THERMODYNAMIC STUDIES ON ADSORPTION OF BOVINE SERUM ALBUMIN (BSA) USING NITROCELLULOSE MEMBRANE

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Abstract—Thermodynamic studies on adsorption of Bovine Serum Albumin (BSA) protein from aqueous solution by using nitrocellulose (NC) membrane was investigate in this study. Nitrocellulose membrane has been choose as adsorbent due to high tensile strength and hydrophilicity, excellent handling in ensuring that membrane will not rip and detergent free. In this study, characterization of NC membrane was studied by using Attenuated Total Reflected and Fourier Transform Infrared Spectroscopy (ATR-FTIR). Moreover, thermodynamic studies on adsorption of BSA protein on NC membrane was investigated and show that this process was not spontaneous reaction and endothermic. The standard Gibbs free energy ($\Delta_r G^\theta$), the change in standard enthalpy ($\Delta_r H^\theta$) and standard entropy ($\Delta_r S^\theta$) was calculated. The result of $\Delta_r G^\theta$ value for 298K, 308K and 318K were 6491.24 J/mole, 3047.25 J/mole and 660.96 J/mole respectively. The results for $\Delta_r H^\theta$ was 64790.17 J/mole and $\Delta_r S^\theta$ shows that 198.24 J/mole. K.

Keywords: Adsorption, BSA protein, Nitrocellulose membrane, Thermodynamic

I. INTRODUCTION

Due to climate nowadays, the health issue in Malaysia keep increasing because of the lack of expert workers in hospital and limitation of medical technology. Therefore, the application of membrane in the medical field especially in the development of diagnostic kit are formed as an alternative kit become more extensively. Moreover, diagnostic kit is the one of important elements in heterogeneous immunoassay is the application of membrane.

Physical process is the alternative ways which employ the adsorption material to bind and isolate the protein to membrane in case of immunoassay protein isolate from solution to membrane. Adsorption can be defined as a mass transfer operation in which substances present in a liquid phase are adsorbed or accumulated on solid phase and thus removed from liquid [1].

Recently, protein adsorption has been extensively investigated owing to its important role in biomedical applications. Adsorption of protein involving complex interactions like, hydrophobic interaction, electrostatic interaction and hydrogen bonding [2].

In the study, NC membrane as an adsorbent was characterized and thermodynamic performance for the adsorption of BSA protein on NC membrane were investigated. The standard Gibbs free energy ($\Delta_r G^\theta$), standard enthalpy ($\Delta_r H^\theta$) and standard entropy ($\Delta_r S^\theta$) were also calculated for analysing adsorption mechanism. Moreover, in this research were conducted to know whether the protein have a strong binding or not with the membrane because poor protein binding will lead to the misleading interpretation of the results.

II. METHODOLOGY

A. Preparation of membrane and protein

NC membranes (HS 135) were manufactured by Millipore Corporation (Bedford, MA) was used as adsorbent. The flat sheet membrane were cut into 20mm X 10mm for characterization. Bovine serum albumin (BSA, A4378) was used as the model protein and was supplied by Sigma (MO, USA) with a molecular weight 66463 Da. For preparation of 0.5 g/L BSA protein, the preparation was started by mixing of 0.125g of powder BSA protein and 500mL of distilled water.

B. Membrane Characterization

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR, Perkin Elmer) was used to monitor and characterize organic thin film systems. The surface of the scanning was wiped by using acetone. Then, put the NC membrane and observed the results in different spots.

The membrane porosity, ξ is defined as the pores volume divided by total volume of the porous membrane and is determined from the weight of a liquid, 2-butanol (>99.0%, Merck). For this analysis, the NC membrane was immersed in the 2-butanol for five minutes and the surface of the membrane was dried by using filter paper. The membrane was weight before and after absorbing the 2-butanol. Therefore, porosity of NC membrane (ξ) based on the dry station of the membrane and it was calculated according to the Eqn. 1[3].

$$\xi = \frac{\frac{(W_B - W_M)}{\rho_B}}{\frac{(W_B - W_M)}{\rho_B} + \frac{W_M}{\rho_P}} \text{ X } 100\% \quad (1)$$

Where ξ is the porosity of the membrane, W_B is the wet membrane weight, W_M is the dry membrane weight, ρ_B is 2-butanol density (0.81 g/cm^3) and ρ_P is the NC density (1.55 g/cm^3). Assume that the density of all materials remaining constant in the wet membranes and there is no air trapped in the membrane pores.

C. Experimental Procedures

The thermodynamic experiments of BSA solutions on NC membranes were carried out in 50 ml test tubes at 298 K, 308 K and 318 K. This experiment started with cut the membrane in 12mm of diameter and immersed the membrane into 3 mL of BSA protein for three hours at initial concentration of 0.5 g/L. Then, 2 mL of bicinchoninic acid (BCA) protein assay kit was added into test tube for 30 minutes at 37°C. The absorbance of the solution was tested by using UV spectrophotometer (HACH) at 562μm wavelength. This experiment was repeated for three times in different temperature.

D. Thermodynamic Studies

The experiment was carried out to measure the nature of the thermodynamic parameters such as changes in standard enthalpy (ΔH°), standard entropy (ΔS°) and standard Gibbs free energy (ΔG°) adsorption of protein at three different temperature. Three parameters were obtained from adsorption experiments at various temperatures (298 K, 308 K and 318 K). The parameters were obtained at different temperatures, were used to calculate important thermodynamics properties, the equations estimating the parameters were express as follows [4,5].

$$k = \frac{C_i - C_e}{C_e} \quad (2)$$

$$\Delta G° = \Delta H° - T\Delta S° \quad (3)$$

$$\ln K = \frac{\Delta S°}{R} - \frac{\Delta H°}{RT} \quad (4)$$

Where k_c is the equilibrium constant, R is the universal gas constant (8.314 J/mol.K), C_i is the initial concentration of adsorbent (mg/mL), C_e is the equilibrium concentration of adsorbent in the solution (mg/mL) and T is the absolute temperature in Kelvin. The values of ΔH° and ΔS° were obtained from the slope and intercept of the plot of ln (k) against 1/T.

III. RESULTS AND DISCUSSION

A. Porosity

NC membrane was tested to determine the porosity of the membrane and this experiment repeated in three times. The weight of wet and dry membrane are listed in Table 1 along with the porosity and standard error of the membrane. The weight of the membrane samples after immersed with 2-butanol are 0.0406g, 0.0397g and 0.0434g. After the membrane was dried till reached equilibrium are 0.0202g, 0.0208g and 0.0224g.

Table 1: Pore size of NC membrane

Sample	Weight of membrane (g)	Porosity (%)
1	0.0202	61.08
2	0.0208	58.51
3	0.0224	59.15
Mean value		59.58
Standard error		0.8485

As can be seen in Table 1, the mean porosity of NC membrane is 59.58% ±0.84. The high porosity will small membrane's pore size will provide high internal surface area. Moreover, high porosity will increase the interconnection between membrane pores increase.

B. ATR-FTIR

FTIR is a useful instrument to identify the chemicals either in organic or inorganic compound. The spectra of FTIR is a tool for identifying the functional groups in a molecule, as each specific bond information on a complex to study the strength and the fraction of hydrogen bonding and miscibility[6]. The spectra of NC membrane were taken in the range of 500 cm^{-1} to 4000 cm^{-1}. Based on Fig. 2., it shows the several distinct and sharp absorptions at 1713 cm^{-1} (indicative of –OH and –NH2 groups), 1243 cm^{-1} (indicative of C=C groups) and 847 cm^{-1} (indicative of C-H groups). –OH group represent hydrophilicity nature of NC membrane will make NC make easy to adsorb protein solution.

Typically, FTIR spectrum for NC shows the –OH group stretching band at 3440.937 cm^{-1}, the $–NO_2$ group stretching vibration at 1643.3 cm^{-1}, the $–NO_2$ group bending vibration at 1274.105 cm^{-1} [6].

C. Thermodynamics Studies

Gibbs free energy change

As a criterion in adsorption, Gibbs free energy change ($\Delta_r G^\theta$) defined as a measure of potential for reversible or maximum work that may be done by a system at constant temperature and pressure and was computed in adsorption by using the following equation [8]:

$$\Delta_r G^\theta = -RT \ln K \quad (5)$$

Where R is the universal gas constant (kJ/mole. K), T is the absolute temperature (Kelvin) and K is the equilibrium adsorption constant. K values indicates the relative distribution of solutes between aqueous and adsorbed phases [8].

As can be seen in Fig. 2, the change in standard Gibbs free energy ($\Delta_r G^\theta$) for 298K, 308K and 318K was 6.49 kJ/mole, 3.05 kJ/mole and 0.66 kJ/mole respectively. In this study, positive values of $\Delta_r G^\theta$ for three different temperature was indicate that BSA adsorption is not spontaneous change in reaction [8]. If the negative $\Delta_r G^\theta$ values was obtained the reaction in spontaneity and favourability of adsorption. Moreover, range of $\Delta_r G^\theta$ value for physical adsorption is 0 to -20 kJ/mole and range of $\Delta_r G^\theta$ for chemisorption belongs to -80 to -400 kJ/mole [8]. Thus, the value of $\Delta_r G^\theta$ indicating that the protein and NC membrane is promoted by physical adsorption.

Standard enthalpy change and standard entropy change

As can be seen in Fig. 3, it shows the Van't Hoff plot that gives correlation constant, R^2 values of 0.9949 and it shows that the adsorption of BSA protein on NC membrane well fitted to the Van't Hoff plot at 0.5 g/L initial concentration. In addition, it shows that the capability of protein to adsorb on NC membrane decrease as the temperature of the reaction increase.

Furthermore, this study was conducted to determine whether the adsorption process was exothermic or endothermic in nature. Fig. 3 shows the adsorption process was endothermic. As the adsorption of BSA protein on NC membrane increase as the temperature increase. The standard enthalpy ($\Delta_r H^\theta$) and standard entropy ($\Delta_r S^\theta$) was calculated by using Eqn. 5 and gives 64.79 kJ/mole and 0.198 kJ/mole. K respectively. Positive value of $\Delta_r H^\theta$ implied that the adsorption was endothermic.

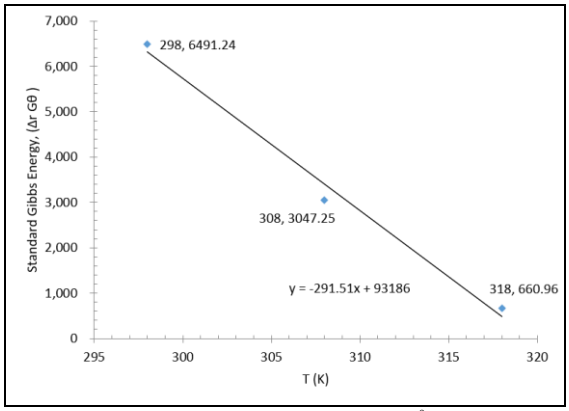

Fig. 2. Graph of standard Gibbs Energy, $\Delta_r G^\theta$ against temperature. Temperature: 298K, 308K and 318K.

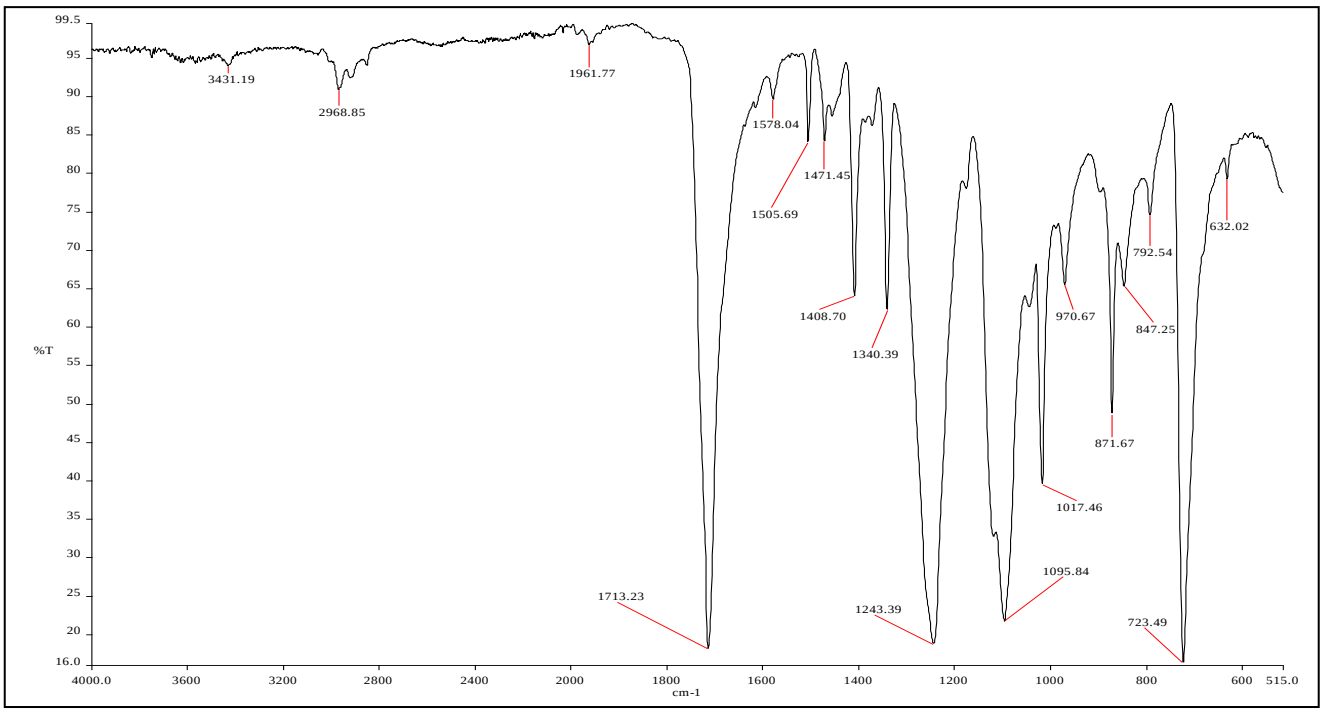

Fig. 1. FTIR spectra for NC membrane

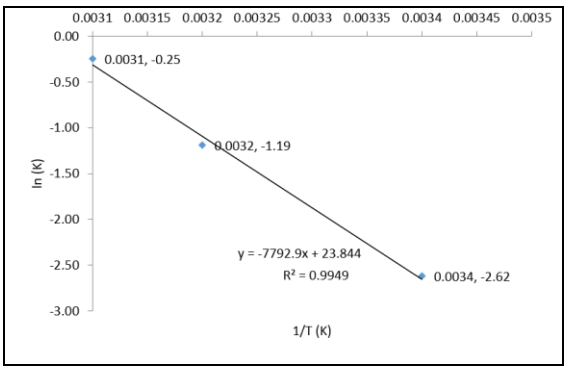

Fig. 3. Van't Hoff plots of BSA adsorption onto NC membrane at different temperature. Initial concentration: 0.5 g/L; Temperature: 298K, 308K and 318K.

IV. SUPPLEMENTARY DATA

With the courtesy of Dr Norhidayah Ideris and Dr S.C. Low, the image of NC membrane (HS 135) observed under magnification of 500.X is provided shown in Fig. 4. The membrane porosity for NC membrane was 79.54 ± 1.91%. This type of structure is suitable for protein adsorption because it can accommodate the large protein molecules within the high surface area of the polymer matrix. Generally, NC membranes consist of a matrix of randomly oriented fibers that are bonded together to form a tortuous maze of highly porous channels [7].

V. CONCLUSION

The NC membrane was found as a potential adsorbent in binding with BSA protein. The NC membrane was characterize by using porosity, ATR-FTIR. The porosity for NC membrane is 59.98% ± 0.8485. The higher the porosity of the membrane should indicate the higher adsorption of protein binding onto membrane. For ATR-FTIR equipment, the spectra of nitrocellulose membrane were taken in the range 500 cm^{-1} to 4000 cm^{-1} and the most intense peaks in this study at 1713 cm^{-1}, 1243 cm^{-1} and 847 cm^{-1}.

The experimental results revealed that the increased temperature condition as decrease the standard Gibbs energy ($\Delta_r G^\theta$). The $\Delta_r G^\theta$ value for different temperatures 298K, 308K and 318K are 6491.24 J/mole, 3047.25 J/mole and 660.96 J/mole respectively. The adsorption of BSA protein on NC membrane were best fitted in Van't Hoff plot with correlation constant, R^2 at 0.9949. From the results obtained, the standard enthalpy ($\Delta_r H^\theta$) was 64790.17 J/mole indicate the adsorption in endothermic process and standard entropy ($\Delta_r S^\theta$) was 198.24 J/mole. K indicates the distribution of BSA protein was adsorbed on NC membrane was more chaotic.

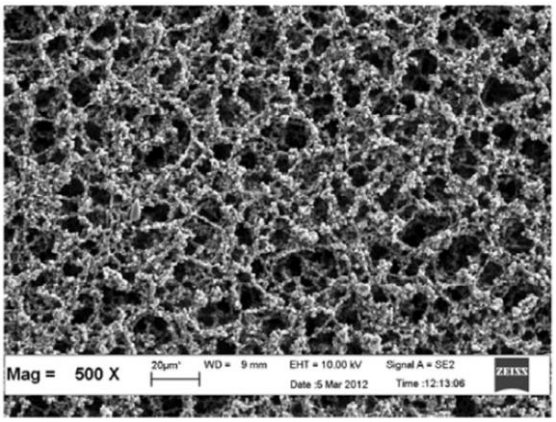

Fig. 4. Morphology of NC membrane (HS-135) by using FESEM. Magnification: 500X [7]

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