Thermodynamic studies on adsorption of BSA using nylon membrane

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***Abstract*—The aim of the study was to investigate the nylon membrane morphology and thermodynamic aspects for adsorption of Bovine Serum Albumin (BSA) as model protein on a nylon membrane. The study focused on the nylon membrane morphology and the effect of temperature on BSA adsorption. The morphology has been studied by using scanning electron microscope (SEM), FTIR-ATR spectroscopy and contact angle analyzer whereas the thermodynamic would be using the dot blotting method. The findings of the studies show that the nylon membrane sample is moderately porous (46.72±0.018%), small pore size (0.159±0.039 µm, 0.181±0.039 µm) with hydrophilic (55.6°) nature that gave decent adsorption of BSA. While the thermodynamic study indicates that the adsorption reaction was exothermic ($\Delta_r H^\circ$ -1.871 kJ/mol), the adsorption may be dominated by physical reaction. The distribution of BSA molecules on adsorbent is less chaotic than the BSA mixture ($\Delta_r S^\circ$ -0.421 kJ/mol.K). In addition, the process is not spontaneous but feasible ($\Delta_r G^\circ$ 126 kJ/mol.K). The findings from the adsorption of BSA may be used to evaluate the modification of nylon membrane needed to obtain optimum adsorption and as the basis for different membrane's performance study in BSA adsorption.**

Keywords*—Nylon membrane, morphology, BSA adsorption, thermodynamic

I. INTRODUCTION

In recent time, a variety of disease have been existed and making a disruption to the earth population. These breakouts initiate a counter action from the people itself in order to survive and be healthy. People and organizations such as World Health Organization (WHO) have been working together in order to find a solution to prepare medical tools that could cure the diseases.

There are a lot of medical methods that utilizes a medical tool such as hemodialysis, plasmapheresis and diagnostic tool kit and it has been used for treatment purposes. Not only that, it can also be a tool to detect disease in the biomedical industry [1]. An antigen such as *Clostridium difficile* and *Giardia lamblia* used a detection method called diagnostic tool kit to detect diarrhea [2,3]. The preparation of diagnostic tool kits divided into two part, the antigen extraction and membrane selection.

Membrane such as nitrocellulose, polyehtersulfone, cellulose acetate and nylon have been used in the preparation of diagnostic tool kit [4]. These diagnostic tool kit applied the concept of filtration, absorption and adsorption as the working mechanism [1]. However, different membrane requires different properties and conditions for the optimum protein adsorption.

Hence, a study on the adsorption of BSA by using nylon membrane was conducted. The BSA protein was used as model protein because it is readily available and can be used on awide selection of membrane for adsorption activity [5].

The adsorption are affected by a lot of factors such as physicochemical condition properties [5] and solution chemistry [6,7]. Whereas, the most common mechanism of adsorption would be physical adsorption [8]. The physical adsorption may be caused by electrostatic interaction, hydrophobic interaction and van der walls force of attraction [9].The interaction may be affected by pH, ionic strength and the temperature.

The thermodynamic study on adsorption of BSA by using hydrophilic polyvinylidene fluoride (PVDF) microfiltration membrane have been carried out [5]. However, adsorption of BSA on nylon membrane had not been clearly specified. Therefore, a study on nylon membrane morphology and thermodynamic of BSA adsorption by using nylon membrane was carried out. The temperature has been examined in the study as the affecting factor to the BSA adsorption. Besides that, the adsorption mechanism also have been analyzed by finding the standard enthalpy ($\Delta_r H^\circ$), standard entropy ($\Delta_r S^\circ$) and Gibbs free energy ($\Delta_r G^\circ$).

II. METHODOLOGY

A. Materials

The ethanol used for the wetting and drying process was obtained from HmbG chemicals (95% ethanol). BSA protein was obtained from Sigma Aldrich with CAS number 9048-46-8. The flat-sheet nylon membrane was obtained from a supplier and used as it is. The nylon membrane was cut into several pieces of 10mm×10mm prior to the experiment. Novagen BCA protein assay kit (4% cupric sulphate, binichloric acid solution) obtained from Merck for the experiment.

B. Methods

The porosity of nylon membrane sample was measured by wetting and drying process. The nylon membrane was first being cut into a size of 20mm x 10mm. Then, the sample was soaked in ethanol solution for 5 minutes. The wet nylon membrane sample was then measured by using the weighing machine. The wet nylon membrane would then be inserted into an oven, operating at the temperature of 37°C. The weight of nylon membrane was measured at every 15 minutes for an hour. The experiment was repeated for 2 times to obtain the mean data. The porosity of the nylon membrane was calculated by using equation 1 [10].

$$\varepsilon = \frac{(W_W - W_d)/\rho_W}{(\frac{W_W - W_d}{\rho_W}) + (\frac{W_W}{\rho_p})} \quad (1)$$

Where the ε is the porosity of nylon membrane sample, W_w is the weight of wet nylon membrane (g), W_d is the weight of the dry nylon

membrane (g), while ρ_W is the density of water (g/cm^3) and the ρ_p is the density of nylon membrane (g/cm^3). The density of nylon membrane was first measured by using micrometer caliper at 1cm×2cm nylon membrane sample. The density of nylon membrane was calculated using equation 2.

$$\rho = \frac{M}{V} \tag{2}$$

Where, the ρ is the density of nylon membrane, M is the mass of nylon membrane (g), and V is the volume of nylon membrane (cm^3).

Then, theimages and pore size of nylon membrane were observed by using scanning electron microscope (TM3000 HITACHI,Japan) at 15kV [11]. The nylon membrane was cut into 10mm×10mm and coated with Au-pd alloy to reduce beam penetration [12]. Both sides of nylon membrane were observed. The SEM images of 500x-15000x magnification had been taken. The images taken by using SEM were then analyzed by using imageJ for the pore size distribution [12].

Nylon membrane sample was cut into a small piece of 20mm diameter and tested by using FTIR-ATR (Perkin Elmer and model Spectrum One) to study the spectra of the nylon membrane. The spectra of FTIR-ATR was recorded at a range of 3450 to 50cm-1 [13]. The spectrum results were observed at 4cm-1 resolutions at 45 incident angle using crystal.

The water contact angle of nylon membrane was measured by using contact angle analyzer (VCA3000,ASTInc.,USA) at ambient temperature, 25° [14].

The adsorption experiment was carried out by first, preparation of 3ml of BSA protein having a concentration of 0.5mg/l [15]. Then, it was tested at 300K, 305k, 310K, 313K and 318K [15] by using a water bath (MaXturday30, Wisd, Korea). The BSA adsorbed was then measured by using UV spectrophotometer at 562nm [16]. The absorbance from UV spectrophotometer was then compared to the standard curve of BSA protein.

The changes in thermodynamic parameter were also determined. The thermodynamic properties such as standard enthalpy ($\Delta_r H^o$), standard entropy ($\Delta_r S^o$) and Gibbs free energy ($\Delta_r G^o$) were calculated by using data from adsorption of BSA at 300 K, 305 K and 310 K. The equation used in the experiment were shown below [17]:

$$K = \frac{C_i - C_e}{C_e} \tag{3}$$

$$\Delta_r G° = -RT \ln(K) \tag{4}$$

$$\Delta_r G° = \Delta_r H° - T\Delta_r S° \tag{5}$$

$$\ln(K) = -\frac{\Delta_r H°}{RT} + \frac{\Delta_r S°}{R} \tag{6}$$

Where the K value is the equilibrium constant and the values of $\Delta_r G°$ and $\Delta_r S°$ are obtained from the slope of plotted graph of ln (K) versus 1/T [18].

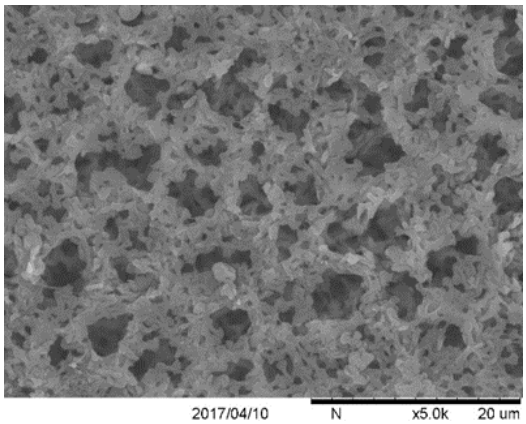
III. RESULTS AND DISCUSSION

A. Characterization of nylon membrane

The porosity of the nylon membrane obtained was 46.72±0.018%. The porosity of nylon membrane sample is close to porosity by Wang et al., where they obtained 52%, 54% and 49% for the porosity of PVDF membrane [19]. These shows that the nylon membrane has adequate porosity. Theoretically, the higher the porosity the better the adsorption due to the higher total surface area [20]. However, the moderate porosity can be backed up with smaller pores size.

The image of nylon membrane was captured using SEM and the pore size distribution was analyzed using imageJ. Figure 1 shows the image of nylon membrane at 5000x magnification.

(a)



(b)

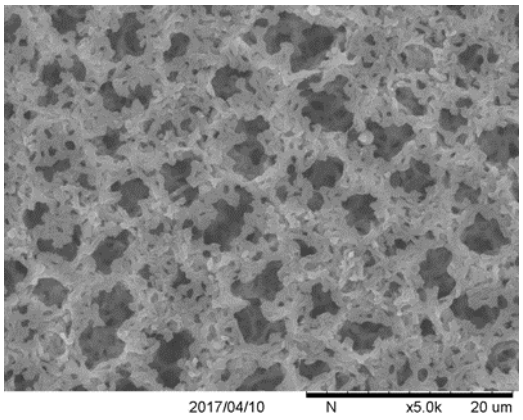


Fig.1. SEM image of nylon membrane sample at 5000x magnification at both side (a) and (b**).**

The SEM image shows that the nylon membrane sample has a symmetrical structure where top and bottom showed almost the same size and porosity [21]. This symmetrical structure of membrane is better for dot plotting while the symmetrical structure of membrane is better for filtration [21]. The study also indicates that the adsorption experiment was a type of dot plotting, the symmetrical structure has improved the BSA adsorption on nylon membrane.

Then, by using the imageJ software, the pore size distribution was analyzed and it resulted to an average pore size, 0.159±0.039 µm for (a) and 0.181±0.039 µm for (b). On the other hand, the number of pore was 3289 for (a) and 2804 for (b). The pore size obtained could be considered as small. According to Low et al., smaller pore size with the higher number of pores have a higher total surface area for adsorption area [1]. Therefore, the smaller pore size of nylon membrane sample would promote decent BSA adsorption. Then, by combining the average porosity of nylon membrane with smaller pore size, the nylon membrane could result to a decent adsorbent for BSA adsorption.

In the study, the nylon membrane composition and structure was also being analyzed by using FTIR-ATR. Figure 2 shows the FTIR-ATR measurement of nylon membrane sample

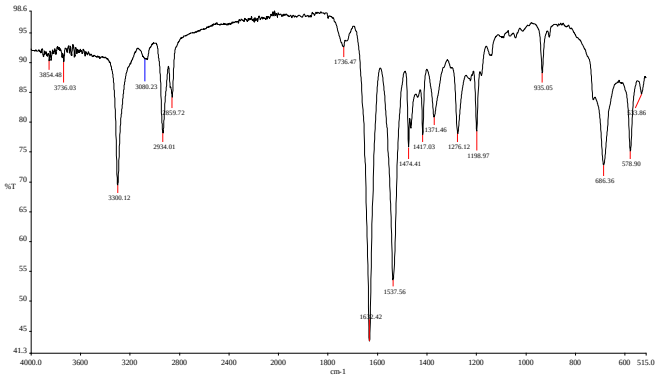


Fig. 2. FTIR-ATR spectra at wavelength of 515-4200cm^{-1}

The peak at 3300.12, 3080.23, 1632.42 and 1537.56 cm^{-1} shows the hydrogen bonding ability of the nylon membrane. It indicates that the higher the peak, the higher the hydrogen bonding ability. According to Joshi et al., high peak of hydrogen bonding indicate that the sample is hydrophilic [22].].Naturally, the nylon membrane sample has notable intensity of hydrogen bonding, hence the sample itself would indicate the presence of some characteristics of hydrophilic. The effect of hydrophilic nature will be discussed in the next section, contact angle. In addition, at the peak of 1198.97 cm^{-1} and 935.05 cm^{-1} it shows that the nylon membrane is highly composed of α-crystals [23]. The α-crystals structure have the zigzag structure while the γ-crystals have the helix structure [24]. The γ-crystals are identified at peak 974 [23] and as analyzed in the FTIR-ATR spectra reading, the nylon membrane has more α-crystals structure than γ-crystals structure. The zigzag structure of α-crystals gives the membrane more stability in thermodynamic change whereas the helix structure of γ-crystals has the meta-stable characteristics [24]. This is due to the thermodynamic stability of α and γ-crystals that were affected by the interaction of intermolecular and intramolecular of nylon membrane molecule because of the competing nature between both agents [25]. According to Galimberti et al., the hydrogen bonding interaction promote the formation of α-crystal structure [25]. Therefore, the nylon membrane has dominance α-crystal over γ-crystals structure due to high hydrogen bonding interaction.

In the present paper, the contact angle of the nylon membrane sample was analyzed by using the angle analyzer. Figure 3 shows the image of contact angle.

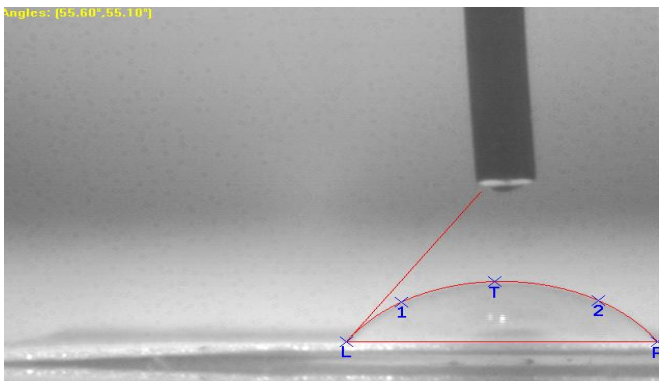


Fig. 3. Water contact angle of nylon membrane sample**.**

The image shows that the water contact angle of the nylon membrane sample is 55.6°. The result obtained by Farahmand et al., shows that the contact angle of nylon membrane obtained was less than 20° and have a hydrophilic nature [26]. Theoretically, a contact angle less than 90° is hydrophilic, whereas more than 90° is hydrophobic. Hence, by comparing with Farahmand et al., and according to the theory, a nylon membrane sample has a hydrophilic nature. According to Feast et al. [27] and Iaraelachvili et al., [28] the protein adsorption was greatly affected by hydrophibic interaction between membrane surface and the protein molecules. This interaction explained by Bayer et al., where according to them, the hydrophilic surface membrane will first develop hydrogen bonds with water molecules. Then, any protein molecule that is expected to adsorp on the surface will have to displace the surface-water molecules bound. This process would require energy and would not occur naturally.

Theoretically, the adsorption reaction is affected by physical and chemical adsorption [8]. The physical adsorption consists of reaction such as van der Waals, electrostatic reaction, and hydrogen bonding [9].In the experiment, the hydrophilic nature is caused by the composition of the nylon membrane. As discussed in the section before, the FTIR-ATR spectra of nylon membrane sample show that the nylon membrane surface functionalities of reaction consist of amine (-NH2) and hydroxyl (-OH). This surface functionalities of reactive cause a polar reaction between water molecules and nylon membrane surface. The different polar of nylon membrane surface molecules and BSA protein molecules would attract them together and form a layer. Hence, the water molecules will be attracted to nylon membrane and diffuse into the membrane thus showing the nature of nylon membrane, hydrophilic.

In the experiment, there was hydrogen bonding interaction between nylon surface and BSA protein molecules. The magnitude of hydrogen bonding could be improved by increasing the polarity of nylon surface. This is a method called coating and it has been used by many researchers. The purpose of this method is to increase the pH of the nylon membrane surface.

However, according to Low et al. when the adsorbent is hydrophilic, the electrostatic interaction will predominate the reaction [20]. This reaction would favor the electrostatic interaction instead of the hydrogen bonding and the opposite charge between nylon membrane and BSA molecules will increase the adsorption whereas if the nylon membrane and BSA molecules hold the same charge, then the adsorptivity will decrease. Hence, by changing the pH of nylon surface, the adsorptivity of BSA may increase or it may also decrease. This is due to the electrostatic interaction that plays a greater role in adsorption of BSA and changing the pH of nylon surface may reduce the electrostatic charge [20].

On the other hand, the adsorption of BSA would reach its best at the isoelectric point (IEP) [20]. The adsorption of BSA protein molecules at this pH is at best due to the interaction such as hydrogen bond and van der Waals that utilize the short-range interaction between nylon surface and BSA molecules will be at the highest [29]. The electrostatic interaction would also play a significant role at the IEP, where the electrostatic interaction can be improved by adding electrolyte to improve ionic strength [20]. At the zero charge, IEP, there will be no competition between protein molecules and it will form a compact layer formation of BSA molecules on the nylon surface. The compact layer would promote the shorter interaction due to shorter range between protein molecule and nylon surface. This condition promotes a better adsorption of BSA.

The thermodynamic study of BSA adsorption was carried out at 300 K, 305 K, 310 K, 313 K and 318 K at the constant concentration of 0.5 mg/ml and at 3 h contact time. Figure 4 shows the BSA concentration adsorbed at the respective temperature.

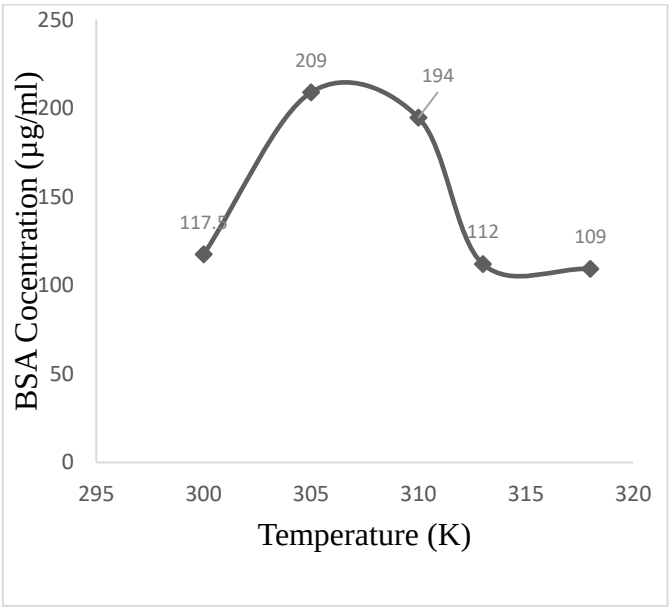


Fig. 4. BSA concentration adsorbed at respective temperature**.**

Then, the value of enthalpy change $\Delta_rH°$ and entropy change, $\Delta_rS°$ for the adsorption process were calculated by using equation 3-6. The value of Gibb's free energy, $\Delta_rG°$ was calculated by using equation 4-5. From the figure 5, the value of $(\Delta_rH°)/RT$ is the gradient of the graph of ln(K) versus 1/T. From the graph plotted the gradient, $(\Delta_rH°)/R$ was valued at 15558 and the y-intercept, $(\Delta_rS°)/R$ was valued at -50.653. Then, the calculated value of enthalpy change of reaction was -1.871 kJ/mol and the value of entropy change calculated was -0.421 kJ/mol.K whereas the value of Gibb's free energy calculated was 126 kJ/mol.

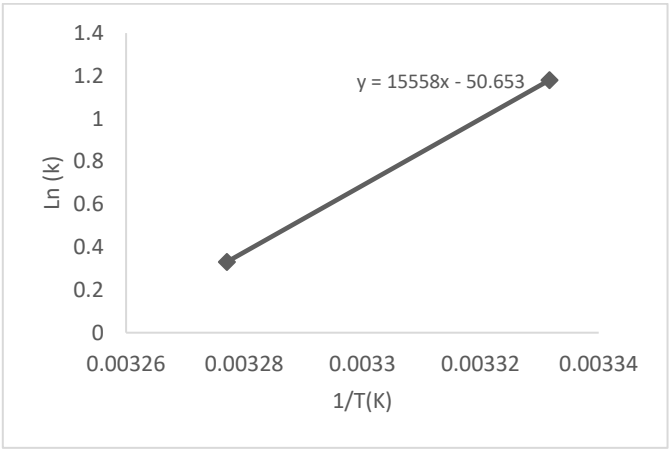


Fig. 5. BSA adsorption on the nylon membrane presented in ln(K) versus 1/T.

The negative value of enthalpy of reaction shows that the reaction is an exothermic reaction. However, according to Zhou et al. when the temperature increases the adsorption capacities also increases [5]. Thus, indicating the process is an endothermic reaction. The theory would contradict by the experimental data obtained due to the value is taken in the experiment was only before the optimum temperature, so the BSA intensity versus temperature curve would definitely have a positive gradient. While in Zhou et al. [5] and Drweesh et al., [30] they have taken into consideration of before and after the reaction and obtained a negative value gradient. The reason of taking value only before the optimum temperature would be because the temperature beyond optimum may not only affected by the adsorption capacity but denature of the protein too. While for lower temperature, it is believed that there is no protein denature but only a slow adsorption would occur.

The adsorption process is chemical adsorption if the enthalpy value is between 40 and 120 kJ/mol [10]. Hence, the -1.87 kJ/mol enthalpy obtained in the experiment shows that the adsorption mechanism was a physical adsorption. Therefore, modification of nylon membrane could be done to obtain a better adsorption rate by improving the physical adsorption.

In the study, the value of entropy obtained was -0.412 kJ/mol.K. The negative value shows that the distribution of BSA protein molecules on the surface is less chaotic compared to the aqueous solution of BSA. This, however, would contradict from the value obtained by Zhou et al., Yi et al. [5] and Drweesh et al. [30]. They define that the entropy value should be positive because of the adsorption reaction, the BSA protein molecules should be in randomness distribution. The reason why the experiment resulting otherwise is because of the ln(k) versus 1/T graph.

Table 1. Gibb's free energy at 300 K, 305 K, 310 K, 313 K and 318 K.

Temperature (K)	$\Delta_rG°$ (kJ/mol)
300	124
305	126
310	128
313	130
318	132

In the experiment, the increase in temperature resulted in increment of Gibb's free energy as shown in Table 1, whereas theoretically, increase in temperature should resulting in decrement of Gibb's free energy. The positive value of Gibb's energy shows that the BSA adsorption is not spontaneous but feasible [5] and according to Nashine et al., the decrement of Gibb's free energy as the temperature increase indicate that the high temperature accelerated the adsorption process [17]. However, since the plotted graph of ln(K) versus 1/T is different as in the entropy and enthalpy determination, the pattern would contradict from the experiment obtained.

IV. CONCLUSION

As for conclusion, the study of characterization of nylon membrane and adsorption study shows that a nylon membrane sample that have an average porosity and have high total surface area with smaller pore size give decent adsorption rate due to stronger combination magnitude of hydrogen bonding, van der Waals and electrostatic interaction between nylon membrane surface and the BSA protein molecules. However, the pore size should be smaller and the porosity should be increased to improve the total surface area and improve the adsorptivity The nylon membrane is also found as a hydrophilic surface that has low hydrophobic interaction. This, however, promotes the electrostatic and van der Waals interaction. In addition, the thermodynamic study shows that, as temperature increases, the Gibb's free energy decreases, the entropy will also come out as positive as the BSA protein molecule

started to bind at the nylon membrane surface that shows the randomness of BSA protein molecules increases. The thermodynamic study also shows that the adsorption of BSA is physical adsorption and it is an endothermic reaction (note that this is a a prediction if the graph of ln(k) versus 1/T is including all the data before and after optimum temperature, otherwise the reaction will be exothermic). The experimental data may be the basis of another study in comparison to the different of the membrane on the same topic and aspects. The findings from the adsorption of BSA may also be used to evaluate the modification of nylon membrane needed to obtain optimum adsorption and as a general basis for different membrane's performance study in BSA adsorption.

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