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### Study on adsorption trends of bovine serum albumin onto metallic surface

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#### ABSTRACT

The hypothesis that the adsorptive affinity of macromolecules to titanium, Ti, is enhanced with the presence of  $\text{Ca}^{2+}$  was tested, by dissolving the adsorbate in Hanks' solution. The study investigated the adsorption ability of Bovine Serum Albumin, BSA (dissolved in Hanks' solution) onto Ti powder. The amount of BSA adsorbed increases with contact time. Saturation of the binding sites of Ti surfaces by BSA took place at 45minutes contact time. The amount of BSA adsorbed onto Ti at 30minutes contact time for all the experimental groups was 1.5times higher than that at 15minutes. The adsorption isotherms of BSA onto Ti exhibited the Langmuir type, with maximum of 0.138mg per gram of Ti powder. The BSA-Ti association constant was 4.62ml/mg. Pretreatment of Ti with calcium, or magnesium alone, resulted in augmented adsorption of BSA onto Ti. No increase in adsorption was observed following pretreatment of Ti with potassium. These results point to the involvement of electrostatic interaction in adsorption of BSA onto Ti. Study of the adsorptive strength indicated strong adsorptive ability of BSA onto Ti, at the eighth rinse no more BSA molecules was adsorbed onto Ti.

**Key words:** Bovine Serum, Titanium, Adsorption, Characteristics

#### INTRODUCTION

Whether as a result of accident, disease, natural defect, or just wear and tear, there are occasions when it is necessary to treat an individual by replacement or enhancement of some parts of the body. While many materials exist with adequate physical properties to perform the desired functions, few can operate without problems when placed within the hostile environment of the body (1). Blood is the first biological fluid the implant comes in contact with when inserted in the body (2). Tang *et al.* (3) observed that the first event at the tissue-material interface, which dictates biocompatibility, is the non-covalent adsorption of plasma proteins from blood onto the surfaces. An alternative method of achieving biocompatibility might be directly incorporate beneficial ions, such as calcium, within the titanium lattice. One method of achieving this is via

ion implantation, a process by which ionized atoms or molecules are accelerated through an electrostatic field into the substrate, altering its chemical, physical and optical properties. The depth of implantation depends on the implantation dose, energy and chemistry of the target. Studies suggest that ion implantation of calcium ion may enhance the responses of bone cells to titanium *in vivo* (4). BSA has been given little attention with respect to its role in the functional properties of whey protein concentrates, and makes up only about 5% of the protein in whey concentrates. It plays a role in mediating lipid oxidation, since BSA has been shown *invitro* to protect lipids against phenolic induced oxidation (5). Once proteins have adsorbed to the surface of the foreign material, host cells no longer see the underlying material, but only the protein coated surface over-layer. This adsorbed protein over-layer rather than the foreign material itself, and then mediates the types of cells that may adhere to the surface, which ultimately can determine the type of tissue that forms in the vicinity. The adsorption of proteins on a solid surface is an important phenomenon taking place as soon as a foreign material is brought into contact with a biological system. Lundstrom (6) developed simple dynamics model for protein adsorption based on the geometric interaction between the adsorbed molecules. Johnson *et al.* (7) reported that protein form generally thin layer on surface like gold and platinum and thicker, more extended layer on (oxidized) metals like titanium and zirconium. Titanium reacts immediately with oxygen when exposed to air forming a 5-6nm surface oxide layer. This layer which increases during prolonged exposure to oxygen consists of titanium dioxide (TiO<sub>2</sub>), but Ti<sub>2</sub>O<sub>3</sub> and other oxides are also present (8, 9).

It is well established that titanium oxide surface bind cation, particularly polyvalent cations (10). Titanium is one of the most widely use dental implant materials. Albumin has been identified in several additional types of dental biofilms such as salivary pellicle on hydroxyapatite (11, 12). On hydroxyapatite ceramics (12) and on denture acquired pellicle (13). Rosenberg *et al.* (14) and Lee *et al.* (15) suggested mechanism of HAS adsorption to biomedical polymers include hydrophobic interaction and electrostatic force. Adsorption of proteins on the surface of biomaterials has been considered to be the most important issue in evaluating their biocompatibility (16).

The aim of this research is to study the kinetics/rate of adsorption of bovine serum albumin onto titanium powder by determining the adsorptive amount and adsorptive strength. The research is also aimed at studying the kinetics of the adsorption characteristics of BSA onto titanium powder, to simulate the *invivo* situation, BSA was dissolved in Hanks

## MATERIALS AND METHODS

### Reagents

Titanium powder, Hanks' solution (HS), Biuret reagent, 0.1% and 0.5% BSA solution, CaCl<sub>2</sub>, KCl, KH<sub>2</sub>PO<sub>4</sub>, MgSO<sub>4</sub>.7H<sub>2</sub>O, NaCl, NaHCO<sub>3</sub>, Na<sub>2</sub>HPO<sub>4</sub>.2H<sub>2</sub>O (Lori and Hanawa, 2001).

### Biuret Reagent

1.50g of hydrated copper sulphate CuSO<sub>4</sub>.5H<sub>2</sub>O, 6.00g of sodium tartarate (NaKC<sub>4</sub>O<sub>6</sub>.4H<sub>2</sub>O) were weighed into a 100ml flask and stirred vigorously. This was then transferred into 1000ml standard flask into which 300ml of 10% w/v sodium hydroxide was added. This was then made up to mark with distilled water.

**Adsorptive amount of BSA onto Titanium**

Titanium powder (5.0, 15.0 and 30.0mg) was added to 1ml BSA solution (0.1% or 0.5%). The solution was then shaken for 15minutes in an incubator at 37°C. The suspensions were allowed to settle and the supernatant solution was collected. The amount of adsorbed BSA was then calculated by subtracting the amount of unabsorbed (free) BSA remaining in the supernatant from the amount of BSA in the control (BSA not adsorbed into Ti powder sample). The procedure was repeated for 30, 45, 60 and 90 minutes contact time.

**Protein Assay**

Aliquot of 0.2ml samples of BSA were placed in 12x100mm test-tubes containing 4.0ml of biuret reagent and the content mixed by inversion. The optical density of the solution was measured at 540nm in a colorimeter. The concentration of the protein in the sample by reference to the calibration curve was calculated according to the standard solution of BSA.

**Adsorptive Strength of Ti onto BSA**

The adsorptive strength was determined by measuring the change in concentration of the supernatant solution after the samples were rinsed with Hanks' solution. 250mg of Ti-powder was added to 10ml BSA solution (0.1%) after incubation at 37°C for an hour, 8ml of the supernatant solution above the Ti was measured out. Then 2ml of HS at 37°C was added to the mixture. The tube was shaken for 1minute to mix the solution and the titanium particles, following which 8ml of the supernatant solution was again removed by the same method. The rinsing procedure described above was performed at 0, 1, 2, 4, and 8 times in different experimental groups. The adsorptive strength was calculated by comparing the protein concentration in the supernatant solution collected for the no rinse and the multiple rinse samples.

**Pretreatment of Titanium Powder**

1g of titanium powder was suspended for 48hours at room temperature in 1ml of Hanks' solution containing 0.1M CaCl<sub>2</sub>, 0.1M MgCl<sub>2</sub> and 0.1M KCl respectively. Titanium powder suspended in HS served as control. The powder was then filtered, washed three times with HS and left to dry at room temperature for 48hours.

**Profile of BSA adsorption onto titanium powder**

250mg of titanium (untreated) was placed in a micro-centrifuge test-tube and suspended in 2.5ml of solution containing 0.2 to 1.4mg/ml purified albumin and shaken constantly for 48hours at 37°C. The suspension was allowed to settle and the supernatant solution was collected. Biuret assay was performed on 0.1ml of the supernatant samples. The amount of protein adsorbed was calculated by subtracting the unabsorbed (free) protein remaining in the supernatant from the amount of protein in the control.

**Effect of Cations on BSA Adsorption onto Titanium**

Untreated titanium, calcium-treated titanium (Ca-Ti), magnesium treated (Mg-Ti) and potassium-treated titanium (K-Ti) powders prepared were suspended in 1.0mg/ml BSA and adjusted to pH 3.0 or pH 7.0 with disodium phosphate buffer. The suspensions were shaken for 2hours at 37°C and the unabsorbed protein was measured as described above.

## RESULTS AND DISCUSSION

### Adsorptive Amount of BSA

As shown in Fig. 1, the amount of BSA adsorbed increased with an increase in the amount of adsorbent. This is so because an increase in the amount of titanium makes available more surfaces for reaction between the BSA molecule and adsorbent. Also as shown in Fig. 2, the amount of BSA adsorbed increased with an increase in incubation time and the concentration of BSA solution. There was a general increase in the amount of BSA adsorbed with respect to time, since adequate time is required to allow the adsorbate molecules move from the bulk solution to the surface of the adsorbent particles. There is however, a general decrease in the trend of the amount of BSA between 45 and 60 minutes indicating monolayer saturation.

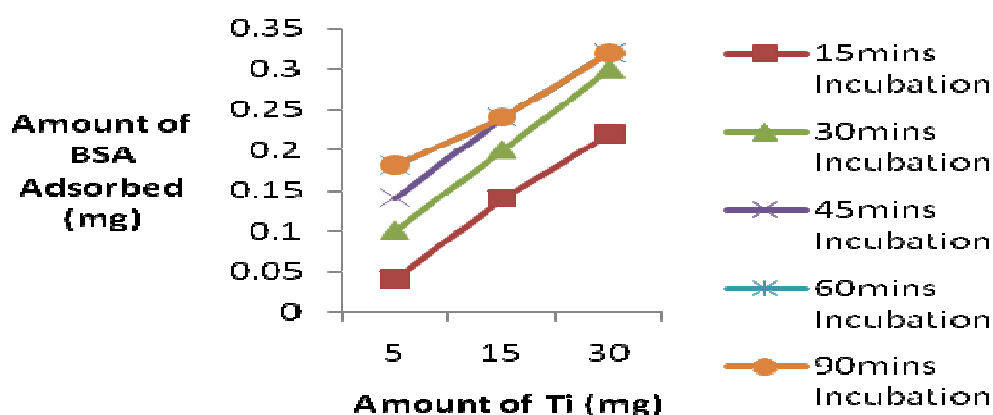


Fig. 1 Adsorption of 0.1% BSA with amount of Ti (mg)

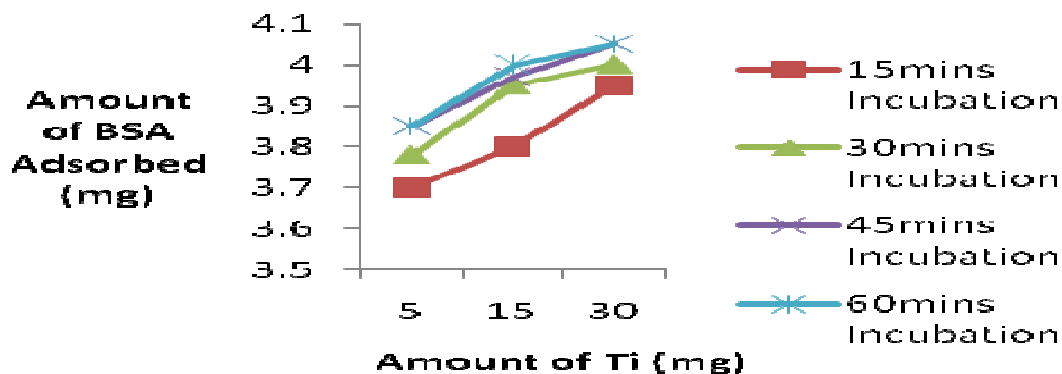


Fig. 2 Adsorption of 0.5% BSA with amount of Ti (mg)

### BSA Adsorption Affinity

The maximum amount of adsorbed BSA onto Ti and affinity between the BSA molecules and Ti were calculated according to the Langmuir adsorption isotherm. The equation of the Langmuir adsorption isotherm being:  $F/B = 1/K_a N + 1/N.F$ . Where B=bound BSA,  $K_a$  =association

constant, F= free BSA, N= maximum amount of BSA adsorbed. This agrees with the regression equation on the graph in Fig. 3.  $Y = 7.2203x + 1.5684$ .

The Langmuir adsorption isotherm showed linearity of the adsorption process as reported in Fig. 4 with a maximum of 0.138mg of BSA adsorbed per gram of titanium powder ( $N=1/\text{slope}$ ). The BSA-Ti association constant is 4.62.

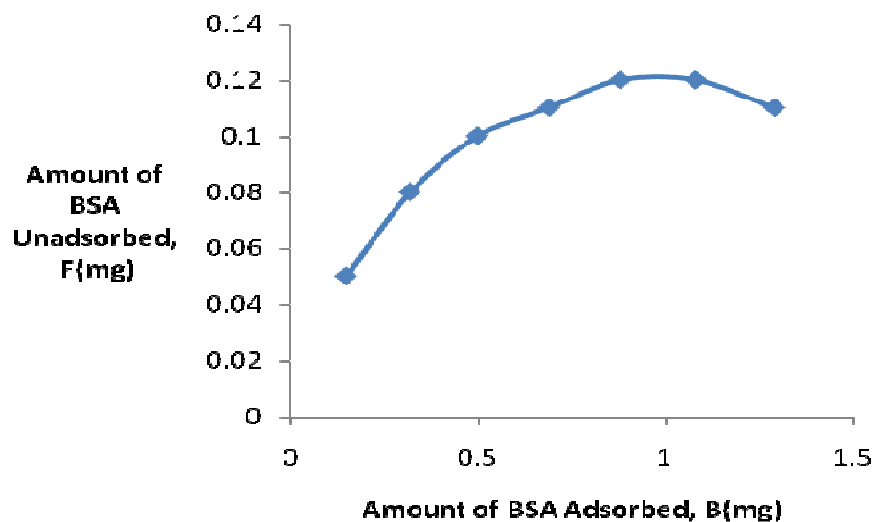


Fig. 3 Adsorption Profile of BSA

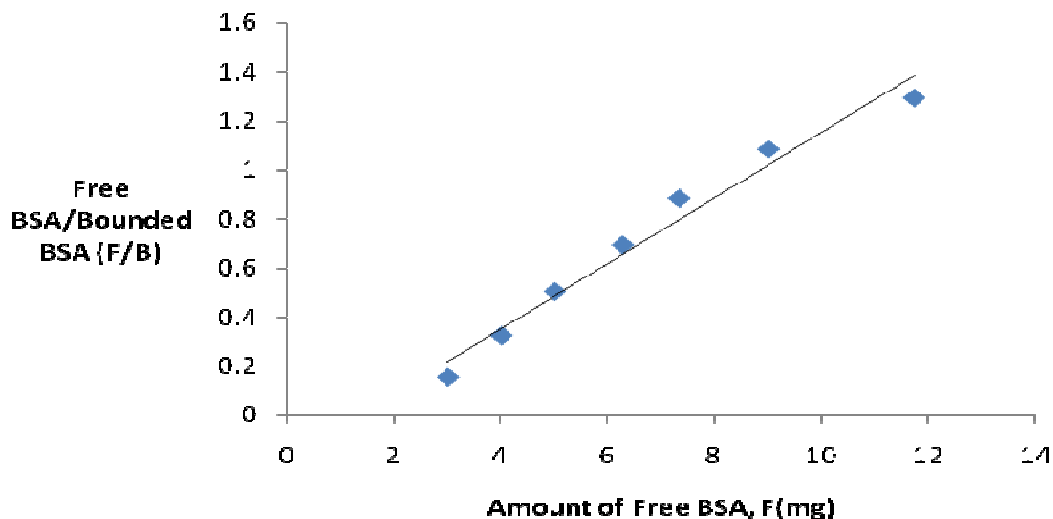


Fig. 4 Adsorption Isotherm of BSA onto Ti Powder

#### Effect of Metal ions on BSA Adsorption

The Fig. 5 show the adsorption of BSA onto Ti-powder pre-treated with  $\text{CaCl}_2$ ,  $\text{MgCl}_2$  and  $\text{KCl}$ . There was a general increase in the amount of adsorbed BSA onto Ti powder following the

suspension of Ti powder when pre-treated with CaCl<sub>2</sub> or MgCl<sub>2</sub> with reference to the control. There was no increase in the adsorption of BSA onto Ti-powder pre-treated with KCl. This observation indicates that Ca<sup>2+</sup> or Mg<sup>2+</sup> ions act through non-specific electrostatic attraction that can be attributed to their electrovalence.

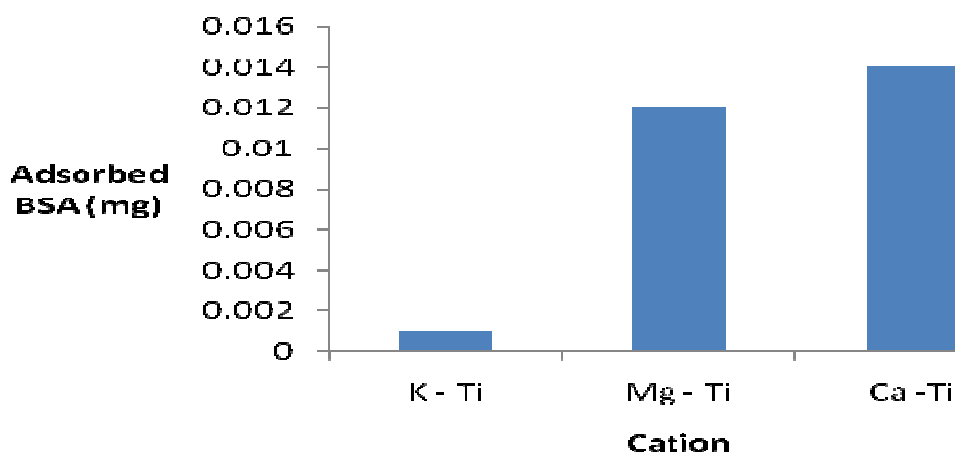


Fig. 5 Effect of cations pre-treated on Ti powder

#### Adsorptive Strength of BSA

The proportion of BSA adsorbed onto titanium was 2.21mg (100%) without rinsing and the proportions of undesorbed BSA were 68.2%, 35.9%, 19.9% and 0.00% with 1, 2, 4, and 8 rinses respectively as shown on fig. 6. The result shows almost a complete desorption of the BSA on Ti surface at the eighth rinse. The apparent collapse of the force of attraction binding the BSA onto the Ti surface indicates that the force of attraction is weak.

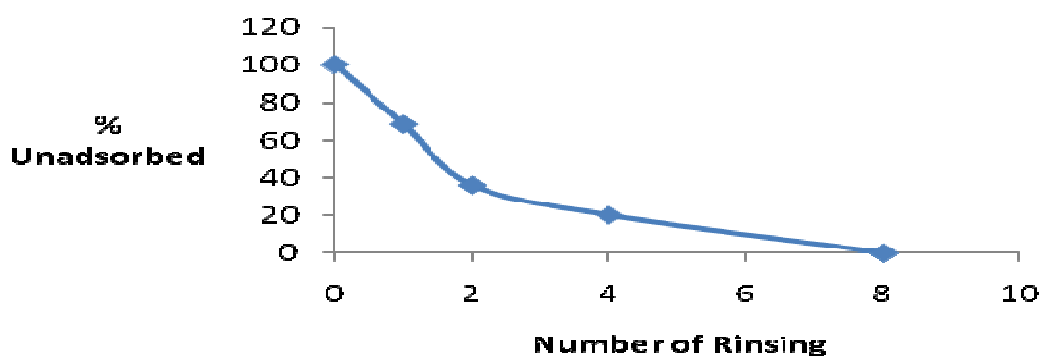


Fig. 6 Percentage Undesorbed After Rinsing

#### CONCLUSION

The adsorption of BSA onto Ti shows a mono-layered adsorption mechanism of the Langmuir type. The results from the rinsing of the BSA from the Ti surface indicate a physisorption process. In light of the results obtain, divalent cations such as calcium and magnesium were seen to enhance BSA adsorption onto Ti surface. The binding of BSA to the calcium and magnesium

treated Ti powders were observed to be much higher than that of potassium, which is also found in physiological fluid showed significant level of increase in the amount of BSA adsorbed compared to that with divalent cations and as such the mechanism of the adsorption process can be attributed to electrostatic attraction that depends on the amount of Ti and also on the concentration of BSA solution.

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