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Kinetics of the Adsorption of Bovine Serum Albumin of White Wine Model Solutions onto Activated Carbon and Alumina

Omoniyi K. Israel[a,*] and Patricia A. Ekwumemgbo[b]

[a]*School of Basic and Remedial Studies, Ahmadu Bello University, Funtua, Nigeria.*
[b]*Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria.*

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ABSTRACT

This study investigates the kinetics of adsorption of bovine serum albumin, BSA, in white wine model solutions onto activated carbon, AC, and alumina, AL. Pseudo-first order and pseudo-second order models were applied to determine the rate and mechanism of adsorption of the white wine protein during the haze removal process. The results showed that the average amount of adsorbed BSA onto AC was 1.10 ± 0.07 times higher than that onto AL. Statistical analysis by two-way ANOVA showed no significant difference in the amount of BSA adsorbed onto the two adsorbents, but a statistically significant difference existed in the amount adsorbed by variation of incubation time. A positive correlation exists between the amounts of BSA adsorbed onto AC and AL. The kinetics of the adsorption were found to be based on the assumption of an intra-particle diffusion-controlled pseudo-second order mechanism, with adsorption rate constants being higher at lower adsorbate concentrations.

KEYWORDS

White wine, haze removal, kinetic modelling, adsorption rate constant.

1. Introduction

Grapes are the most common fruit used to make wine; wine is also made from rice, pears, apples, berries or other products. Wine naturally contains about 85 to 89 % water, 10 to 14 % alcohol, less than 1 % fruit acids and hundreds of aroma and flavour components in very small amounts.[1] Its large acceptability emanates from its low alcohol content. Though wine proteins are of a heterogeneous group, not all cause heat instability and hazing. The protein profile and content of wine vary among different wine types. A wine's taste, aroma, sparkle and haze are qualities that depend on the protein type inherent in the product used to make it, and also on the proteins that belong to yeast strains added for its fermentation.[2] Protein may be removed from fluids by adsorption onto inorganic silica-oxide cogels. Preferred cogels are silica alumina and silica magnesia, activated so that the cogel surface is in acidic form, with Ho values (measure of the acidity of the solid composition) being less than the pH of the protein-containing fluid which, in turn, is less than the isoelectric point of the protein. This method is particularly effective for removing haze-forming proteins from wine.[3] Protein instability in white wines has been linked to protein fractions with low molar masses (12 to 30 kDa) and low isoelectric points, pI (4.1–5.8). A solution of proteins in this isoelectric point range exhibits minimum conductivity, osmotic pressure and viscosity, causing it to have the greatest tendency to coagulate.[4]

Typical wines have a pH of 3.0 to 3.5. The proteins thought to cause haze formation are believed to have a net positive charge in wine. Positively charged proteins will interact to a greater extent with the negatively charged (acidic) adsorbent surface.[5] Research has shown that activated carbon, AC, is non-toxic when used in food processing and it has a net negative charge when activated at low temperature (<500 °C), making the surface relatively hydrophilic.[6] In order to reduce the proteins in white wine model solutions, three proteins, namely bovine serum albumin (BSA), ovalbumin (OVA) and lysozyme (LSZ) were adsorbed onto zirconium oxide and sodium bentonite surfaces. The protein adsorption capacity of zirconium oxide was observed to be lower than that of sodium bentonite.[5] Zirconium oxide showed adsorption selectivity and a preference for removing the unstable proteins, thus stabilizing white wine.

Protein adsorption appears to be mainly irreversible in many cases.[7] In some, conformational changes occur in the protein molecule during or after the adsorption. Model calculations indicate that the kinetics of exchange reactions could be faster than spontaneous desorption.[8] This study aims to evaluate the adsorption affinities of activated carbon and alumina in removing haze-forming proteins from model wine solutions. Since sorption kinetics can be used to predict the rate of protein removal from aqueous solutions,[9] this work will aid in the design of appropriate sorption treatment plant. The study also applied two kinetic models: pseudo-first order[10] and pseudo-second order[11] for determining the specific rate constants of adsorption of BSA onto AC and AL.

2. Experimental

2.1. Materials

Coconut shells were obtained from Zaria market in Kaduna State, Nigeria. Alumina (BDH Chemicals Ltd., Poole, England) having particle size ranging from 10–15 μm in size was used. Bovine serum albumin, BSA (Sigma-Aldrich Co., St. Louis, MO, USA), which is an acidic protein, with molar mass 67.0 kDa and isoelectric point, pI, 4.7 was used as the wine protein since unstable proteins or membrane fouling could be related to the molar mass range between 20 and 70 kDa.[12]

2.2. Preparation of Activated Carbon

The coconut shells were dried to a constant mass in an air circulating oven at 100–105 °C for 6 h and pulverized with a Wiley pulverizer to 150 μm mesh size. These samples were carbonized in a furnace at 500 °C for a residence time of 5 min. Approximately 2 g of the carbonized particles were activated with

*To whom correspondence should be addressed. E-mail: israelflourish@yahoo.com

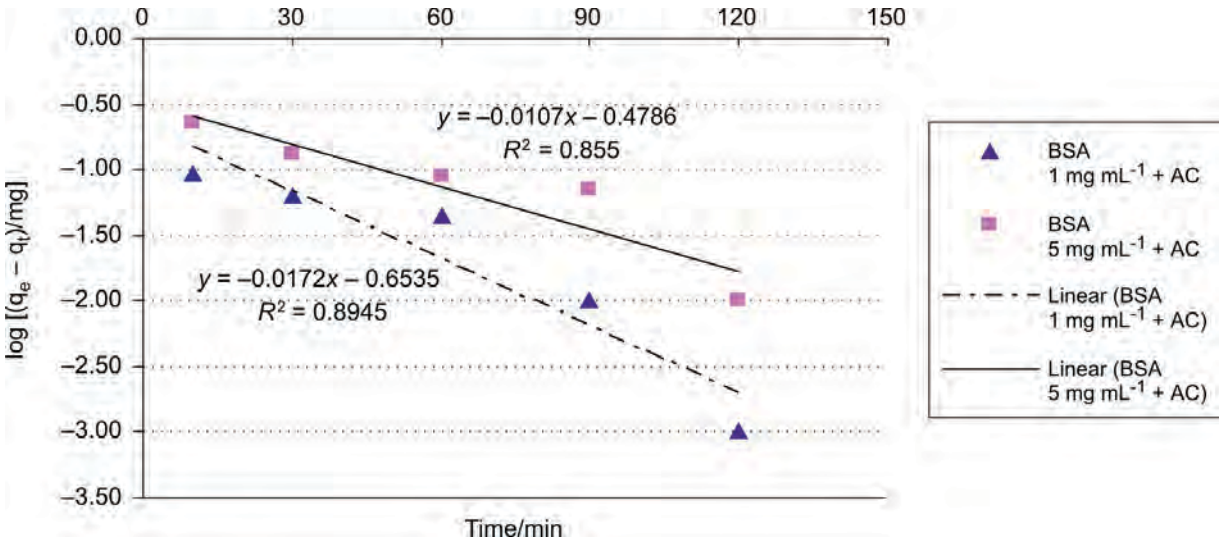

Figure 1 Lagergren plots for BSA adsorption onto activated carbon.

2.00 mL of 0.10 mol L^{-1} H_3PO_4 solution at 800 °C for a period of 5 min. The activated samples were then washed with 0.50 mol L^{-1} ethanoic acid and thoroughly rinsed with distilled/deionized water and dried.[13]

2.3. Protein Adsorption Study

Exactly 1.0 mL of 1.00 mg mL^{-1} or 5.00 mg mL^{-1} BSA solution was used as the white wine model system. This was added to 50 mg of AC or AL in a polypropylene (PP) centrifuge tube. The mixtures of each experimental group were continuously shaken in a shaker at 25 °C, for residence times of 10, 30, 60, 90 and 120 min respectively. The samples were further microcentrifuged for 2 min and 0.5 mL of the supernatant was assayed for protein concentration using the Bradford method at a UV absorption band wavelength of 595 nm.[14] The mass of adsorbed protein was calculated by subtracting the mass of unadsorbed (free) protein remaining in the supernatant from the mass of protein in the control (protein solution not suspended in adsorbent sample).

3. Results

The results are expressed as the average of triplicate determinations ± the standard deviation. The results were compared using SAS statistical software, and a 2-way ANOVA was applied using Duncan's post-hoc multiple range test (DMRT) at $P > 0.05$ in order to assess differences in the mean values of BSA adsorbed due to the effects of adsorbent type and incubation time.[15] Pearson's correlation was used to establish the relationship between the amounts of adsorbed BSA by AC and AL.[16]

The time profiles for the adsorption of BSA onto AC and AL indicated that the masses of BSA adsorbed onto AC increased steadily from 0.392 ± 0.002 mg to 0.484 ± 0.004 mg in the 1.0 mg mL^{-1} solution group, and from 3.46 ± 0.01 mg to 3.69 ± 0.02 mg in the 5.0 mg mL^{-1} solution group. The masses adsorbed onto AL increased steadily from 0.346 ± 0.003 mg to 0.446 ± 0.001 mg in the 1.0 mg mL^{-1} solution group, and from 3.24 ± 0.06 mg to 3.50 ± 0.03 mg in the 5.0 mg mL^{-1} solution group. The results showed that the average mass of adsorbed BSA onto AC was 1.10 ± 0.07 times higher than that onto AL. Statistical analysis by two-way ANOVA showed no significant difference in the mass of BSA adsorbed onto the two adsorbent types but a statistically significant difference in the mass adsorbed by variation of incubation time. A positive correlation was obtained for the adsorption processes of AC and AL. Saturation of the adsorption process took place at 90 min for AL and 120 min for AC. Activated carbon has a characteristic hydrophobic surface while alumina (Al_2O_3) has a hydrophilic surface; the preferential adsorption of proteins on hydrophilic surfaces is generally governed by electrostatics.[17] The mechanism of adsorption of BSA onto AC and AL is speculated to be through a specific electrostatic attractive force between the negative charges on BSA and localized positive charges on the AC and AL surfaces, though the net surface charge of both is negative at the working pH. A number of factors are important in determining the amount of protein on surfaces. These include the magnitude and sign of charge of both the protein and the surface and the degree of hydration of the protein.[18]

Three kinetic models were applied for determining the rate and mechanism of adsorption of BSA onto AC and AL. These are based on the assumption that the adsorption follows a pseudo-first order rate equation and was further investigated by a pseudo-second order rate equation. The pseudo-first order equation is generally expressed as[10]

$$\frac{dq_t}{dt} = k_{ad}(q_e - q_t) \tag{1}$$

After integration and applying boundary conditions $t = 0$ to $t = t$ and $q_t = 0$ to $q_t = q_t$, the integrated form of Equation (1) becomes the Lagergren equation,[10] given as

$$\log\,(q_e - q_t) = \log q_e - \left(k_{ad}/2.303\right)t \tag{2}$$

where q_e and q_t are the masses of BSA adsorbed at equilibrium and time t respectively, t is time, and k_{ad} is the specific rate constant in min^{-1}. A plot of log $(q_e - q_t)$ *versus* t will give a linear relationship from which k_{ad} and q_e can be determined from the slope and intercept of the graph, respectively.

The Bhattacharya and Venkobacharya equation is also a pseudo-first order equation given as

$$\log[1-(U)T] = \left(k_{ad}/2.303\right)t \tag{3}$$

where

$$(U)T = \frac{C_i - C_t}{C_i - C_e} \tag{4}$$

C_i and C_t are initial concentration and concentration at time t, respectively, k_{ad} is the rate constant and C_e is the concentration at equilibrium.

As presented in Figs. 1 and 2, straight lines with correlation

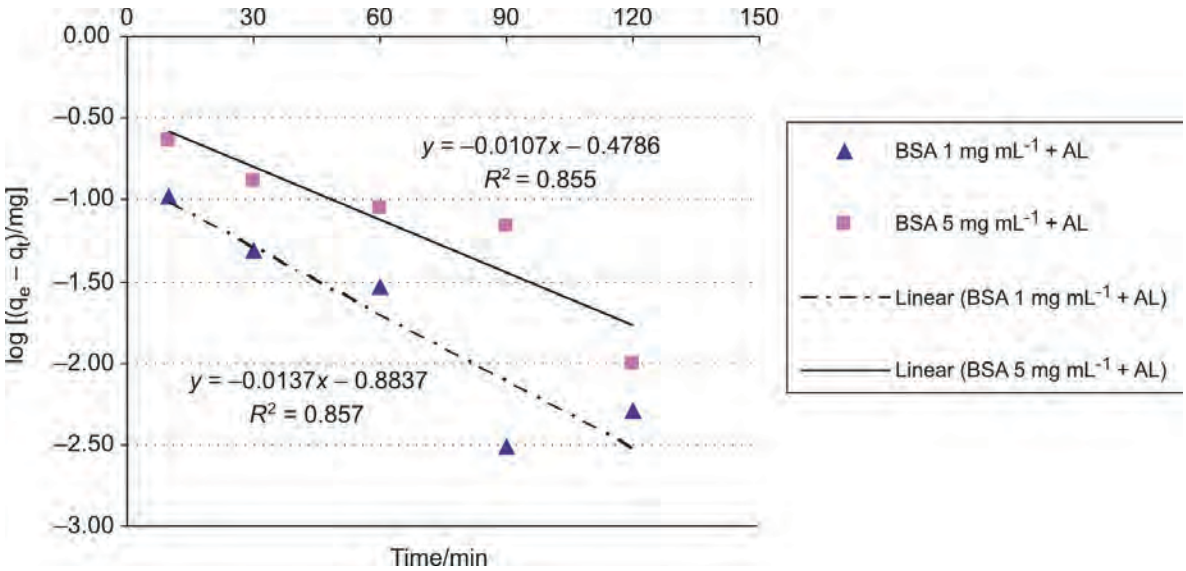

Figure 2 Lagergren plots for BSA adsorption onto alumina.

coefficients, R^2, in the range 0.855–0.894 indicate acceptability of the model for the two adsorbents.

The Bhattacharya and Venkobacharya plots (Figs. 3 and 4) also gave linear relationships with correlation coefficients in the range 0.923–0.979. This further reaffirms the fit of the experimental data to pseudo-first order kinetics, as suggested by the Largergren model.[10] The high values of $(U)T$ from Equation (4) show that BSA molecules have greater accessibility to the adsorbent surfaces. The kinetic theory behind Figs. 3 and 4 is that it could be used to explain the sorption process in terms of adsorption being controlled by film diffusion or particle diffusion.[19] The study of the kinetics of adsorption of mucin onto commercially pure titanium confirmed the presence of an intra-particle diffusion process as the rate-determining step.[20] The linearity of Figs. 3 and 4 indicates that the adsorption of BSA onto AC and AL is controlled by particle diffusion. Linearity of the diffusibility plot showed that the pseudo-first order equation proposed was adequate in describing the adsorption study.

The pseudo-second order equation is given in Equation (5) as[11]

$$\frac{dq_t}{dt} = k_{ad}(q_e - q_t)^2 \qquad (5)$$

Integrating Equation (5) gives

$$\frac{t}{q_t} = \frac{1}{h} + \frac{1}{q_e} \cdot t$$

where $h = k_{ad}.q_e^2$ (mg g^{-1} min^{-1}) can be regarded as the initial adsorption rate.

If the pseudo-second order kinetics are applicable to the experimental data, a plot of t/q_t *versus* t will be linear (see Figs. 5 and 6).

The adsorption rate constant is higher at lower adsorbate concentration as shown in Table 1. This implies that at high concentration the average distance between BSA molecules diminishes to a point where each affects the charge distribution of the BSA molecules close to it. The coefficient of determination, R^2, was chosen as the error function for the kinetic model analysis. The coefficient of determination for the pseudo-first order kinetic model was smaller than for the pseudo-second order model, having $R^2 > 0.998$, indicating that the pseudo-second order equation is more appropriate in describing the adsorption. The specific rate constants for adsorption of BSA onto AC were found to be greater than those of AL. This result could be attributed to the larger pore size of AC and conforms to the report of Welch[3] that the approximate diameter of typical wine proteins is 30 to 50 Å and a cogel with substantial porosity having diameters greater than 60 Å should be chosen for use, after

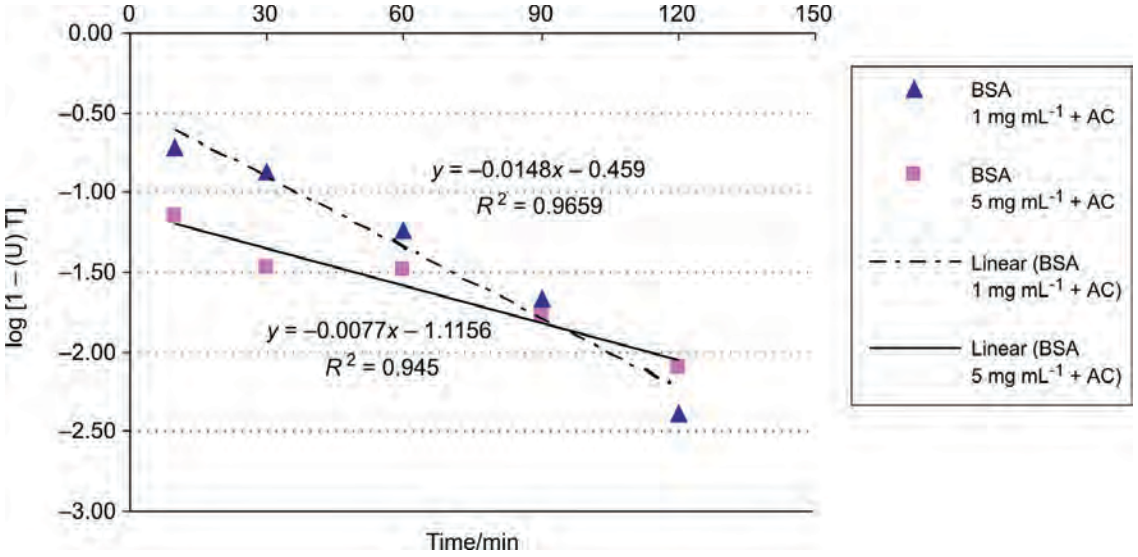

Figure 3 Bhattacharya and Venkobacharya plots for BSA adsorption onto activated carbon.

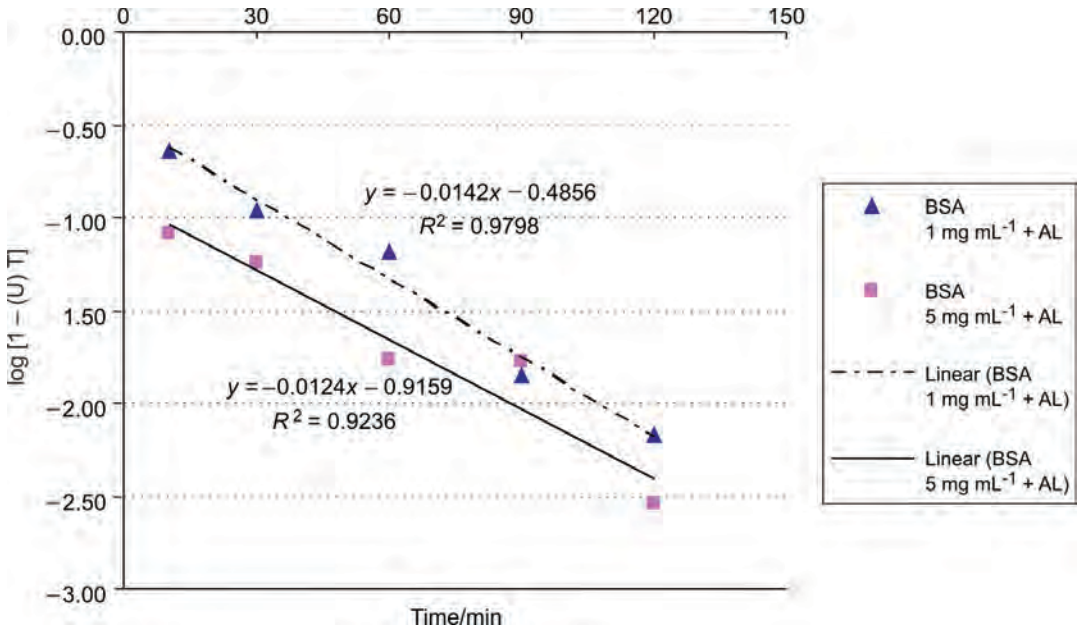

Figure 4 Bhattacharya and Venkobacharya plots for BSA adsorption onto alumina.

Table 1 The rate constants, k_{ad}, for the adsorption of BSA onto AC and AL.

Adsorbent and concentration of BSA/mg mL^{-1}	Lagergren k_{ad}/min^{-1}	Bhattacharya and Venkobacharya k_{ad}/min^{-1}	Pseudo-second order equation k_{ad}/min^{-1}
AC (1.0)	3.92×10^{-2}	3.22×10^{-2}	42.76×10^{-2}
AC (5.0)	1.61×10^{-2}	1.61×10^{-2}	38.49×10^{-2}
AL (1.0)	2.99×10^{-2}	3.22×10^{-2}	50.44×10^{-2}
AL (5.0)	2.46×10^{-2}	2.76×10^{-2}	30.55×10^{-2}

appropriate activation. Based on the result obtained in this analysis, AC is a better adsorbent than AL. The use of AC is therefore recommended; this will help to minimize environmental impacts of agricultural wastes. Obviously this is not a solution to the huge amount of agricultural wastes produced, but it is a way to obtain economic benefit from a waste product.

4. Conclusion

The kinetics of the adsorption were found to be based on the assumption of an intra-particle diffusion-controlled pseudo-second order mechanism, with adsorption rate constants being higher at lower adsorbate concentration. From the kinetic model analyses using coefficients of determination, the pseudo-second order model was the more appropriate for the description of BSA transport from the bulk white wine model solutions onto the surfaces of AC and AL. Other chemical components, responsible for the wine's aroma, complexity and colour, need to remain so that the wine's sensory characteristics are substantially unaltered by the treatment. Investigation on the effect of the adsorption on these sensory characteristics is being carried out.

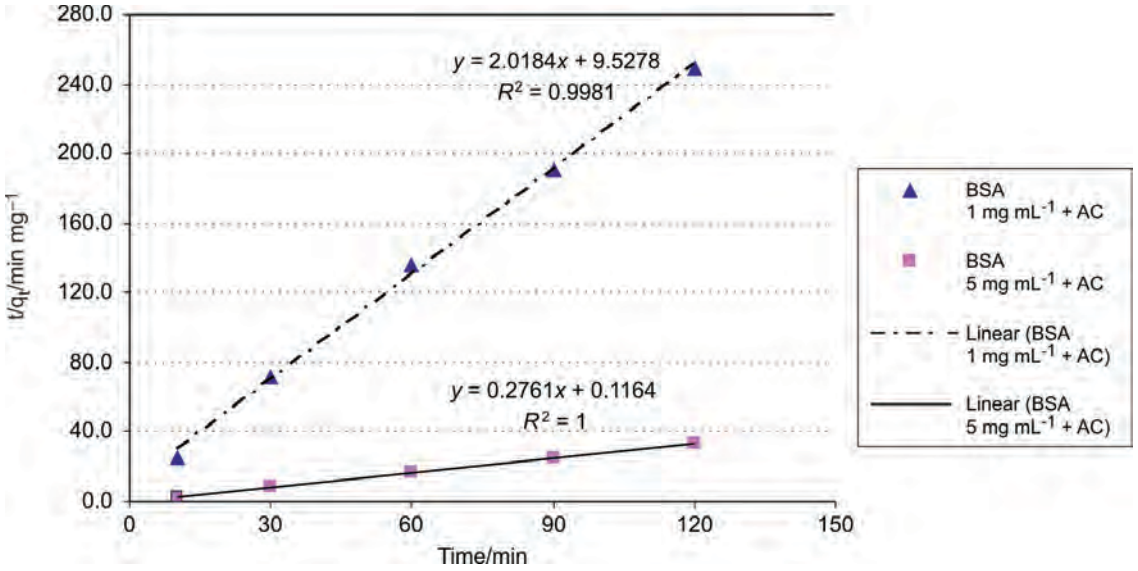

Figure 5 Pseudo-second order adsorption kinetics of BSA onto activated carbon.

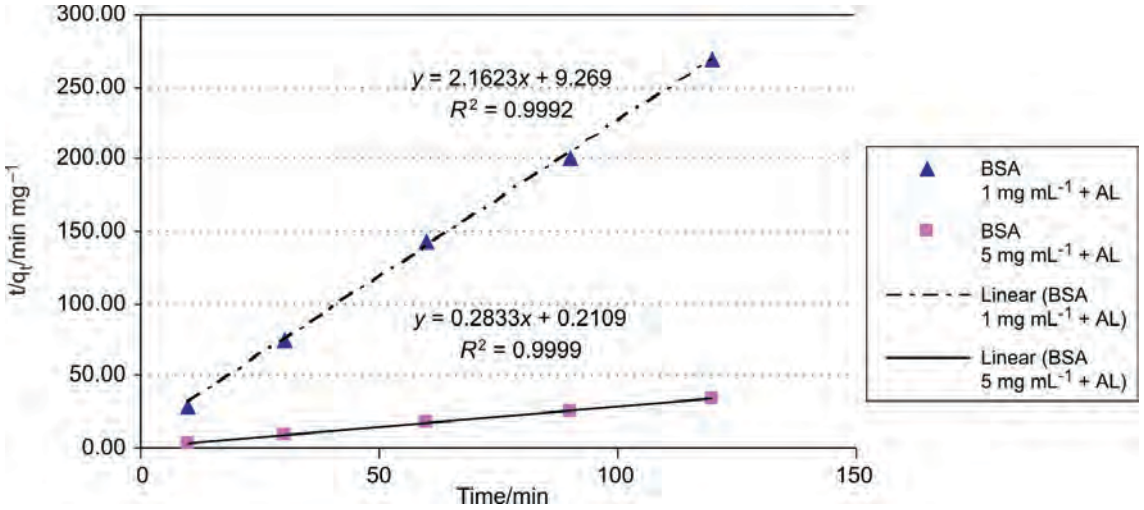

Figure 6 Pseudo-second order adsorption kinetics of BSA onto alumina.

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References

1 L.F. Bisson and C.E. Butzke, Microsoft Encarta 2009 [DVD]. Microsoft Corporation, Redmond, WA, USA. (Accessed 3 January 2010).

2 S.L. Brown, V.J. Stockdale, F. Pettolino, K.F. Pocock, M. de Barros-Lopes, P.J. Williams, A. Bacic, G.B. Fincher, P.B. Hoj and E.J. Waters, *Appl. Microbiol. Biotech.,* 2006, **73**, 1363–1376.

3 W.A. Welsh, *Adsorption of proteins from fluids.* http://www.freepatentsonline.com/4684530.html (Accessed 25 June 2008).

4 J.C. Hsu, D.A. Heatherbell, J.H. Flores and B.T. Walson, *J. Enology Viticulture,* 1987, **38**, 17–22.

5 V. Pachova, M. Ferrando, C. Guell and F. Lopez, *J. Food Sc.,* 2001, **67**, 2116–2121.

6 L. Bell, *Applied Microbiol.,* 1983, **45**, 784–791.

7 S.W. Kim and R.G. Lee, *Appl. Chem. Protein Interf. (Adv. Chem. Series),* 1975, **4**, 145–218.

8 I. Lundstrom, *Prog. Coll. Polym. Sci.,* 1985, **70**, 76–82.

9 M. Horsfall, Jr. and A.I. Spiff, *Bull. Chem. Soc. Ethiop.,* 2005, **19**, 89–102.

10 S. Lagergren, *Handlinger,* 1898, **24**, 147.

11 Y.S. Ho, D.A. John-Wase and C.F. Foster, *Water Res.,* 1995, **29**, 1327–1335.

12 C.E. Gimba and N.A. Bahago, *Chem. Class J.,* 2004, **1**, 208–213.

13 F.N. Salazar, J.P.F. Bruijn, L. Seminario, C. Güell and F. Lopez, *J. Food Eng.,* 2007, **79**, 1329–1336.

14 M.M. Bradford, *Analyt. Biochem.,* 1976, **72**, 248–254.

15 F. Wilcoxon, *Biometr. Bull.,* 1945, **1**, 80–83.

16 M. Hollander and D.A. Wolfe, *Biometrika.,* 1999, **2**, 15–19.

17 K. Kandori, F. Aya and I. Tatsuo, *Phys. Chem.,* 2000, **2**, 2015–2020.

18 T.H.W. Diana and E. Graham, *Biomater.,* 1996, **17**, 859–864.

19 Y.S. Ho and G.A. Mckay, *Trans. I. Chem. E.,* 1998, **76**, 313–321.

20 K.I. Omoniyi, A.J. Lori and O.J. Ogbodobri, *Continent. J. Applied Sci.,* 2007, **2**, 7–11.