Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

Available online at www.jcbsc.org

Section A: Chemical Sciences

CODEN (USA): JCBPAT

Research Article

Computation and modeling of the flux of gamma globulin molecules onto titanium surface

P. A. Ekwumemgbo[1]*, J. A. Kagbu[1], A. J. Nok[2] and K. I. Omoniyi[1]

[1]*Department of Chemistry, Ahmadu Bello University, Zaria - Nigeria.*

[2]*Department of Biochemistry, Ahmadu Bello University, Zaria - Nigeria.*

Received: 8 December 2011; Revised: 19 January 2012; Accepted: 20 February 2012

ABSTRACT

Titanium (Ti) is widely employed for various biomedical applications due to its biocompatibility. Studies of γ-globulin adsorption onto Ti are important for the development of biocompatible devices. This work examines the rate of adsorption of γ-globulin onto Ti surface. The plot of γ-globulin adsorption onto Ti versus time shows that the initial γ-globulin adsorption increased linearly with time with the rate constant obtained 0.032min^{-1} while the correlation coefficient (R^2) is 0.9999. The experimental adsorption rate is limited by the availability of γ-globulin molecules at the adsorbing surface, resulting in a situation in which, most γ-globulin molecule that collides with the surface sticks to it. In order to predict the factors that determine the rate of adsorption, the flux of γ-globulin molecules onto Ti surface was computed by developing a model similar to implantation of Ti into the body for the transport-limited of γ-globulin adsorption onto Ti from flowing γ-globulin solution. It was deduced that the rate of this adsorption is determined by the wall shear rate, the diffusivity of γ-globulin and the bulk amount of γ-globulin solution. An adsorption model was developed in order to seek the factors that determine the intrinsic (qualitative) adsorption and desorption rate constants. The experimental adsorption data was likened to a simple γ-globulin adsorption model, which comprises of adsorption and desorption processes. The model incorporated the rate of diffusion through the boundary (unstirred) layer of constant thickness by solving for the Fick's law. It was deduced that the intrinsic adsorption and desorption rate constants of γ- globulin onto Ti surface.

Keywords: Rate, adsorption, computation, modeling, gamma globulin, titanium

INTRODUCTION

The problem of finding acceptable materials for use in contact with tissue is highly relevant to present day clinical practice. The difficulty of this problem reflects the complex nature of tissue-biomaterial (implant) interactions which are influenced by properties of the tissue, properties of the biomaterial,

and by the transport of fluids around the implant. Implants designed with each of these aspects in mind may produce a "biocompatible environment," analogous to the nonthrombogenic environment thought to be required for blood compatibility with implants[1].

Titanium is employed for various biomedical applications requiring enhanced blood compatibility, which is partly due to its extremely stable oxide layer[2]. Biomaterials play an important role in bioengineering applications such as regenerative medicine, tissue engineering, biosensing, and in orthopedics[3]. The ability of cells to adsorb, proliferate, and differentiate on the biomaterial surface is crucial for the formation of new bone tissues and the subsequent integration of the implant in the body[4].

Gamma globulin (γ-globulin) also called immunoglobulin is a class of blood plasma proteins[5-6] most notably including the antibodies that help fight infections and diseases[7]. γ-globulin adsorption plays a major role in determining the biocompatibility of biomaterials. The first stage of implant integration is the adsorption of protein followed by cell attachment[8]. Protein adsorption behavior is at the heart of many of today's research fields including biotechnology and materials science. With understanding of protein−surface (biomaterial) interactions, the quest for a suitable biomaterial would be achieved[9].

Several works have been presented on the interaction of γ-globulin on biomaterials. For instance Kinetics of γ-globulin adsorption onto Ti was studied, the adsorption was observed to be pseudo first order and intra-particle diffusion process was discovered to be the rate determining step[10]. In another work, adsorption of γ-globulin from solution was studied by the pendant drop technique as a model for the solid-liquid system[11]. It was concluded that it adsorbs in a constant manner on interfaces for example Ti at equilibrium[10], the surface tension components of the protein layer onto the hydrophobic surface was presented. The range of hydrophobic surfaces for which such constants behavior might be expected has been deduced and a new model for protein adsorption was proposed[11]. The present work seeks to monitor the rate of γ-globulin adsorption onto Ti surface with time, compute the flux of γ-globulin molecules to the Ti surface in order to seek the factors that determine the rate of adsorption of γ-globulin to Ti and develop an adsorption model that will help predict the factors that determine the intrinsic (qualitative) adsorption and desorption rate constants.

EXPERIMENTAL

Materials: UV-Visible Spectrophotometer (Jenway 64050), Gallenkamp thermostated shaker with incubator (England), thirty five mesh (500 µm) commercially pure Ti grade (II) powder obtained from BDH chemicals Ltd (Poole, England), bovine serum albumin (BSA) and Commercial pure grade γ-globulin from bovine blood. All reagents used were of Analar grade obtained from Sigma Aldrich and double distilled water was used for their preparation.

Biuret assay: Biuret assay standard calibration curve was prepared by reading the absorbance of 1.00 - 10.00 mg/ml standard concentrations of bovine serum albumin (BSA) at 540 nm using the UV-Visible Spectrophotometer. The results obtained were used in plotting the calibration curve from which the concentration of γ-globulin in every test sample was calculated.

Rate studies: The rate of γ-globulin adsorption onto Ti was studied by monitoring the adsorption of γ-globulin onto Ti surface with time. The adsorption rate parameters employed in this study were optimized[10]. Exactly 1.00 ml of 0.50% (w/v) γ-globulin solution (optimum amount of adsorbate) was added to 40.00 mg Ti powder (optimum amount of adsorbent) and pH adjusted to 7.00 (optimum pH) with phosphate buffer solution. The solution was shaken for 5 minutes while incubating in a 37^{o}C shaker (optimum temperature); the suspension was allowed to settle and the supernatant collected. The experiment was repeated at varying incubation periods of 10, 15, 20, 25, 30, 35, 40 minutes and at 120 minutes (optimum incubation period) respectively. Biuret assay was performed on the

supernatant samples and the amount of γ-globulin adsorbed was calculated by reference to the standard calibration curve of bovine serum albumin (BSA)[10].

RESULTS AND DISCUSSION

Determination of the rate of gamma globulin adsorption onto titanium: The rate of γ-globulin adsorption onto Ti surface requires that the amount of adsorbed γ-globulin is known as a function of time. Therefore Fig.No.1 is the plot of γ-globulin adsorption on the hydrophobic Ti surface as a function of time. The rate constant obtained is 0.032 minutes^{-1} while the correlation coefficient (R^2) is 0.9999. Since the initial γ-globulin adsorption on the hydrophobic Ti surface increased linearly with time, it is assumed that this adsorption might be limited by transport of γ-globulin through an unstirred boundary layer close to the surface. In the case of transport-limited adsorption and for very slow or minimal desorption, the experimental adsorption rate is only limited by the availability of γ-globulin molecules at the adsorbing surface, resulting in a situation in which, every γ-globulin molecule that collides with the surface sticks to it. Therefore it is imperative to compute the flux (continuous flow) of γ-globulin molecules onto Ti surface.

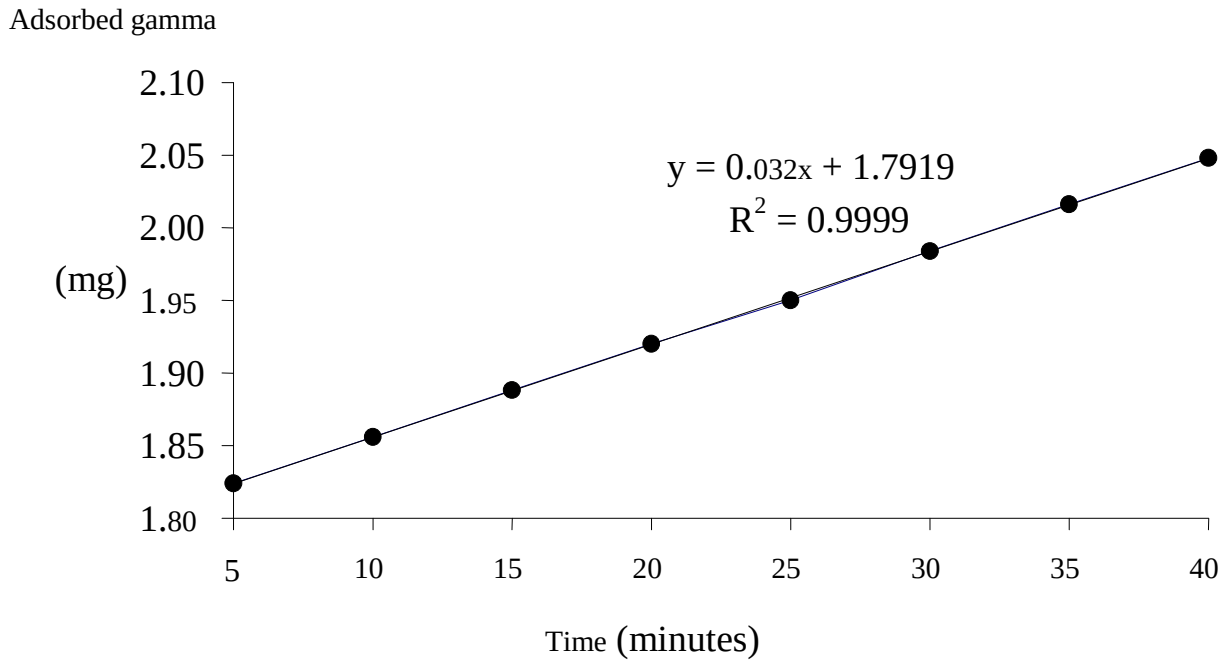

Fig.No.1: Amount of gamma globulin as a function of time
Adsorbed onto titanium

Computation of the flux of gamma globulin molecules onto titanium surface: In order to predict the factors that determine the rate of adsorption of γ-globulin onto Ti, a model similar to implantation of Ti into the body was developed for the transport-limited of γ-globulin adsorption onto Ti from flowing γ-globulin solution. Assuming that laminar flow[12-13] of γ-globulin existed on the Ti surface; an equation for the flowing homogeneous γ-globulin solution could be expressed[14-15] as:

$$\frac{\partial c}{\partial t} = \gamma y \left(1 - \frac{y}{b}\right)\frac{\partial c}{\partial x} = D_{glo}\frac{\partial^2 c}{\partial y^2} \qquad \ldots (1)$$

With the following boundary conditions:

t = 0, C = 0 for all y, x

x = 0, C = C_0 for all y, t

$$y = \frac{b}{2}, \qquad C = 0 \text{ for } t < \frac{x}{v_m} \qquad \text{and}$$

$$C = C_0 \text{ for } t \geq \frac{x}{V_m}$$

$$y = 0, C = 0 \text{ for all } x > 0, t \geq 0$$

Where C is the amount of γ-globulin, t is the time, x is the direction of flow, y is the distance from the surface, b is the thickness of the reaction vessel, C_o is the bulk γ-globulin solution, V_m is the maximum velocity of the flow at y = b/2, D_{glo} is the diffusion coefficient of γ-globulin and γ is the wall shear rate given by:

$$\gamma = \frac{6q}{bw} \qquad \ldots (2)$$

Here q is the volumetric flow rate of γ-globulin solution and w is the width of the reaction vessel. As time increases, a concentration boundary layer (unstirred layer) forms close to the surface. Its thickness, delta (δ) is:

$$\delta = C\,[D_{glo}\,x]^{1/9} \qquad \cdots (3)$$

The profile of the amount of γ-globulin across this layer develops rather rapidly. At longer times and at y/b << 1, the time dependent term in the equation 1 may be neglected and the equation assumes this following form:

$$\gamma y \frac{\partial C}{\partial x} = D_{glo} \frac{\partial^2 C}{\partial y^2} \qquad \ldots (4)$$

$$\frac{\gamma}{D_{glo}} \frac{\partial c}{\partial x} = \frac{1}{y} \frac{\partial^2 C}{\partial y^2} \qquad \ldots (5)$$

$$C(x, 0) = X(\alpha)Y(y) \qquad \ldots (6)$$

$$\frac{\partial C}{\partial x} = Y(y)X^1(\alpha) \qquad \ldots (7)$$

$$\frac{\partial C}{\partial y} = X(x)Y^1(y) \qquad \ldots (8)$$

$$\frac{\partial^2 C}{\partial y^2} = XY^{11}(y) \qquad \ldots (9)$$

Substituting equations (7) and (9) into equation (5) gives equation (10)

$$\frac{\gamma}{D_{glo}} Y(y)\, X^1(x) = \frac{1}{y} XY^{11}(y) \qquad \ldots (10)$$

$$\frac{\gamma}{D_{glo}}\frac{1}{X}X^{1}(x)=\frac{1}{y}\frac{1}{Y}Y^{11}(y) \quad \ldots(11)$$

With the boundary conditions

$$at\ x=0, C=C_0\ for\ all\ y$$

$$as\ y\ \rightarrow\ \infty, C=C_0\ for\ all\ x$$

$$at\ y=0, C\ for\ x\ >0$$

The analytical solution for this classical problem proves that the continuous flow of γ-globulin molecules to the surface, is equal to the rate of adsorption of γ-globulin onto the surface, $d\gamma_{glo}/dt$, under the assumption that all γ-globulin reaching the surface adsorb to it[16] could be expressed as:

$$\frac{d\gamma_{glo}}{dt}=\left[\gamma\left(\frac{4}{3}\right)^{-1}.\ 9^{-\frac{1}{9}}\left(\frac{\gamma}{Dglo.X}\right)\right]^{\frac{1}{9}}.Co \quad \ldots(12)$$

Where γ_{glo} is the adsorbed amount of γ-globulin, from the equation 12 it could be deduced that, in the case of the transport-limited γ-globulin adsorption and very slow or minimal desorption, the adsorbed amount of γ-globulin onto the surface initially rises linearly with time as observed in Fig.No.1 and the rate of adsorption is determined by the wall shear rate (γ), the diffusivity of γ-globulin (D_{glo}) and the bulk amount of γ-globulin solution (c_o)[16].

Modeling of the rate of gamma globulin adsorption onto titanium: This adsorption model was developed in order to predict the factors that determine the intrinsic (qualitative) adsorption and desorption rate constants. The experimental adsorption data were likened to a simple γ-globulin adsorption model, which comprises of adsorption and desorption processes. The model incorporates the rate of diffusion through the boundary (unstirred) layer of constant thickness by solving for the Fick's law which qualitatively defines diffusion[17-18]. The model that is well suited to describe the adsorption process of γ-globulin from flowing γ-globulin solution is that the rate of γ-globulin adsorption to the surface, $d\boldsymbol{\gamma}(t)/dt$, filled with identical, independent binding sites could be written according to[16] as:

$$\frac{d\gamma(t)}{dt}=K_{ad}\left(\gamma_{max}-\gamma(t)\right)+C(o,t)-K_{de}+[\gamma(t)] \quad \ldots(13)$$

Where K_{ad} is the intrinsic adsorption rate constant, K_{de} is the intrinsic desorption rate constant, γ_{max} is the maximum adsorbed amount of γ-globulin, $\gamma(t)$ is the adsorbed amount per unit surface at time t, $[\gamma_{max} - \gamma(t)]$ is the remaining unoccupied γ-globulin binding sites at time t and C(0, t) is the amount of γ-globulin right next to the adsorbing surface (at distance y = 0 from the adsorbing surface). With reference to Fick's diffusion law[18-19] the diffusion equation expressed as:

$$\frac{\partial C[x,t]}{\partial t}=D_{glo}\frac{\partial^2 C[x,t]}{\partial y^2} \quad \ldots(14)$$

is solved numerically with the boundary conditions, in which the rate of adsorption is linearly related to the gradient of the amount of γ-globulin right at the surface.

$$D_{glo}\frac{\partial C\,[0,t]}{\partial y}=\frac{d\gamma}{dt} \qquad \ldots(15)$$

Secondly that the bulk amount of γ-globulin at a distance delta from the surface is equal to the bulk amount of γ-globulin at distance greater than delta from the surface

$$C[0,t]=\ C_{glo} \qquad \ldots(16)$$

Since a linear amount of γ-globulin gradient in the "unstirred" layer forms within a minute, the boundary condition in equation 15 is re-cast in the following form:

$$\frac{dy(t)}{dt}\ =\ D_{glo}\ \frac{\partial C\ (0,t)}{\partial x}=\ D_{glo}\ \left[\frac{C_{glo}-C(0,t)}{\delta}\right] \qquad \ldots(17)$$

Or

$$C(o,t)=C_{glo}\ -\ \frac{\delta}{D_{glo}}\ \left(\frac{dy(t)}{dt}\right) \qquad \ldots(18)$$

Substituting equation 18 into equation 13, the same could be rewritten as:

$$\frac{d\gamma\ (t)}{dt}=K_{adap}\left[\gamma_{max}-\ \gamma\ (t)\right]+\ C_{glo}\ -K_{deap}\ +\ \gamma\ (t) \qquad \ldots(19)$$

Where K_{adap} and K_{deap} are the apparent adsorption rate constant and desorption rate constant, defined respectively as:

$$K_{adap}=\frac{K_{ad}D_{glo}}{D_{glo}+\ \delta_{ad}\left[\gamma_{max}-\gamma(t)\right]} \qquad \ldots(20)$$

$$K_{deap}\ =\frac{K_{de}D_{glo}}{D_{glo}+\delta_{de}\left[\gamma_{max}-\gamma(t)\right]} \qquad \ldots(21)$$

The adsorption model allows the intrinsic adsorption and desorption rate constants to be defined as empirical exponential functions of amount of γ-globulin on the surface.

$$K_{ad}=\ K_1\int_c^{-\infty} \qquad \ldots(22)$$

$$K_{de}=\ K_{-1}\int_c^{-\beta} \qquad \ldots(23)$$

Where k_1 is the initial intrinsic adsorption rate constant, k_{-1} is the initial intrinsic desorption rate constant and alpha and beta are the "cooperativity" adsorption and desorption constants, respectively.

CONCLUSION

γ-globulin could bind (stick) onto Ti surface and the rate of adsorption of γ-globulin onto Ti is determined by the wall shear rate, the diffusivity of γ-globulin and the bulk amount of γ-globulin

solution, while the intrinsic adsorption and desorption rate constants of γ-globulin could be defined as (empirical) exponential functions of amount of γ-globulin on the Ti surface.

ACKNOWLEDGEMENT

The authors are grateful to Mac-Arthur foundation for grant offered for the completion of the research under the auspices of Ahmadu Bello University, Zaria, Kaduna State, Nigeria dissertation completion grant.

REFERENCES

1. T.A. Horbett, *Biomaterials;* 1982, **199,** 244.
2. P.S. Chandra, *J. Biomater. Appl.;* 2003, **17**, 319.
3. B. Kasemo, *Biol .Surf. Sci*.; 2002, **500**, 677.
4. T.J. Dolatshahi-Pirouz, D.C. Kraft, F. Morten, P. Kingshott, J.L. Hansen, A.N Larsen, C. Jacques, F. Besenbacher, *Amer. Chem. Soc.;* 2010, **4 (2)**, 2882.
5. E.M. Marieb, K. Hoehn, Human Anatomy & Physiology (7th Ed.). San Francisco, CA: Pearson Benjamin Cummings, 2007.
6. E.M. Marieb, K. Hoehn, Human Anatomy & Physiology (8th Ed.). San Francisco, CA: Pearson Benjamin Cummings, 2010.
7. N. Foster, What is Gamma Globulin? Wisegeek clear answers for common questions, 2011.
8. P. Roach, D. Farrar, C.C. Perry, *J. Amer. Chem. Soc*.; 2005, **127 (22),** 8173.
9. P. Roach, D. Farrar, C.C. Perry, *J. Amer. Chem. Soc.;* 2006, **128 (12),** 3945.
10. P.A. Ekwumemgbo, J.A. Kagbu, A.J. Nok, I.K. Omoniyi, *Rasayan J. Chem.;* 2010, **3(2),** 231.
11. R.D. Bagnall, *J. Biomed. Mat. Res.;* 2004, **11,** 978.
12. Technology Assessment Team, An Overview of Laminar Flow Ventilation for Operating Theatres, 2001.
13. J. Ostrower, *Flight Intern.;* 2011, 978.
14. B.K. Lok, Y.L. Cheng, C.R. Robertson, *J. Coll. and Interf. Sci*.; 1983, **91 (1),** 103.
15. M.L. Yarmush, D.B. Patankar, D.M. Yarmush, *Mol. Immune.;* 1999, **33 (15),** 1214.
16. .H. HO, P. Dryden, D.W. Britt, Department of Bioengineering, University of Utah, Salt Lake City, UT, 2006.
17. N.B. Hannay, Solid-State Chemistry Prenticle-Hall, Inc. Englewood Cliffs, New Jersey, 1967.
18. Arizona Board of Regents, *blowers.chee.arizona.www.edu/cooking/mass/fickslaw.html*, 2007.
19. T. Tsang, *J. Appl. Phys.* 2009, **32 (8),** 1520.