium particles. It was noted that the difference in the σ_{app} values between solid line and dotted line increased gradually with increasing pH from pH 4 to pH 7. This result suggested that the number of titratable free ionic groups on HSA or on titanium, which release protons in the pH range of 4 to 7, decreased probably due to interactions between HSA and titanium.

HSA is composed of 585 amino acids (molecular weight: 66,500) and it has 105 units of β -, γ -carboxyl groups and 60 units of ϵ -amino groups as the main acidic and basic ionic groups¹¹⁾. The dissociation constants (pK_a) of β -, γ -carboxyl (Asp and Glu) and ϵ -amino (Arg, Lys), main acidic and basic groups on HSA, were 4.0 and 9.4, respectively²³⁾. In the pH range of 4 to 7, carboxyl groups dissociate with the increase in pH ($-\text{COO}^-$, molar ratio: $\alpha = 0.5$ to 1.0), whereas amino groups remain protonated form ($-\text{NH}_3^+$, $\alpha = 1.0$). On the other hand, since it is assumed that the pK_a of acidic and basic OH groups on titanium are situated at 1.4 pH unit away from the pzc_{app} (4.4) of titanium²⁴⁾, a major fraction of acidic OH groups are present as the dissociated form, releasing protons ($-\text{TiO}^-$), whereas the ratio of deprotonated form ($-\text{TiO}^-$) of basic OH groups to the neutral form ($-\text{TiOH}$) increase with the increase in pH over the pH range of 4 to 7. Accordingly, proton-releasing titratable ionic groups in the pH range of 4 to 7 seem carboxyl groups of HSA, and acidic and basic OH groups on titanium particles. At this point it has been known that the curve for the σ_{app} of protein-adsorbed solid particles was more affected by the degree of shift in acid-base equilibria on the adsorbed protein molecule rather than by solid surface nature^{13),20)}. These findings suggest that dissociation of part of carboxyl groups on HSA is prevented probably due to the interaction with basic OH groups on titanium surfaces in the titration system of the HSA-adsorbed

Fig. 5 Curve for the σ_{app} of HSA-adsorbed titanium particles fouled at Γ of 2.2 mg/m^2 as a function of pH. Titration was conducted in the presence of 10^{-1} M KNO_3 . Dotted line denotes the calculation curve derived summing of the σ_{app} of titanium and $Z_{\text{H}^+ \text{ app}}$ of 2.2 mg of HSA.

titanium particles.

3. 4 Uptake and release of proton upon adsorption

To elucidate the types of interactions between HSA and titanium, we designed the experiments to measure the variation in pH of solutions (ΔpH) as a result of adsorption. It is assumed that positive value of ΔpH is attributed to release of OH^- (or uptake of H^+) during the adsorption process, and negative value of ΔpH is attributed to release of H^+ to the solution. **Figure 6A** shows ΔpH as a function of pH_{ini} . The ΔpH was zero at pH 5.2, below which it was slight positive value and above which negative value. Based on these data, the magnitude of variation in H^+ concentration (ΔH^+) was calculated (**Fig. 6B**). At pHs less than 5.2, ΔH^+ became large negative values with decreasing pH, indicating that uptake of H^+ occurred upon adsorption. On the other hand, in the pH_{ini} range of 5.2 to 9.2, the absolute values of positive ΔH^+ were extremely low, indicating that only a slight release of H^+ occurred. It is of interest that the pH giving $\Delta\text{H}^+ = 0$ was observed near the pzc_{app} (5.4) of HSA.

3. 5 Interactions between HSA and titanium

The main possible interaction types between protein and metal surface are known to be electrostatic (ion-ion and dipole-dipole) interactions, hydrogen bonding, and ligand exchange^{8),11),15)}. Considering the results in **Fig. 5** and **Fig. 6**, we proposed the probable reactions between HSA and titanium according to the investigation by Oliva et al¹¹⁾.

At pH 7.4, human blood pH, the predominant acidic and basic OH groups on titanium are $-\text{TiO}^-$ ($\alpha = 1.0$) and $-\text{TiOH}$ ($\alpha = \text{ca. } 1.0$), respectively, while the main functional groups on HSA are $-\text{COO}^-$ ($\alpha = 1.0$) and $-\text{NH}_3^+$ ($\alpha = 1.0$). Judging from the fact that the ΔH^+ was slight positive value (**Fig. 6B**), some possible reactions are proposed as follows:

Fig. 6 The ΔpH (A) and $\Delta[\text{H}^+]$ (B) upon HSA adsorption on titanium particles as a function of pH_{ini} .

Although type of reaction involving carboxylic groups is lesser than those involving amino groups, the large number of carboxylic groups (105 units) can contribute to many points of attachment with titanium surfaces via hydrogen bonding. It is likely that reaction (4) is responsible for the shift in the σ_{app} curve of HSA-adsorbed titanium to the positive side as shown in Fig. 5. Presumably, reactions (1) to (4) would dominate the adsorptive interactions in the pH_{mi} range of 5.4 to 9.2.

In the pH range of 2.8 to 5.4, titanium surfaces were positively and negatively charged within ca. $\pm 0.1 \text{ C/m}^2$ on the acidic and basic sides of its pzc_{app} . In this pH region, it is thought that $-\text{Ti}_2\text{OH}$ and $-\text{Ti}_2\text{O}^-$, and $-\text{TiOH}$ and $-\text{TiOH}_2^+$ coexisted as the acidic and basic OH groups, respectively. On the other hand, the main functional groups on HSA are $-\text{COO}^-$ ($\alpha = 0.2$ to 1) and $-\text{NH}_3^+$ ($\alpha = 1.0$). Since polarity of dissociated carboxyl groups is higher than protonated amino groups, carboxyl groups are thought to be highly reactive with the basic OH groups on titanium. On the acidic side of the pzc_{app} (5.4) of HSA, the ΔH^+ was large negative values (Fig. 6B), indicating uptake of H^+ or release of OH^- . Apart from reactions (1) to (4), other most probable reactions are as follows:

The strength of binding force per bond increases in the order

Fig. 7 Effect of the pH of cleaning solution on the HSA removal from titanium particles. Batchwise cleaning experiment was conducted at 40 °C for 2 h with shaking (140 oscillations per min). The pH of cleaning solution was adjusted with 0.1 M HNO_3 and KOH solution. HSA from titanium particle, the 0.5-g portion of HSA-adsorbed titanium particles were prepared at pH_{mi} 7.4 ($\Gamma = 2.2 \text{ mg/m}^2$).

hydrogen bonding < electrostatic interaction < ligand exchange. In addition, there are stronger electrostatic interactions between carboxyl groups on HSA and basic OH groups on titanium compared with those between amino groups and OH groups on titanium^(8),12). However, the S_i values after cleaning with 0.1 M NaOH were almost equal to each other irrespective of the adsorption pH (Fig. 4B). It is presumed that, although the global binding strength of HSA to negatively charged titanium surfaces is relatively low on the basic side of the pzc_{app} of HSA, strongly bound HSA molecules coexist in the adsorbed layer due to a multipoint attachment or possibly a variety of surface interactions.

3. 6 Effect of the pH of cleaning solution on the HSA removal

Figure 7 shows the effect of the pH of cleaning solution on the removal efficiency of HSA from titanium particles ($\Gamma = 2.2 \text{ mg/m}^2$ at pH_{mi} 7.4). The removal efficiency was considerably higher when using the alkali cleaning solution than when using the acidic cleaning solution. At pHs above pH 10, the removal efficiency increased markedly with increasing pH. A small amount of HSA was removed from titanium by a cleaning with acidic solution of pH 1. In the pH range of 2 to 10, only a small or no HSA removal was observed. These phenomena indicate the availability of OH^- ions in removing HSA from titanium surfaces. Adsorption of OH^- ions at the HSA-titanium contact region can make their surfaces more negative, thereby diminish or minimize the magnitude of forces of interactions between HSA and titanium (reactions 1 to 9) but not completely. It was noted that the HSA removal occurred markedly at alkaline pH region above the pK_a values of side-chain ϵ -amino groups (pH 9.4), phenolic OH groups (pH 10.4), and guanidyl groups (pH >12). In alkaline pH regions above 10, the $Z_{\text{H}^+ \text{app}}$ of HSA is expected to become large negative value. As well as the electrostatic repulsion between HSA and titanium, the swollen structure of HSA arising from intramolecular repulsion is the driving force to transport HSA from titanium surfaces to the solution.

4. Conclusions

In this study, the factors determining the Γ_{sat} value HSA on titanium and the type of interaction between HSA and titanium were investigated. It was confirmed that the $Z_{\text{H}^+ \text{app}}$ and $[\eta]$ of HSA were the most important factors determining the Γ_{sat} value. HSA was adsorbed on titanium surfaces even under unfavorable electrostatic conditions through a variety of interactions between ionic groups on HSA and surface OH groups on titanium. The high removal efficiency of HSA from titanium surfaces was obtained by cleaning with alkaline solutions, particularly of pHs above 11 which is higher than the pK_a values of basic side-chain functional groups on HSA.

(Received January 5, 2010 ; Accepted April 25, 2010)

References

- 1) A. Klinger, D. Steinberg, D. Kohavi, M. N. Sela ; *J. Biomed.Mater. Res.*, **36**, 387 (1997).
- 2) M.C.Sunny, C. P. Sharma ; *J. Biomater. Appl.*, **6**, 89 (1991).
- 3) B. Wälivaara, B. O. Aronsson, M. Rodahl, J. Lausmaa, P. Tengvall ; *Biomaterials*, **15**, 827 (1994).
- 4) E. M. Hetrick, M. H. Schoenfish ; *Chemical Society Reviews*, **35**, 780 (2006).
- 5) F. David; Titanium in medicine: material science, surface science, engineering, biological response and medical applications, p.13

- (Springer Verlag, 2001).
- 6) J.C. Wataha ; *The Journal of Prosthetic Dentistry*, **83**, 223 (2000).
 - 7) H. P. Boehm ; *Disc. Faraday Soc.*, **52**, 264 (1971).
 - 8) K. Takahashi, S. Fukuzaki ; *Biocontrol Sci.*, **13**, 9 (2008).
 - 9) M. Textor, C. Sittig, V. Frauchiger, S. Tosatti, D. M. Brunette ; *Titanium in medicine: material science, surface science, engineering, biological response and medical applications*, p.171 (Springer Verlag, 2001).
 - 10) C.A. Haynes, W. Norde ; *Colloids surface B, Biointerfaces*, **2**, 517 (1994).
 - 11) F.Y. Oliva, L. B. Avalle, O. R. Cámara, C. P. De Pauli ; *J. Colloid Interface Sci.*, **261**, 299 (2003).
 - 12) K. Imamura, M. Shimomura, S. Nagai, M. Akamatsu, K. Nakaniishi ; *J. Biosci. Bioeng.*, **106**, 273 (2008).
 - 13) H. Urano, S. Fukuzaki ; *J. Biosci. Bioeng.*, **90**, 105 (2000).
 - 14) J. Lyklema ; *J. colloid interface sci.*, **99**, 109 (1984).
 - 15) S. Fukuzaki, H. Urano, K. Nagata ; *J. Ferment. Bioeng.*, **80**, 6 (1995).
 - 16) S. Fukuzaki, H. Urano, M. Hiramatsu, A. Takehara ; *Biocontrol sci.*, **6**, 87 (2001).
 - 17) O. H. Lowry, N. J. Rosebrough; A. L. Farr, R., J. Randall ; *J. Biological Chem.*, **193**, 265 (1951).
 - 18) K. Monkos ; *Biochim. Biophys. Acta*, **1700**, 27 (2004).
 - 19) C. Tanford, J. G. Buzzell, D. G. Rands, S. A. Swanson ; *J. Am. Chem. Soc.*, **77**, 6421 (1955).
 - 20) S. Fukuzaki, H. Urano, K. Nagata ; *J. Ferment. Bioeng.*, **81**, 163 (1996).
 - 21) W. Norde, J. Lyklema ; *J. Colloid Interface Sci.*, **66**, 257 (1978).
 - 22) H. Oshima ; *Adv. Colloids Interface Sci.*, **62**, 189 (1995).
 - 23) C. Tanford ; *J. Am. Chem. Soc.*, **72**, 441 (1950).
 - 24) P. W. Schindler, H. Gamsjäger ; *Colloid Polymer Sci.*, **250**, \$ 759 (1972).
 - 25) K. Imamura, Y. Kawasaki, T. Nagayasu T. Sakiyama, K. Nakaniishi ; *J. Biosci. Bioeng.*, **103**, 7 (2007).