



## KINETICS OF GAMMA GLOBULIN ADSORPTION ONTO TITANIUM

Patricia Adamma Ekwumemgbo<sup>1\*</sup>, James Adagadzu Kagbu<sup>1</sup>, Andrew Jonathan Nok<sup>2</sup>, and Kehinde Israel Omoniyi<sup>3</sup>

<sup>1</sup>Department of Chemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

<sup>2</sup>Department of Biochemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

<sup>3</sup>Ahmadu Bello University, School of Remedial Studies Funtua, Kaduna State, Nigeria.

\*Email: pat\_adamma@yahoo.com

---

### ABSTRACT

Kinetics of gamma globulin adsorption onto Ti was studied by monitoring the rate of gamma globulin adsorption onto Ti with time under optimum conditions of incubation period, amount of Ti, amount of globulin, temperature of incubation and pH values which are 120 minutes, 40.00 mg, 0.50%, 37°C, pH 7.0 and 120 minutes respectively. The adsorption was observed to be pseudo first order with the first order rate constant ( $K^1$ )  $3.3 \times 10^{-3}$ , the second order rate constant ( $K$ )  $8.25 \times 10^{-5}$  mg/mlmin and the degree of fitness of the data ( $R^2$ ) 0.9997. The adsorption data were fitted with some kinetics models. The linear correlations of the pseudo first-order models were statistically significant at 95% confidence level and indicated the applicability of these models and the pseudo first order nature of the adsorption process; however, Natarajan and Khalaf pseudo first-order model provided the best description of the data while intra-particle diffusion process was discovered to be the rate determining step. These findings are insight into the mechanism of gamma globulin adsorption onto Ti when used for biomedical implantation.

**Keywords:** Adsorption, Gamma globulin, Titanium, Incubation, Kinetics, First-order

---

### INTRODUCTION

Gamma globulin is one of the blood proteins found in body fluids; it is a blood product used for the treatment of patients with primary immune deficiency where the primary problem is that such patients do not produce enough gamma globulin to prevent infection<sup>1</sup>. Globulin is synthesized by an individual in response to a foreign substance introduced into the body called antigen<sup>2</sup>, and it is naturally designed with extraordinary specificity and binding affinity for a given antigen<sup>3</sup>.

Titanium and Titanium-based alloys of biomedical grade are well-known biomaterials that show excellent performance as dental, orthopaedic and blood contacting materials such as artificial hearts valves, stents and pacemakers. When a biomaterial is implanted into the human body, it is surrounded by the blood proteins and adsorption of these proteins on the implanted biomaterial begins to take place immediately. How much of these proteins adsorb on the surface of the biomaterial will help to decide the biocompatibility of the material<sup>4,5</sup>.

Most adsorption kinetics processes are usually controlled by different mechanisms, of which the most limiting are the diffusion mechanisms, attributed to rapid external diffusion or boundary layer diffusion and surface adsorption<sup>6,7,8,9</sup>. It has been confirmed that in protein adsorption studies the potential of mean-force between the adsorbing protein and the adsorbent-protein surface changes as a function of time due to changes of the adsorbent layers as the proteins adsorb<sup>10</sup>. Based on this, adsorption kinetics have been utilized to predict rate determining steps of adsorption processes<sup>11,12</sup>.

A lot of studies on the adsorption of blood proteins onto surfaces have been carried out, for example studies on the conformational changes during adsorption of myoglobin (MGB) onto ultrafine silica and Zr particles<sup>13</sup>; adsorption of myoglobin (MGB), lysozyme (LSZ) and bovine serum albumin (BSA) onto various colloidal synthetic hydroxyapatite<sup>14</sup>; adsorption of immunoglobulin (IgG), BSA, MGB

and LSZ onto synthetic calcium hydroxyapatite particles<sup>[3]</sup>, adsorption of immunoglobulin onto various synthetic calcium hydroxyapatite particles<sup>15</sup>, and adsorption of fibronectin molecules onto mica and oxidized Ti substrates<sup>16</sup>. However, to the knowledge of the authors, the kinetic of gamma globulin adsorption onto Ti has not yet been reported elsewhere. The aim of this work therefore, is to determine the rate of gamma globulin adsorption onto Ti; determine the rate determining step and seek the best kinetic model that best describes this adsorption process.

## EXPERIMENTAL

### Materials

The materials used for this study were UV-Visible Spectrophotometer (Jenway 64050), Gallenkamp thermostated shaker with incubator (England); thirty five mesh (500  $\mu\text{m}$ ) commercially pure Ti grade (II) powder, bovine serum albumin (BSA) and commercial pure grade gamma 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 gamma globulin in every test sample was calculated.

### Optimisation of kinetics parameters

Incubation period was optimised by weighing 5.00 mg of Ti powder; this was added to 1.00 ml of 0.10% globulin solution. The solution was shaken for 15 minutes while incubating at 37°C. The suspension was allowed to settle and the supernatants were collected. Biuret assay was performed on the supernatant samples and the amount of globulin adsorbed was calculated using the calibration curve of bovine serum albumin (BSA). The experiment was repeated at incubation periods of 30, 45, 60, 120, 180 and 240 minutes respectively.

The amount of adsorbent (Ti) was optimised by weighing separately 5.00 mg, 10.00 mg, 15.00 mg, 20.00 mg, 25.00 mg, 30.00 mg, 35.00 mg, 40.00 mg, 45.00 mg and 50.00 mg of Ti powder, these were added to 1.00 ml of 0.10% globulin solutions respectively. The solutions were shaken for 120 minutes while incubating at 37°C and biuret assay was performed on the supernatant.

The optimum amount of globulin bound onto Ti was investigated by preparing 0.10%, 0.20%, 0.30%, 0.40% and 0.50%, 0.60%, 0.70%, 0.80%, 0.90% and 1.00% (w/v) standard solutions of globulin. 1.00 ml of each standard solution was added to 40.00 mg of Ti. The solutions were shaken for 120 minutes while incubating at 37°C and biuret assay was performed on the supernatant.

Incubation time was optimised by weighing accurately 40.00 mg of Ti powder (optimum amount of adsorbent); this was added to 0.50% globulin solutions. The solutions were shaken for 120 minutes while incubating at 31°C and biuret assay was performed on the supernatant. The experiment was repeated at incubation temperature of 33°C, 35°C, 37°C, 39°C and 41°C respectively.

Optimisation of pH of globulin adsorption onto Ti was performed by weighing accurately 40.00mg of Ti powder; this was added to 1.00 ml of 0.50% globulin solutions and pH adjusted to 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 with phosphate buffer solution respectively. The solutions were shaken for 120 minutes while incubating at 37°C and biuret assay performed on the supernatant.

### Kinetics studies

The kinetics of globulin adsorption onto Ti was studied by monitoring the rate of globulin adsorption onto Ti with time. 1.00 ml of 5.00% (w/v) 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°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 globulin adsorbed was calculated by reference to the standard calibration curve of bovine serum albumin (BSA).

## RESULTS AND DISCUSSION

### Optimum incubation period

Figure 1 is the plot of bound globulin against incubation time in which the amount of globulin adsorbed onto Ti surface increased with increase in incubation period until at 120 minutes where increase in incubation period did not result to further increase in adsorption of globulin molecules. Therefore, the optimum incubation period of globulin adsorption onto Ti surface is 120 minutes.

### Optimum amount of titanium

Figure 2 is the plot of the amount of globulin adsorbed onto different amounts of Ti at 120 minutes incubation period. The result shows that the amount of globulin adsorbed onto Ti surface increased with increase in the amount of Ti surface until at 40.00 mg of Ti. The same amount of globulin was adsorbed onto 40.00 mg, 45.00 mg and 50.00 mg of Ti respectively. This implies that the optimum amount of Ti onto which globulin could adsorb is 40.00 mg under the conditions tested.

### Optimum amount of globulin adsorbed onto titanium

Figure 3 is the plot of the amount of globulin adsorbed onto 40.00 mg of Ti versus initial amount of globulin solutions at 120 minutes incubation period, it is observed that the amount of globulin adsorbed onto Ti surface increased with increase in the amount of globulin until at 0.50% (w/v) globulin solution. The same amount of globulin was adsorbed onto 40.00 mg of Ti when 0.50%, 0.60%, 0.70%, 0.80%, 0.90% and 1.00% globulin solutions were used for the adsorption studies respectively. This implies that the optimum amount of globulin which could be used for the adsorption studies to yield optimum adsorption of globulin onto 40.00 mg of Ti is 0.50% (w/v) globulin solution.

### Optimum incubation temperature

Figure 4 is the plot of the amount of globulin adsorbed onto 40.00 mg of Ti at 120 minutes incubation period in 0.50% globulin solution at various temperatures. The result shows that the amount of globulin adsorbed onto Ti surface increased with increase in temperature until at 37°C where increase in temperature resulted to gradual decrease in the adsorption of globulin molecules onto Ti surface. Therefore, the optimum temperature of globulin adsorption onto Ti surface is 37°C.

### Optimum pH

Figure 5 is the plot of the amount of globulin adsorbed onto 40.00 mg of Ti at 37°C and at 120 min incubation period in 0.50% globulin solution at various pH values of 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 respectively. It is observed that the amount of globulin adsorbed onto Ti surface increased with increase in pH until at pH 7.0 where increase in pH resulted to gradual decrease in the adsorption of globulin molecules onto Ti surface. Therefore, the optimum pH of globulin adsorption onto Ti surface is pH 7.0.

### Kinetics of the adsorption process

Quantitative analysis of adsorption kinetics requires that the amount of adsorbate bound onto the adsorbent is known as a function of time. Kinetics of globulin adsorption onto Ti was studied by monitoring the rate of globulin adsorption with time under optimum conditions of incubation period, amount of Ti, amount of globulin, temperature of incubation and pH values which are 120 minutes, 40.00 mg, 0.50%, 37°C, pH 7.0 and 120 minutes respectively, with the amount of Ti at least thirty five fold excess over the amount of globulin. In a system where A and B react to produce P, the equation for the reaction could be represented thus:



If the initial concentration of the reactant A is much larger than the concentration of B, the concentration of A will not change appreciably during the course of the reaction, which implies that the concentration of A will remain almost constant. Thus, the rate law with reference to the concentration of B could be written as:

$$-d[B]/dt = K^1[B]; \text{ Where } K^1 = K^1[A] \quad (2)$$

Equation (2) represents the differential form of the rate law. Integration of this equation and evaluation of the integration constant C produces the corresponding integrated law. Substituting  $[B] = c$  into equation (2) yields:

$$-dc/c = K^1 dt \quad (3)$$

Integrating equation (3) gives:

$$\ln c = -K^1 t + C \quad (4)$$

The constant of integration C could be evaluated by using boundary conditions. At  $t = 0$  and concentration of B =  $c_0$ . Therefore

$$C = \ln c_0 \quad (5)$$

Where-

$$\ln c = \ln c_0 - K^1 t \quad \text{Or } c = c_0 e^{-K^1 t} \quad (6)$$

If the decrease in concentration of B is followed by photometric measurement then Beer's law would be obeyed by the reaction system. Therefore-

$$\text{Abs} = \log P_0/P = -\log T = \epsilon.c.d \quad (7)$$

Where T is transmittance, Abs is absorbance,  $\epsilon$  is molar absorptivity, c is concentration of the adsorbate in solution,  $P_0$  is the incident radiant power, P is the transmitted radiant power and d is path length of light. The relationship between  $K^1$  and  $\ln(\text{Abs})$  could be obtained using the following equations,

$$\ln(\text{Abs}) = -K^1 t + \ln(\epsilon.d) \quad (8)$$

Or

$$\ln(\text{Abs}) = -K^1 t + C \quad (9)$$

A plot of  $\ln(\text{Abs})$  versus time yields a straight line whose slope is the pseudo-first order rate constant  $K^1$ . The value of  $K^1$  is divided by the known, constant concentration of the more concentrated reactant (A) to obtain the true second order rate constant K.

$$K = K^1 / [A] \quad (10)$$

In adsorption studies interest is centred on the amount of adsorbate bound onto the adsorbent, where absorbance (Abs) is proportional to free (unadsorbed) adsorbate while bound (globulin) adsorbate is the free globulin subtracted from globulin not suspended in Ti powder (control). In bound globulin (BD) was plotted against time to obtain the pseudo first order plot of the adsorption of globulin onto Ti as shown in Figure 6. The rate ( $K^1$ ) obtained is  $3.30 \times 10^{-3} \text{ min}^{-1}$  while the degree of fitness of the data ( $R^2$ ) is 0.9997. The linearity of this plot indicates that the adsorption is first order with respect to the amount of globulin bound onto Ti. Therefore, the rate equation could be represented with equation 11.

$$d[\text{glo}]/dt = [\text{glo}][\text{Ti}] \quad (11)$$

$$K = K^1 / [\text{Ti}] \quad (12)$$

Where [glo] is amount of globulin, [Ti] is amount of Ti,  $K^1$  is Pseudo first order rate constant, K is second order rate constant calculated from equation 10.

$$K = 0.0033/40.00 = 0.0000825 \text{ mg/mlmin}$$

$$K = 8.25 \times 10^{-5} \text{ mg/mlmin}$$

### Weber-Morris intra-particle diffusion model

Intra-particle diffusion model is a graphical method to prove the occurrence of intra-particle diffusion and to determine if it is the rate determining step in adsorption process. The diffusion model of the adsorption of globulin to Ti was characterised using the relationship between specific sorption (qt) and the square root of time ( $t^{1/2}$ )<sup>8,17,18</sup> as shown below:

$$qt = Kid t^{1/2} + C \quad (13)$$

Where qt is the amount of gamma globulin adsorbed per unit mass of titanium at time t, Kid is the intra-particle diffusion rate constant ( $\text{mg/mlmin}^{-1/2}$ ), C is the intercept and t is time in minutes. The values of qt were correlated with  $t^{1/2}$  as illustrated in Figure 7. The values of qt were found to be linearly correlated with values of  $t^{1/2}$  as evidenced by  $R^2$  values which is equal to unity. From the plot, the value of intra-particle diffusion rate constant (Kid), is  $0.0882 \text{ mg/mlmin}^{-1/2}$ . This result indicate the presence of intra-particle diffusion process as one of the rate determining steps<sup>6,8,17,18</sup> besides, many other processes controlling the rate of adsorption, all of which may be operating simultaneously<sup>19</sup>. In adsorption process, it has been proved that when the adsorbate species are transported from the bulk of the solution to the solid phase through intra-particle diffusion/transport process, it is referred to as the rate determining step<sup>7,8</sup>.

### Kinetics models

The data obtained were studied by applying kinetic models proposed by Lagergren for first and second order kinetics<sup>20</sup>, Bhattacharya and Venkobachar model<sup>21</sup>. The linear form of these models is given below:

#### Lagergren first-order model

$$\text{Log}(q_e - qt) = \text{log } q_e - (K_{ad}/2.303)t \quad (14)$$

#### Lagergren second-order model

$$(1/q_e - qt) = (1/q_e) - Kt \quad (15)$$

#### Bhattacharya and Venkobachar model

$$\text{Log}[1 - U(T)] = (K_{ads}/2.303)t \quad (16)$$

#### Natarajan and Khalaf model

$$\text{Log}[c_i/c_t] = (K_{ads}/2.303)t \quad (17)$$

Where  $q_e$  is the amount of gamma globulin adsorbed per unit mass of the Ti at equilibrium,  $q_t$  is the amount of gamma globulin adsorbed per unit mass of Ti at time t respectively,  $U(T) = [(C_i - C_t)/(C_i - C_e)]$  function of time,  $C_e$  is the amount of gamma globulin adsorbed per unit mass of the Ti at equilibrium,  $C_i$  is the amount of gamma globulin at time zero (initial amount),  $C_t$  is the amount of gamma globulin at time t respectively,  $k_{ads}$  is Bhattacharya and Venkobachar first order adsorption rate constant ( $\text{min}^{-1}$ ),  $K_{ADS}$  is Natarajan and Khalaf first order adsorption rate constant ( $\text{min}^{-1}$ ), and t is time in minutes. The values of  $\text{log}(q_e - qt)$ ,  $1/(q_e - qt)$ ,  $\text{log}[1 - U(T)]$  and  $\text{Log}(C_i/C_t)$  were correlated with time as shown in Figure 8, 9 10 and 11 respectively.

The rate constants obtained from the Lagergren first-order model, Lagergren second order model, Bhattacharya and Venkobachar, Natarajan and Khalaf plots are  $-0.0096 \text{ min}^{-1}$ ,  $0.0801 \text{ mg/mlmin}$ ,  $-0.0096 \text{ min}^{-1}$  and  $-0.0016 \text{ min}^{-1}$  respectively, while the degree of fitness of the data ( $R^2$ ) are 0.9879,

0.9422, 0.9879 and 0.9996 respectively as presented in Table 1. The rate constants and degree of fitness ( $R^2$ ) values obtained for Lagergren first-order model and Bhattacharya and Venkobachar models were found to be approximately equal. The linear correlations for the pseudo first-order models which are Lagergren first-order model, Bhattacharya and Venkobachar, Natarajan and Khalaf models were found to be statistically significant at 95 % confidence level and indicate the applicability of these kinetic models and the pseudo first order nature of the adsorption of gamma globulin onto Ti. Natarajan and Khalaf pseudo first-order model provided the best description of the data obtained as shown in the highest value of degree of fitness among the models used in the study.

### CONCLUSION

Gamma globulin could adsorb onto Ti surface, the adsorption was observed to be pseudo first order, the linear correlations for the pseudo first-order models were found to be statistically significant at 95% confidence level which indicates the applicability of these kinetic models and the pseudo first order nature of the adsorption process. However, Natarajan and Khalaf pseudo first-order model provided the best description of the data obtained among the models employed. Weber-Morris intra-particle diffusion model indicated the presence of intra-particle diffusion process as one of the rate determining steps. These findings are insight into the mechanism of the biocompatibility of Ti when used as biomaterial.

### ACKNOWLEDGEMENTS

The authors are thankful to Prof. T. Hanawa of the Institute of Biomaterials and Bioengineering Tokyo Medical and Dental University, Tokyo, Japan and Prof. J. A. Lori of Ahmadu Bello University, Zaria, Nigeria through whom the bovine serum albumin, gamma globulin and titanium used for this research were obtained.

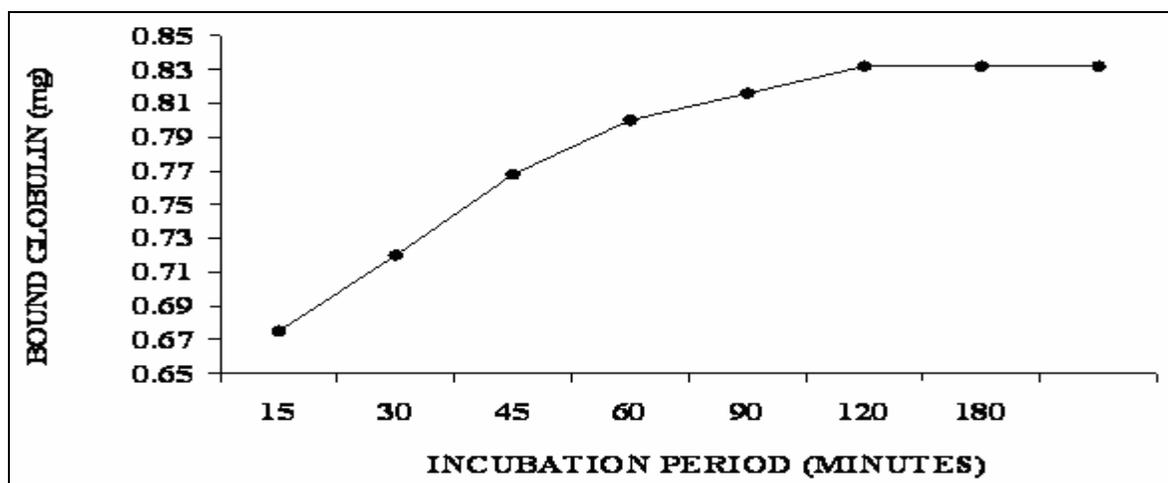


Fig.-1: Optimisation of incubation period of globulin adsorption onto titanium

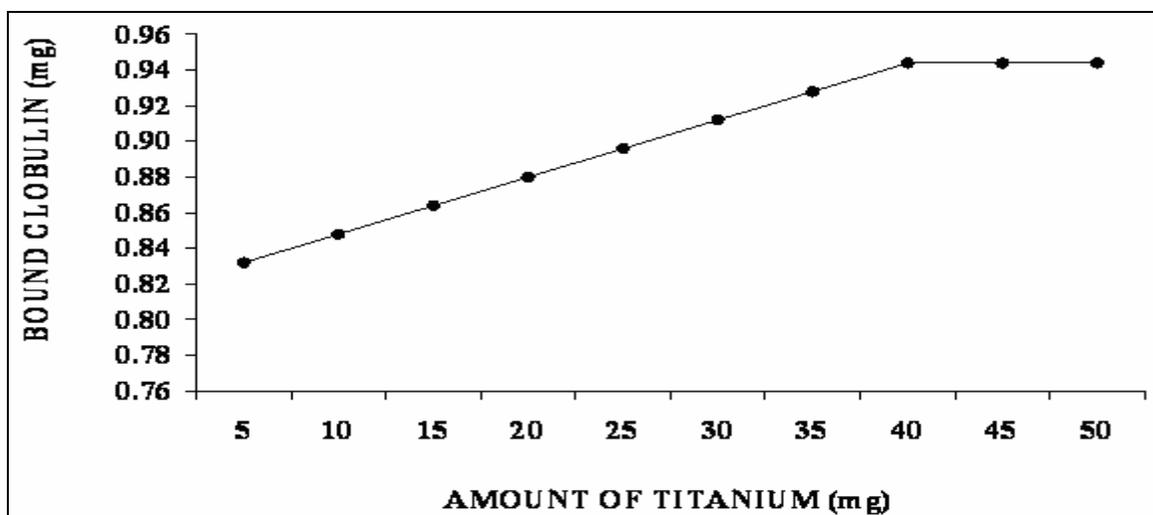


Fig.-2: Optimisation of amount of titanium

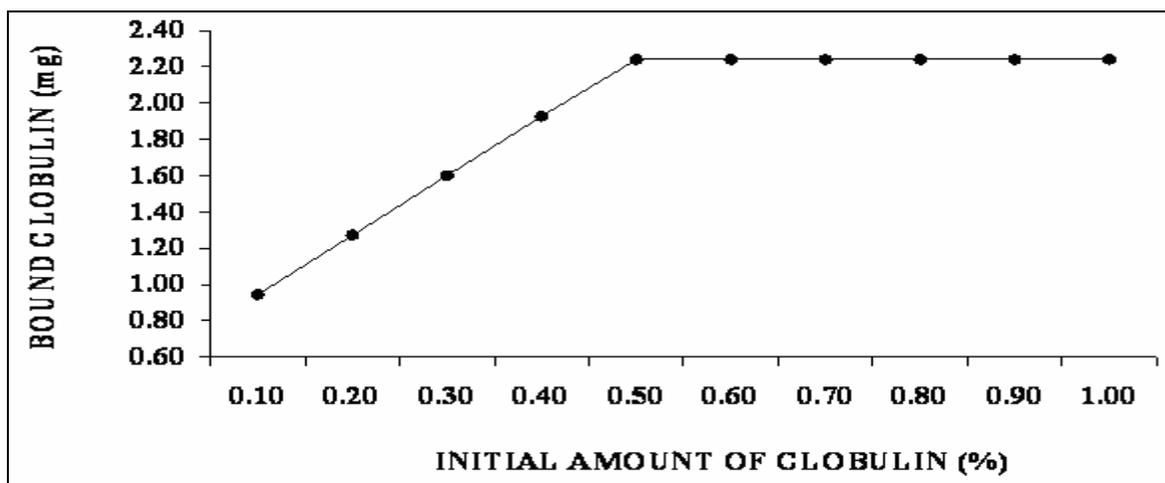


Fig.-3: Optimisation of amount of globulin adsorption onto titanium

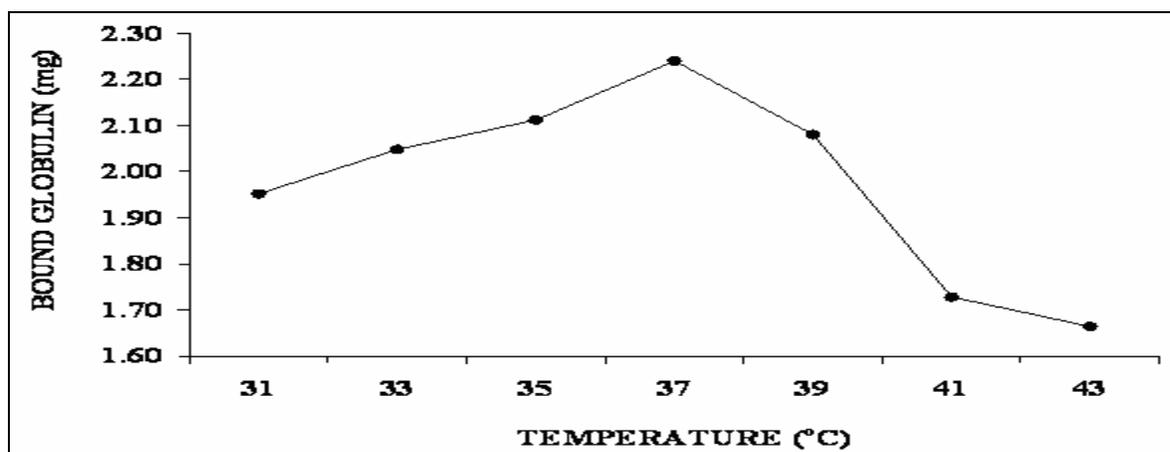


Fig.-4: Optimisation of temperature of globulin adsorption onto titanium

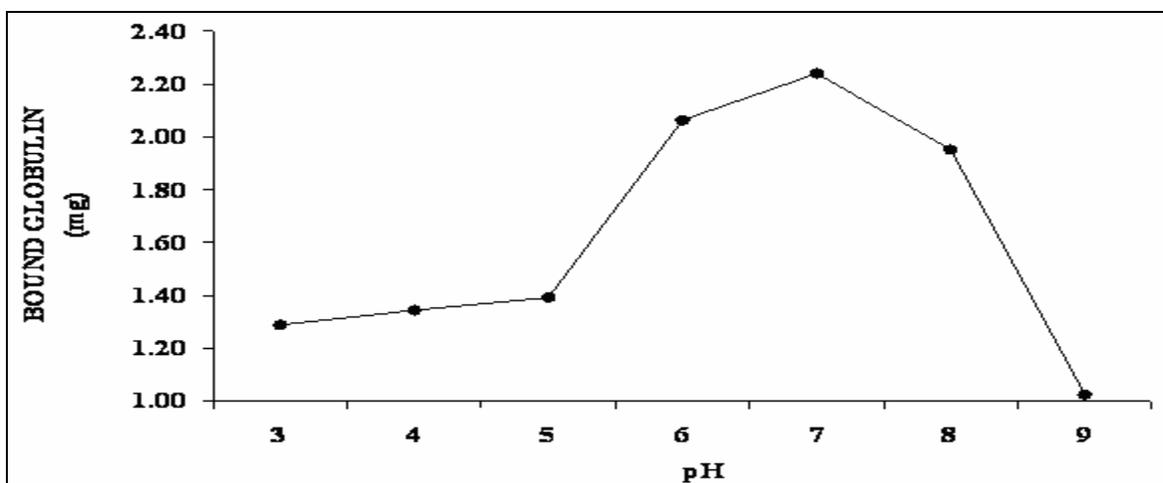


Fig.-5: Optimisation of pH of globulin adsorption onto titanium

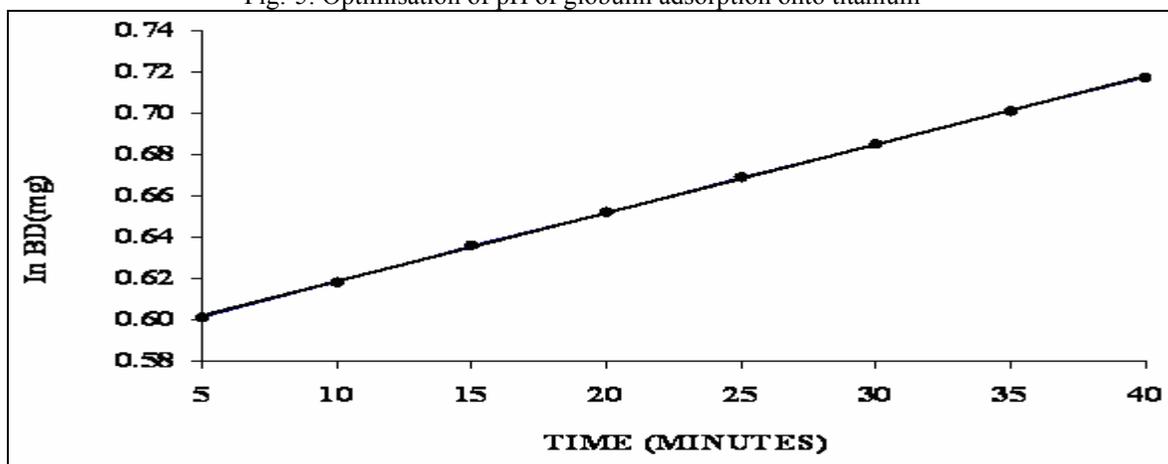


Fig.-6: Pseudo first order plot of globulin adsorption onto titanium

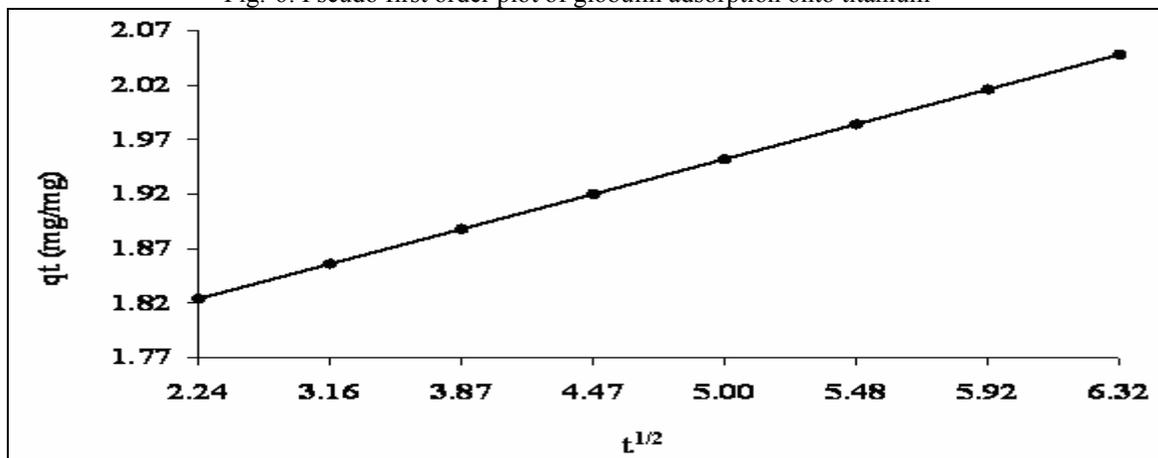


Fig.-7: Weber-Morris plot of globulin adsorption onto titanium

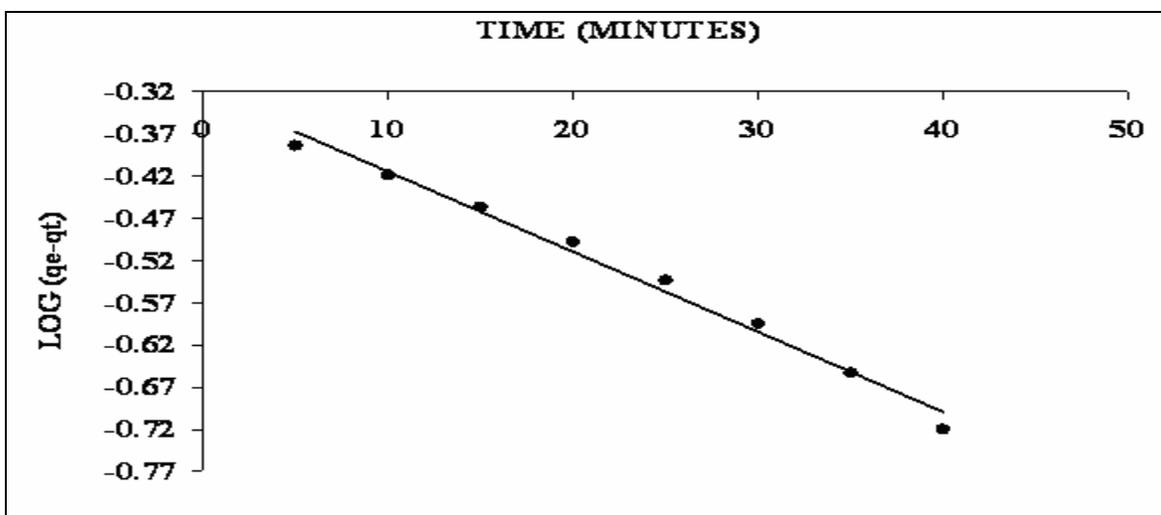


Fig.-8: Lagergren first order plot of globulin adsorption onto titanium

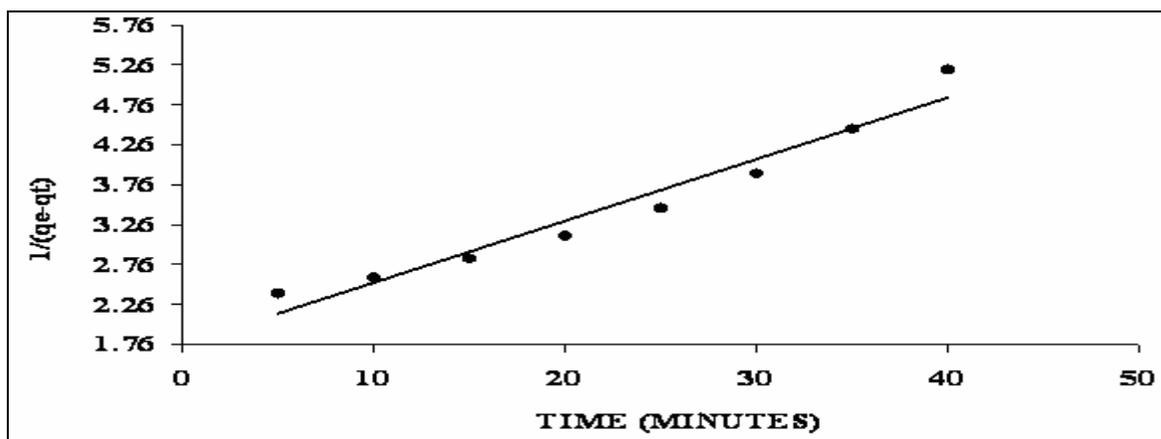


Fig.-9: Lagergren second order plot of globulin adsorption onto titanium

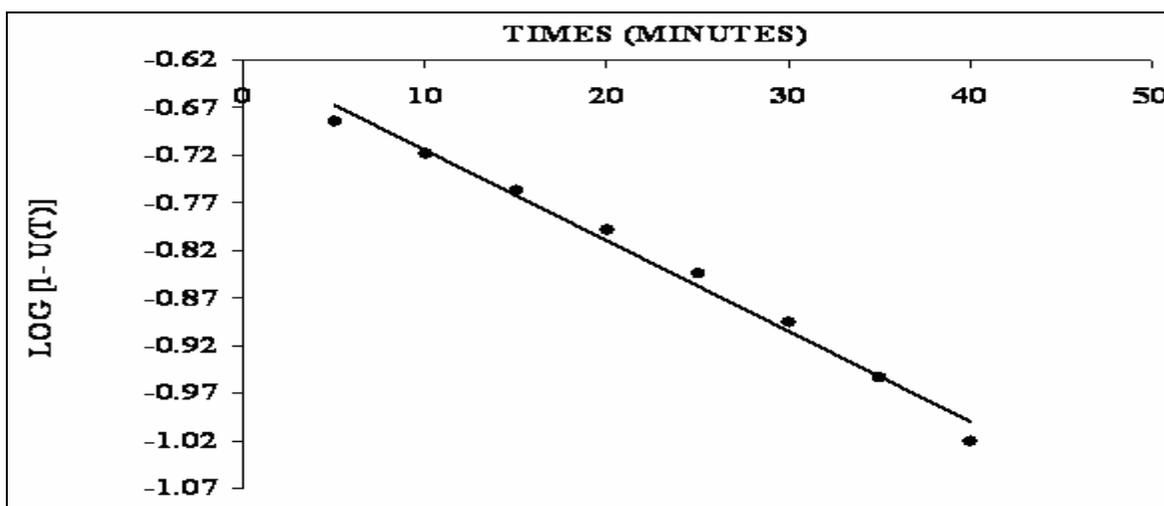


Fig.-10: Bhattacharya and Venkobachar plot of globulin adsorption onto titanium

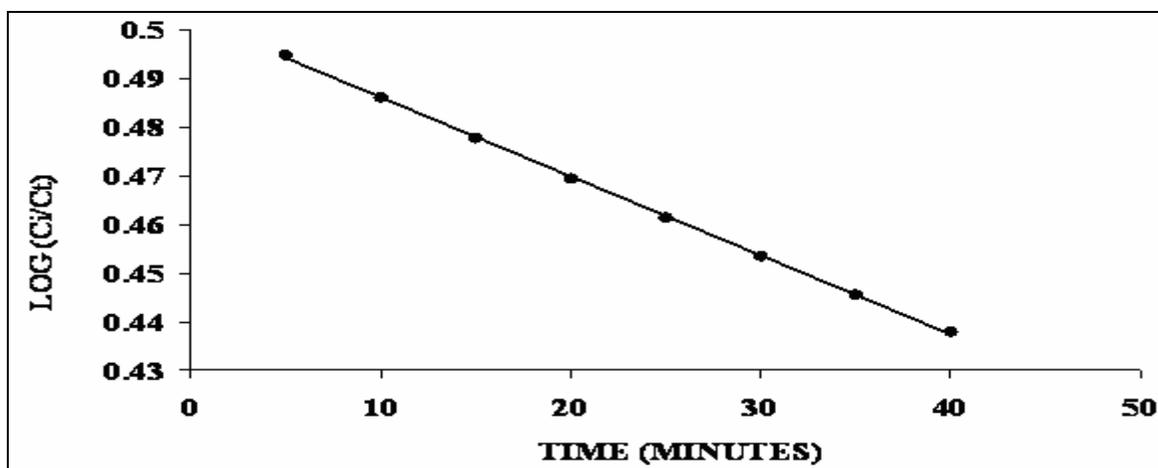


Fig.-11: Natarajan and Khlaf first order plot of globulin adsorption onto titanium

Table -1: Rate Constants and R<sup>2</sup> values for the kinetic models

Kinetic models	Rate constants and R <sup>2</sup> values	
	Lagergren first-order model	k <sub>ad</sub>
	R <sup>2</sup>	0.9879
Lagergren Second order model	K	0.0801 mg/mlmin
	R <sup>2</sup>	0.9422
Bhattacharya and venkobachar	k <sub>ads</sub>	-0.0096 min <sup>-1</sup>
	R <sup>2</sup>	0.9879
Natarajan and Khalaf Equation	K <sub>ADS</sub>	-0.0016 min <sup>-1</sup>
	R <sup>2</sup>	0.9996

## REFERENCES

1. Canadian Immunodeficiencies Patient Organisation (CIPO), *A Guide to gammaglobulin also known as Intravenous Immunoglobulins (IgIV)*, **3**, (2006).
2. C.E. Giacomelli, *Encyclopedia of Surface and colloid science*. Hubbard, A.T., ed. Dekker, NewYork, **418**, (2002).
3. K. Kazuhiko, M. Kaori and I. Tatsu, *Journal of colloid and interface science*, **273**, 406(2004).
4. P.I. Albrektsson, H.A. Branemark, B. Kasemo, I. Larssons, D.H. McQueen and R. Skalak, *Acta. Orthop. Scad.*, **11**, 1(1983).
5. T. Albrektsson, H.A. Hansson and B. Ivarsson, *Biomaterials*, **6**, 97(1985).
6. G. Mckay, M.S. Otterburn and A.G. Sweeny, *Water Res.*, **14**, 10(1980). S.G. McKay, *Chem. Eng. Res. Des.*, **61**, 29(1983a).
7. N. Deo and M. Ali, *Indian J. Environ. Protec.*, **13**(7), 496, **13**(8), 570(1993).
8. S. Ong, C. Seng and P. Lim, *Electron. J. Environ. Agric. Food Chem*, **6**(2), 1764(2007).
9. F. Fang, J. Satulovsky and I. Szleifer, *Biophysical Journal*, **89**, 1516(2005).
10. W.J. Weber Jr., *Physiochemical processes for water quality control*, Wiley Interscience, London, 199(1972).
11. E.F. Smith, H.B. Mark and P. Carthy, in *Chemistry of Wastewater Technology*, Inc. Ann Arbor, Michigan, (1978).
12. Kondo and J. Mihara, *Journal of Colloid Interface Science*, **177**, 214(1996).
13. K. Kazuhiko, F. Aya and I. Tatsu, *Physical Chemistry*, **2**, 2015(2000).
14. K. Kandori, K. Miyagawa and T. Ishikawa, *J. of colloid interface science*, **273**, 406(2004).

15. H. Zhang, K. Bremmell, S. Kumar and R.S. Smart, *Journal of Biomedical Material Research*, **68**(3), 479(2004).
16. K. Nagarethinam and M. Mariappan, *Water, Air and Soil Pollution*, **138**, 289(2002).
17. K. Nagarethinam and R. Gurusamy, *Water, Air and Soil Pollution*, **163**, 185(2005).
18. G. McKay, *Biochem. Eng. J.*, **27**, 187(1983b).
19. S. Lagergren, *Stoffe. Stoc. Ak. Handl.Bihay*, **24** (1), 39(1998).
20. A.K. Bhattacharya and C. Venkobachar, *J. Am. Civ. Enbb.*, **110**, 110(1984).
21. N. Kannan and A. Vanangamudi, *J. Environ. Protec.*, **11**(4), 241(1991).

(Received: 9 April 2010

Accepted: 2 June 2010

RJC-542)

## Highlights of RASĀYAN

- It is a full text open access international journal of Chemical Sciences. Covers all fields related to Chemistry.
- Research papers will be published on the website and also in the printed version simultaneously.
- Manuscript is reviewed and published very quickly.
- Full text of the article is available on the site <http://www.rasayanjournal.com> all over the world. **Reprints may be downloaded directly from the website.**
- Papers can be submitted through e-mail to [rasayanjournal@gmail.com](mailto:rasayanjournal@gmail.com).

## Adopt **GREEN CHEMISTRY** Save Our Planet.

We publish papers of Green Chemistry on priority.

If you think that you may be a potential reviewer in field of your interest, write us at [rasayanjournal@gmail.com](mailto:rasayanjournal@gmail.com) with your detailed resume and recent color photograph.