

Kinetic Study of the Adsorption of Mucin onto Titanium Surface

¹J.A. Lori, ²M.B. Ayeni, ¹E.E. Jasper and ¹E.J. Ekanem

¹Department of Chemistry Ahmadu Bello University, Zaria, Nigeria

²Department of Science Laboratory Technology, School of Applied Sciences,
Kogi State Polytechnic, Lokoja, Nigeria

Abstract: The aim of this study was to investigate the kinetics of adsorption of mucin onto titanium surface. Mucin adsorbed onto untreated, calcium and potassium treated titanium surfaces in vitro tests were monitored using uv-visible spectrophotometer the effect of contact time was determined by the "limited bath technique." The parameters used in this study such as the amount of titanium, concentration of mucin solution, reaction temperature and pH were optimised by preliminary experiments as 0.02 mg, 0.1 mg/mL, 35°C and 6.4, respectively. The amount of mucin adsorbed increased with time until equilibrium was attained after about 115 min with the maximum concentration of mucin adsorbed being 0.0603 mg/mL for Ca-Ti followed by that of onto K-Ti implying that the adsorption is not influenced by the presence of Ca²⁺ ions. Analysis of kinetic data obtained with Lagergren, Bhattacharya/Venkobacharya and Flick's models revealed that the adsorption is first order with respect to mucin molecules and that diffusion is the predominant mechanism for the adsorption.

Key words: Adsorption, biomaterial, kinetics, Mucin, rate of adsorption, Titanium

INTRODUCTION

Implants are frequently used by medical and dental professions to anchor artificial limbs, teeth etc. Titanium implants have been used extensively in the medical and dental fields with excellent results because it is judged to be wholly-bio-compatible. The success of titanium as an implant material is indexed by its interaction with surrounding tissue and fluids following surgical implantation (Lori and Nok, 2004). Biocompatibility is controlled by the initial interfacial reactions that occur between the implants and the tissues, especially on the protein adsorption processes that take place at the interface (Lee and Kim, 1974).

Proteins are biological macromolecules vital to cell structure and function. The ability to incorporate proteins onto synthetic materials offers the promise of new devices of high potential impact on the quality of human life. Mucin is a glycoprotein with repeated amino acid sequences rich in serine, threonine and proline (Brockhause and Kuhn, 1997), present in the secretion of the gastrointestinal (pancreatic, small intestine and salivary), respiratory and reproductive tracts (Rosalind and Stuart, 1980). There are records of high affinity of protein in saliva and human fluids for titanium (Ellingsen, 1991).

Several studies on titanium-protein interactions have been undertaken for the development of body implants. Studies on the mechanism of adsorption of mucin, albumin, and fibrinogen, on titanium surface have been

reported (Lori and Nok, 2004). Kinetics study of the adsorption of lactalbumin on titanium has also been reported (Bentaleb *et al.*, 1998). Kothari *et al.* (1995) investigated the adsorption of several serum proteins on titanium oxide. It has been reported however, that adsorption of protein on certain body implants could stimulate the growth and accumulation of micro-organisms on dental surfaces (Lori and Nok, 2004). This necessitates the aim of this research study to kinetically study the adsorption of mucin onto the Ti surface, as it will provide parameters that will be useful for the development of implants exhibiting improved biocompatibility. The aim was achieved by investigating the rate of adsorption, rate constant, and effect of pH on rate of adsorption and rate constant, effect of Ca²⁺ and K on rate of adsorption and rate constant for the adsorption of mucin onto titanium.

EXPERIMENTAL METHODS

These studies were conducted in the Inorganic and Analytical Chemistry Research Laboratories of Ahmadu Bello University, Zaria-Nigeria from 2008.

Reagents and solutions: All reagents were of analytical grade unless otherwise stated. Also deionised water was used in the preparation of solutions unless otherwise stated. Phosphate Buffer Saline Solutions (0.02 M, pH 3 - pH 8) were prepared using calculation methods of Henderson-Hasselbalch equation (Po and Senozan, 2001).

Optimization of variables:

Optimization of Ti metal: Titanium metal (crushed sponge) powder (BDH Chemicals Ltd., Poole, England) was used. Titanium masses ranging from 0.02 to 0.10 g were weighed into centrifuge tubes, 10 mL of 0.1 mg/mL mucin solution at pH 4.7 (the determined isoelectric pH of mucin) was poured into each of the tubes and allowed to incubate at 25±1°C in a thermostated bath for 2 h with constant stirring. The tubes were centrifuged at 656 g for about 5 min and 0.1 mL of the supernatant assay for protein using Bradford method at 595 nm uv-visible absorption band wavelength (Bradford, 1976). The concentration of free protein (mucin not bound to Ti) corresponding to the absorbance measured was calculated according to the standard calibration curve of bovine serum albumin (sigma). The amount of bound mucin to Ti was then calculated by subtracting the amount of unadsorbed mucin in the supernatant from the amount of mucin in the control experiment.

Optimization of the concentration of mucin solution:

The optimum mass of titanium (0.02 g) obtained was weighed into centrifuge tubes. 10 mL of a respective mucin solution at pH 4.7 with concentrations ranging from 0.02 to 1.0 mg/mL was poured into each tube. The tubes were incubated at 25±1°C in the thermostated bath for 2 h with constant stirring. The tubes were then centrifuged at 656 g for about 5 min. Free protein estimation and the concentrations of bound mucin were calculated as in 2.2.1.

Optimization of temperature for the adsorption of mucin onto Ti:

Titanium (0.02 g) was weighed into each of centrifuge tubes. A 10 mL aliquot of 0.1 mg/mL (optimum concentration of mucin solution obtained) was poured into each tube, incubated at a respective temperature ranging from 25 to 45°C in the thermostat bath. The adsorption at a particular temperature was determined after for 2 h with constant stirring. The tubes were then centrifuged at 656 g for about 5 min. Free protein estimation and the concentration of bound mucin were calculated as above.

Optimization of pH for the adsorption of mucin:

Titanium metal (0.02 g) was weighed into each of nine centrifuge tubes. Mucin solutions (0.1 mg/mL) were made with the phosphate buffer saline at the pH ranging from 3.0 to 8.0. Mucin solutions (10 mL in each case) were measured and poured into tubes containing the 0.02 g optimum mass of titanium metal. The tubes were incubated at 35°C in the thermostated bath for 2 h with constant shaking, centrifuged at 656 g for about 5 min. Free protein estimation and the concentration of bound mucin were calculated as above except that the blank used was prepared from 0.1 mL phosphate buffer saline at a particular pH.

Pre-treatment of Ti: Titanium metal (1 g each) was weighed into two different test tubes and suspended for 24 hours at 25°C in 1 mL of 0.1 M calcium chloride and 0.1M potassium chloride prepared. Titanium metal suspended in de-ionised water served as control. The titanium metal from each tube was then washed thrice with water and left to dry at room temperature.

Kinetic studies: The effect of contact time was determined by the "limited bath technique". The optimum mass of titanium metal (0.02 g) was weighed for each of treated (Ca-Ti and K-Ti) and control (Un-Ti) and added to 10 mL of 0.1 mg/mL mucin solution prepared in Phosphate Buffer Saline (PBS) pH 6.4 under shaking. The temperature of the solution was held constant at 35°C in a thermostated bath. After 5 min intervals in the range 0-130 min, 0.1 mL of the supernatant was taken for spectrophotometric measurements in duplicate using the method of Bradford, (1976). Free protein estimation and concentration of bound mucin were calculated as above except that the blank used was prepared from 0.1 mL of the Phosphate Buffer Saline (PBS) of pH 6.4.

Adsorption rate constants: The time- concentration profile characteristics of the adsorption of mucin onto Ti surfaces were tested with Lagergren first-order (Lagergren, 1898), Bhattacharya/Venkobacharya (Ho and McKay, 1998) and the intra-particle diffusion (Weber and Morris, 1963) models. The linear form of the first order equation of Lagergren is generally expressed as follows:

$$\log (q_e - q_t) = \log (q_e) - (k/2.303) t \quad (1)$$

where q_e and q_t are the adsorption concentrations (mg/mL) at equilibrium and time t , respectively, t is the adsorption time (min), K_{ad} , (mg/mL min) is the adsorption rate constant of the first order adsorption. If first order kinetics is applicable, the plot of $\log (q_e - q_t)$ versus t should give a linear relationship from which the order parameters can be determined from the slope and intercept of the adsorption plot. If the intercept is equal to $\log q_e$, then the adsorptions is first order.

The adsorption rate constant K_{ad} , of the first order adsorption for the different surfaces using Bhattacharya/Venkobacharya equation (Bereket *et al.*, 1997) is generally expressed as follows:

$$\ln (1 - u_t) = - (K_{ad}) t \quad (2)$$

$$u_t = C_o - C_t / C_o - C_e \quad (3)$$

where K_{ad} is rate constant of the first order adsorption in (mg/ml min) and C_o , C_e and C_t are concentrations (mg/mL) of mucin in solution at initial, equilibrium and time t (min), respectively. The plots of $\ln (1 - u_t)$ versus t should give straight lines for the different surfaces for first order equations.

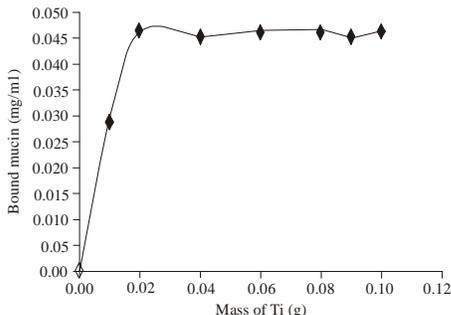


Fig. 1: Optimization of mass of Ti

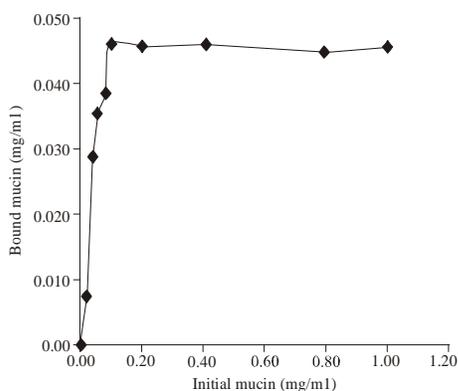


Fig. 2: Optimization of the concentration of mucin in the adsorption of mucin onto Ti

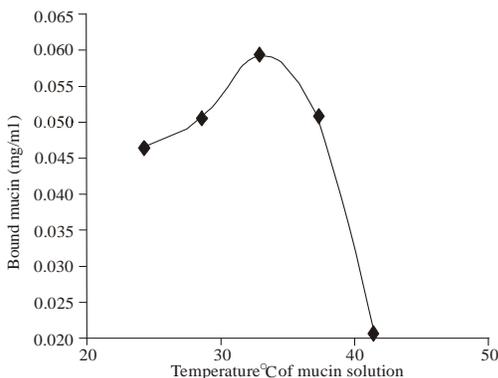


Fig. 3: Optimization of temperature for the adsorption of mucin onto Ti

Adsorption of sorbate on sorbent proceed in several steps involving transport of the solute molecules from the aqueous phase to the surface of the solid particulate and diffusion of the solute molecules into the interior of the pores. The linear driving force concept was equally used to analyze the kinetic data by a diffusion-controlled adsorption model. The intra-particle diffusion rate constant is given by the equation:

$$q_t = k_p t^{1/2} + C \quad (5)$$

where k_p is the intra-particle diffusion rate constant in (mg/mL min), C is the boundary layer thickness and q_t is the amount of mucin adsorbed (mg/mL) at time t (t is the time in minutes). If the plot of q_t versus t results in a linear relationship with zero intercept, the adsorption process is particle- diffusion controlled.

RESULTS AND DISCUSSION

Optimization of Ti metal: The adsorption of mucin onto titanium surface depends on the efficiency of the reaction at the interface between the solid- and liquid-phase of the reactors, the amount of titanium, concentration of mucin solution, reaction temperature and pH had major effects and had to be optimized. The influence of the mass of titanium was studied in the range of 0.01-0.1 g. The optimization profile is shown in Fig. 1. The optimum mass of Ti was 0.02 g. The amount of mucin adsorbed decreased as the mass of Ti increased. The decrease in the amount adsorbed with increasing mass of Ti is due to the concentration gradient between solute concentration in solution and the solute in the surface of the Ti. This can be explained by the fact that a fixed mass of Ti can only adsorb a certain amount of protein.

Optimization of the concentration of mucin solution: The plot of the concentration of mucin adsorbed (bound mucin) as a function of initial concentrations of mucin is presented in Fig. 2. The optimum concentration adsorbed was 0.1 mg/mL of mucin.

Optimization of temperature for the adsorption of mucin onto Ti: The results of the effect of temperature on the adsorption of mucin onto titanium metal are shown in Fig. 3. The optimum temperature of the adsorption process is 35°C which is consistent with the physiological temperature of 37°C.

Optimization of pH for the adsorption of mucin: The pH optimization profile is shown in Fig. 4. The optimum pH for the adsorption process was pH 6.4. The net charge of a given protein depends on its isoelectric pH and the pH of the environment. The isoelectric pH of mucin determined in this work is 4.7. At higher pH values mucin undergoes a neutral-acid transition and becomes negatively charged; while at lower pH values mucin undergoes a neutral-basic transition and becomes positively charged. At pH 6.4 the protein and the titanium (whose surface is in oxide form) are both negatively charged; so the electrostatic repulsion barrier should be overcome mainly by hydrophobic interaction. The nature of the hydrophobic interaction is however not apparent since titanium oxide surface is hydrophilic. The maximum amount of mucin was adsorbed at pH 5 because of its

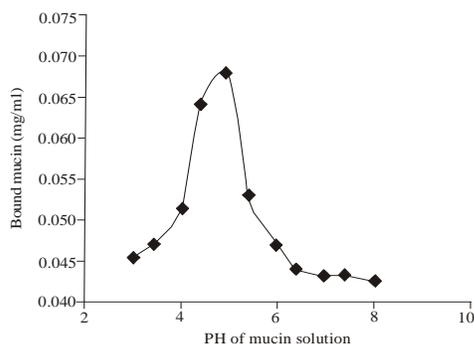


Fig. 4: Optimization of pH for the adsorption of mucin onto Ti

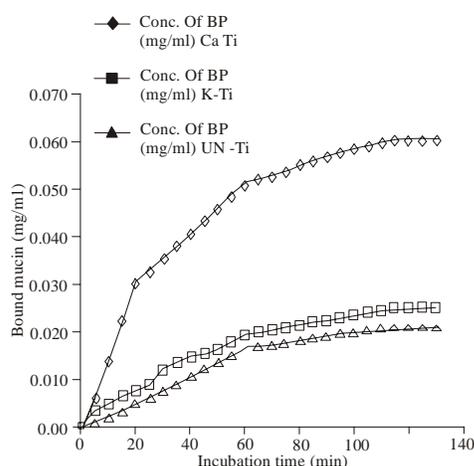


Fig. 5: Effect of incubation time on the rates of adsorption of mucin onto Ca-Ti, K-Ti and Un-Ti

closeness to its isoelectric pH. The amount of mucin adsorbed decreased beyond pH 7.4 meaning that the protein began to denature at higher pH values.

Kinetic studies: The rates of adsorption profiles are shown in Fig.5. Adsorption processes attained equilibrium at 115 min. There were rapid increases in the rates of adsorption initially from 0 to 60 min but which began to slow down till equilibrium was attained for the three types of titanium surfaces. The initial rapid increases in rates could be attributed to the concentration gradient existing at the start of the adsorption process between solute concentration in solution and that at the Ti surface. As the surface coverage increased the protein concentration gradient reduced and gave way to slower adsorption. The kinetic regimes described by protein adsorption curves reveal transport-limited and adsorption-limited regimes. During the initial stage of the adsorption, diffusion is the rate-limiting step at the short times. Following the sufficient adsorption to the surface, an adsorption-limited became prominent. The adsorption-limited regime is divided into two regions, one in which

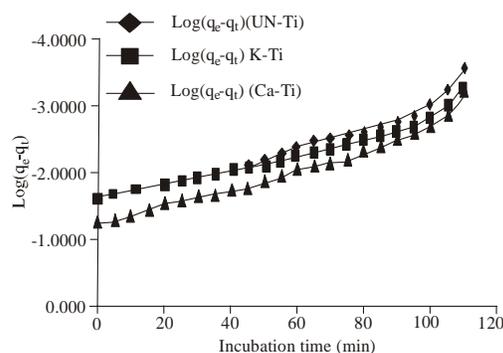


Fig. 6: Lagergren plots for determining the effect of incubation time and pH on rate constants for the adsorption of mucin onto Un-Ti, Ca-Ti and K-Ti

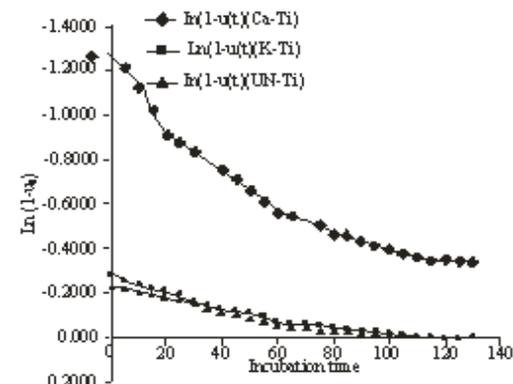


Fig. 7: Bhattacharya/Venkobacharya plots for determining the effects of incubation time and pH on rate constants for the adsorption of mucin onto Un-Ti, Ca-Ti and K-Ti

there is a linear decrease in adsorption rate and a second region in which there is a non-linear approach to saturation is seen.

The amount of mucin adsorbed at equilibrium (q_e) for Un-Ti, Ca-Ti and K-Ti were 0.0209, 0.0603 and 0.0249 mg/mL, respectively. These were equivalent to 20, 60 and 24% of the initial mucin solution 0.1 mg/mL used. Amounts adsorbed at 1 h of the adsorption process were 0.0150, 0.0509 and 0.0192 mg/mL for Un-Ti, Ca-Ti and K-Ti, respectively. These correspond to 71.8, 84.4 and 77.1% of the total protein adsorbed at equilibrium. This means that much of the adsorption occurred at 1 h of the adsorption process.

Mucin is negatively charge at the pH of the experiment. Though the amount of protein adsorbed at equilibrium onto Ca-Ti was up to 3 times that for untreated titanium (Un-Ti), equilibrium of the adsorption process is not influenced by the presence of the calcium ions. The amount of mucin adsorbed by the potassium treated titanium (K-Ti) simply suggests that calcium ions only open up more binding sites for protein adsorption onto Ti surface.

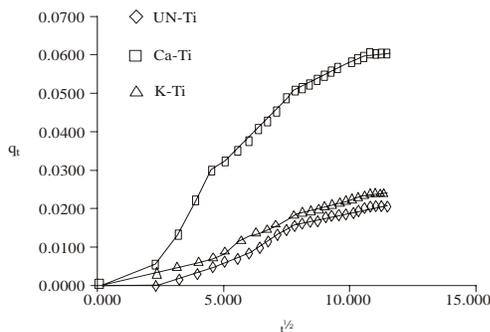


Fig. 8: Flick's plot for determining intra-particle diffusion rate constant for the adsorption of mucin onto Un-Ti, Ca-Ti and K-Ti

Adsorption rate constants: The adsorption rate constant profiles are shown in Fig. 6-8. An error function is required to evaluate the fitness of each kinetic obtained from the optimization process employed. In the present study, the linear coefficient of determination, R^2 , was used. The values for rate constants, log of the concentrations at equilibrium (q_e), intercepts and R^2 for each equation were fitted to the experimental data and. The highest values of R^2 were obtained when the experimental data were fitted into the intra particle diffusion equation ($R^2 = 0.955-0.980$).

The result presented in Fig. 6 was obtained when the data from adsorption experiments were analysed using Lagergren equation for the first-order reaction. When the values of $\log(q_e - q_t)$ were plotted against time, the linearity of the plots were high, rate constants were calculated from the slopes and intercepts were almost the same as the log of the concentration at equilibrium (q_e), meaning first-order reaction (Lagergren, 1898). The first order rate constants were 3.45×10^{-2} , 2.99×10^{-2} and 2.99×10^{-2} (mg/mL min) for Ca-Ti, K-Ti and Un-Ti, respectively. The k_{ad} value for Ca-Ti was the highest, followed by K-Ti and was least for Un-Ti. The adsorption rate constant shown in Fig. 7 was obtained when the data was analysed using Bhattacharya/Venkobacharya equation. When $\ln(1 - u_t)$ was plotted against time (min), straight line plots were obtained for the three types of Ti surfaces. The range of the linearity of the plots were given as $R^2 = 0.930-0.947$ and the first order rate constants obtained as 4.606×10^3 , 1.612×10^2 and 4.606×10^3 (mg/mL min) for Ca-Ti, K-Ti and Un-Ti. The result profiles presented in Fig. 8 were obtained when the data were analysed using Flick's intra-particle diffusion equation. These gave straight lines for the treated and untreated Ti and the intercept almost zero meaning that the adsorption process is limited by the internal diffusion process (Bhattacharyya and Sharma, 2004). This is an indication that the particle diffusion mechanism was predominant. The rate constants were calculated from the slopes, intercepts gave the boundary layer thickness and

the range of R^2 was 0.955-0.980. The intra-particle diffusion rate constants were obtained as 0.005, 0.001 and 0.001 (mg/mL min) for Ca-Ti, K-Ti and Un-Ti, respectively.

CONCLUSION

Based on the data obtained from these studies, the optimum values of reaction parameters including mass of Ti metal, concentration of mucin solution, pH and temperature respectively were obtained as 0.02 g, 0.1 mg/mL, 6.4 and 35°C . The amount of mucin adsorbed at equilibrium from the initial mucin solution (mg/ml) onto Ca-Ti, K-Ti and Un-Ti surfaces were found to be 0.045 ± 0.006 , 0.017 ± 0.003 and 0.014 ± 0.00 (mg/mL), these being equivalent to 60, 24 and 20%, respectively. The rates of adsorption increased within the 1 h of the adsorption process and thereafter began to decrease in a steady pattern till equilibrium was attained. The results showed that 84, 77 and 70% of total protein adsorbed at equilibrium occurred at 1 h of the adsorption process for Ca-Ti, K-Ti and Un-Ti respectively.

Adsorption rate constants values obtained using Lagergren first order rate equation were 3.45×10^2 , 2.99×10^2 and 2.99×10^2 (mg/mL min) for Ca-Ti, K-Ti and Un-Ti respectively.

Using Bhattacharya/Venkobacharya first order rate equation, adsorption rate constants values obtained were 4.606×10^3 , 1.612×10^2 and 4.606×10^3 (mg/mL min). Intra-particle diffusion rate constants values obtained using Flicks diffusion equation were 0.005, 0.001 and 0.001 mg/mL min for Ca-Ti, K-Ti and Un-Ti, respectively. These results revealed that the adsorption reaction is a first-order reaction with respect to the mucin molecules in solution and that the kinetic regimes described by protein adsorption curves shown the transport-limited and adsorption-limited regimes. During the initial stage of the adsorption, diffusion was the rate-limiting step at the short times. Following the sufficient adsorption to the surface, an adsorption limited process was the event. The amount of mucin adsorbed on Ca-Ti was 3 times that absorbed by the Un-Ti and that large amount of protein adsorbed occurred within 1 h of the adsorption process. From these it can be concluded that much mucin is adsorbed onto calcium treated titanium but the adsorption is due to diffusion.

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