A kinetics study of fibrinogen adsorbed on titanium powder

S. K. Omotugba¹*, J. A. Lori², E. J. Ekanem² and A. J. Kagbu²

²Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

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A kinetics study of fibrinogen adsorbed on titanium has been investigated by means of spectrophotometer of the type developed for micro-enzyme linked immune-sorbent assay determination. Implants invoke inflammatory responses from the body and an attempt to alleviate the problem caused by the adsorption of protein on artificial implants mostly used in the medical, informed this study. The experimental isotherm data were analysed using the Langmuir equation and the amount of fibrinogen adsorbed increase in contact time and reached close to saturation after 120 min for the initial protein concentration 0.1, 0.2, 0.4 and 0.6 mg/ml used in this study. The adsorption process was found to be of first order with respect to the bulk concentration, and in the transient region, the adsorption process is independent of the concentration of protein in the bulk and the actual rate constants for each fibrinogen concentration investigated are, 0.01706, 0.01856, 0.01840 and 0.01841 cm/s.

Key words: Titanium, fibrinogen, implants, kinetics rate, adsorption.

INTRODUCTION

Protein adsorption, defined as a non-covalent bonding of a protein to a surface, is important in many applications in biotechnology and in particular, it plays a key role in the biocompatibility of medical implants. The composition and the evolution with time of adsorbed protein layers at solid interfaces are of great biological and industrial significance (Bentaleb et al., 1998). Blood plasma contains a large number of proteins having different affinities for a sorbent. Among these, fibrinogen is of most interest because of the role it plays in the thrombin-blood coagulation cascade (Agashe, 2005). The adsorption of this protein (fibrinogen) as one component generally is transient and is progressively replaced by molecules having a higher affinity for the same adsorbent. The residence time for protein adsorption becomes a crucial parameter, because the rate of adsorption decreases with increasing contact time of the molecules with the adsorbent surface (Slack and Horbett, 1988 and 1989). Understanding the factors governing protein adsorption on artificial materials and their displacement from the interface provide parameters that can be used for the production of materials exhibiting improved biocompatibility (Ball et al., 1996). Thus the study of protein adsorption on metals must be taken seriously (Lori et al., 2001). However, Andrade (1985) and Kasemo (1983) reported that in order to be able to characterize the protein adsorption, one must seek for the information about the kinetics of adsorption, conformational changes and a number of physical parameters for the process. Hence, in this present study, particular attention was paid to the kinetics of the adsorption of fibrinogen on titanium which makes it complimentary to the previous studies. The ability of titanium to allow the growth of bone cells coupled with its uncorrosive nature and spontaneous generation of an oxide surface layer when exposed to air or aqueous media (Ana et al., 1999), placed it at an advantage for use as an implant in this study as the oxide layer creates a surface for protein adsorption.

MATERIALS AND METHODS

Chemicals and adsorbent

Fibrinogen (MW: 340,000 Kg, mol⁻¹), was purchased from BDH.
chem. Protein were dissolved in 0.028 M PBS buffer in the presence of 2 M NaCl, with the pH adjusted to 7.4 and 5.1 respectively (Crisson 2000 pH meter and hand held Hanna ion specific H193710 pH).

All chemicals were of analytical grade and used without further purification. Buffer solutions were prepared with deionised water and stored at 4°C until used. Fibrinogen was radio-labelled according to the modification of the McFarlane method (1958) using iodine monochloride as the oxidizing agent and Na$_{125}$I for the labelling. The solution was diluted to the desired protein concentrations in PBS buffer before used. The aliquots were thawed at room temperature to avoid denaturising of the protein. Concentrations of the labelled protein solutions were determined by absorbance at 595 nm with a spectrophotometer.

Thirty-Five mess (500 µm) titanium metal (crushed sponge) powder was obtained from BDH (chemicals Ltd Poole, England). The titanium powder was chemically analysed by atomic absorption spectroscopy (Buck scientific 200A) and found to consist of about 99.7% Ti.

**Experimental procedure**

All the experiments were performed in 5 ml of polycarbonate eppendorf tubes at 37°C.

**Adsorption experiments**

Adsorption experiments were performed by adding 2 ml of solution containing various amount of fibrinogen, 0.2 mg of titanium powder in twelve 5 ml clean eppendorf tubes; three for each time interval of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 min. The surface-to-volume ratio was maintained constant in all experiments. After incubation, the test tubes were centrifuged for about 5 min and the absorbance at 595 nm of 100 µl sample of supernatants were determined. The activity difference before and after adsorption allowed a precise estimation of the protein adsorbed. For the adsorption kinetics, the experiments were stopped at different times whereas the adsorption isotherm was performed by varying the bulk concentration and the protein concentration over a period of 120 min.

**RESULT AND DISCUSSION**

**Kinetics of protein adsorption**

The kinetics model applied in the present investigation to describe the adsorption rate was based on the assumption that adsorption of protein onto metal surface is a pseudo first order reversible process. The modification of linear driving force concept for pseudo first order rate equation (Michael and Ayebaemi, 2005.) was used to obtain the fractional attainment of equilibrium. The equation is expressed as:

\[ \ln (1 - F) = -K_1t \]

where \( F \) is the fractional attainment of equilibrium and \( K_1 \) is the overall rate constant. The fractional attainment is the ratio of the amount of fibrinogen adsorbed from the iodine-labelled fibrinogen after a certain time to that removed when adsorption equilibrium is attained. In order to quantify the changes in sorption as a function of time, the rate of protein adsorption was applied to the experimental data. A plot of fractional attainment of equilibrium (\( F \)) against time of the experimental data for various concentration of fibrinogen onto titanium was made.

\[ F = \frac{C_o - C_t}{C_o - C_e} \]

where \( C_o \) = initial concentration, \( C_t \) = concentration at a certain time, \( C_e \) = concentration of proteins in aqueous phase at equilibrium.

It is evident from Figure 1 that the values of \( F \) decrease with contact time. This indicates that increasing the contact time above the equilibrium time do not have any significant increased sorption on the titanium. The plot further confirmed that, the adsorption rates were rapid and that increasing the initial protein concentrations resulted in a decrease in the initial rate especially for low protein concentration. Furthermore, the plot confirms that optimum adsorption was attained within 60 min. After this period the adsorption rate became almost constant probably due to surface saturation of the titanium. A long contact time necessary to reach equilibrium indicates that the predominant mechanism is physical adsorption and invariably, it will be reversible. A relatively short contact time indicates that chemisorptions is the predominant mechanism. The contact time obtained in this study therefore, shows that both physisorptions and chemisorptions may be involved.
The adsorption isotherm

The adsorption isotherm was determined by bringing the titanium particles in contact with fibrinogen solutions of various concentrations (0.1, 0.2, 0.4 and 0.6 mg/ml) for 2 h (Figure 2). Thereafter, it was observed that the optimum amount (0.0139 µg/µl) for fibrinogen solution of pH 7.4 adsorbed was reached within 60 min of contact. The monolayer shaped adsorption isotherm obtained after 2 h of contact demonstrates a relatively strong affinity of the fibrinogen molecule for this sorbent. The fibrinogen molecule and the oxide surface of the titanium are both negatively charged at pH 7.4, and thus the electrostatic repulsion barrier should be overcome mainly by hydrophobic interaction (Bentaleb et al., 1998). The inflection on the shaped adsorption isotherm shows that a surface transition phenomenon occurs.

Effect of contact time and initial concentration of fibrinogen

Effect of contact time and initial fibrinogen concentration on adsorption by titanium surface are presented in Figure 3. The amount of fibrinogen adsorbed increased with increase in contact time and reached close to saturation after 120 min for the initial protein concentrations 0.1, 0.2, 0.4 and 0.6 mg/ml used in this study. The time to attain saturation state is independent of initial protein concentrations, but in the first 25 min, the initial rate of adsorption was greater for higher initial fibrinogen concentration, this can be attributed to the driving force of the concentration gradient because an increase in protein concentration accelerate the diffusion of protein from the solution onto adsorbent. Hence, the optimum fibrinogen adsorbed 0.0070, 0.0098, 0.0115 and 0.0139 µg/µl as the initial fibrinogen concentration and was increased from 0.1 to 0.6 mg/ml. These values are similar though smaller to those reported by Lori and Nok (2004). The rate of change of the adsorbed fibrinogen was calculated and a plot of these values against the time for the adsorption process is presented in Figure 4. The different curves represent the amount of fibrinogen that remained and adsorbed at a given time on the titanium surface. The data fit into an exponential decay function:

\[ \Gamma^*(t) = 0.01218e^{-K_1t} + \Gamma \]

where \( \Gamma \) represents the amount of irreversibly adsorbed fibrinogen and \( K_1 \) is the rate constant. The fitting of the experimental data were adjusted by the above expression using non linear curve fit program in graph pad prism for windows (version 4.0, 2003), the good agreement between the curve adjustments and the experimental data is materialized by the solid lines in Figure 4 (Bentaleb et al., 1998).

The evolution of the rate constants \( K_1 \) as a function of the labeled fibrinogen concentration (\( C_{\text{bulk}} \)) in solution is demonstrated in Figure 5. The linear dependence \( K_1 \) with \( C_{\text{bulk}} \) indicates that the proteins adsorption process is of first order with respect to the fibrinogen in solution as found by Bentaleb et al. (1998) and further proof is the half life which is almost constant within 95% confidence interval. The rate constants for the different concentration of protein followed the same trend as Michelle (2001) though with little significant at \( P > 0.05 \) when tested using the Kolmogorov and smirnov statistical tools.
Conclusion

The present study has provided important information to the understanding of negatively charged fibrinogen molecules adsorbed on to titanium surface. The adsorption process does not strictly follow the Langmuir model though the adsorption reaction is a first order reaction with respect to the molecules in the bulk solution. In the initial region and for the explored fibrinogen concentration range, the protein adsorbed rates appeared to be independent from protein concentration however, the adsorption kinetics is significantly more rapid at the early stage of adsorption process. The approach to saturation for data obtained is found to be exponential indicating that the adsorption process is not strictly irreversible in nature. These data are of great importance for the development of implants.

REFERENCES