Thermodynamics Studies of Protein Adsorption on PVDF Membrane

Amirah Asyiqin bt Mohd Rashid, Dr. Norhidayah bt Ideris

Faculty of Chemical Engineering, Universiti Teknologi Mara, 40450 Shah Alam, Selangor, Malaysia

Abstract- The application PVDF membrane in immunoassays nowadays is significant especially for the use of detecting of contagious disease such as typhoid and many types of influenza. Therefore, the reason of conducting this study is to characterize the Polyvinylidene Fluoride (PVDF) membrane in terms of morphology, polymorph and hydrophobicity by using several methods and equipment which are Field Emission Scanning Electron microscopy (FESEM), Fourier-Transform Infrared Spectroscopy (FTIR), water contact angle using Video Contact Angle system and porosity test. This study is also aimed to investigate the thermodynamic aspect for adsorption of bovine serum albumin (BSA) as a model protein on PVDF membrane.

Keywords- PCDF membrane, BSA protein, Protein adsorption, thermodynamics

INTRODUCTION

Nowadays, the application of membrane in the field of biomedical is widely spreading especially in the development of diagnostic kits. Since there is scarcity of medical expertise and medical resources, the demand for diagnostic kits has increased greatly. Diagnostic kits use in this research is based on the concept of homogenous and heterogeneous immunoassay. One of the important elements in heterogeneous immunoassay is the application of membrane. For this purpose, the adsorption mechanisms of protein adsorption on the surface of PVDF membrane need to be studied. A protein, bovine serum albumin (BSA) is used as a model protein to evaluate the adsorption on the PVDF membrane surface.

METHODOLOGY

Materials

Immobilon-PSQ, PVDF microporous membrane was used. The characterization of the membrane includes porosity test, water contact angle (VCA-3000, AST products Inc., USA), Fourier Transform Infrared Spectroscopy (Perkin Elmer and FTIR- Spectrum One spectrometer,USA.

The protein adsorption on PVDF membrane experiment was done using BSA as a model protein and Bicinchoininic acid (BCA) as the working reagent. The concentration of BSA solutions was measured by UV spectrophotometer (UV-2550, Japan) at λ=562 nm.

Experimental Procedures

Membrane characterization

The membrane porosity, ε was simply defined as the pores volume divided by the total volume of the porous membrane. It can be determined by weighing the liquid 2-butanol (≥99.0%) that occupied the membrane sample pores when it was immersed into the butanol for 30 seconds, after which the membrane was dried using filter paper and heated in oven at 37ºC for 1 hour. The membrane was weighed before and after it was dried. Porosity of membrane was calculated using Eq.(1) [2].

$$\varepsilon = \frac{(W_B - W_M)/\rho_B}{(W_B - W_M)/\rho_B + W_M/\rho_P} \times 100\% \quad (1)$$

Where ε is the porosity of the membrane, W_M is the dry membrane weight, W_B is the wet membrane weight, ρ_B the 2-butanol specific gravity (0.81 g/cm^3) and ρ_p is the PVDF specific gravity (1.78 g/cm^3).

Water contact angle was performed using Video contact Angle System (VCA-3000, AST products Inc., USA). The angle obtained was used to determine the hydrophobicity of the PVDF membrane [5]. Fourier Transform Infrared Spectroscopy (Perkin Elmer, FTIR- Spectrum One spectrometer,USA) was used to determine IR spectra of the membrane. The α and β forms can be characterized by the absorption peak at 763 cm^{-1} and 840 cm^{-1}, respectively [6].

Protein immobilization and thermodynamics studies

Three PVDF membranes, 12mm in diameter were placed in test tubes containing 3mL of 0.5mg/mL BSA protein solution. The test tubes were shaken in waterbath for 3 hours at 298K to allow for protein binding. Then, the membranes were took out and placed in other test tubes that contained deionized water to be shaken for another 15 minutes. Later, the membranes were put into other test tubes that contained 2 mL of BCA reagent which was initially green in color. The test tubes were shaken in waterbath for another 30 minutes at 37ºC. The color of BCA reagent changed to purple. A high intensity of purple color indicates higher concentration of

protein that bind on the membrane. Later, the BCA solution was put in a cuvette for analysis with spectrophotometer at 562nm (this step must be done in less than 10 minutes). The spectrophotometer readings were recorded. The procedures for this adsorption experiment were repeated for temperature at 208K and 318K. The equations that were used to estimate the value of the three thermodynamic parameters which are enthalpy change ($\Delta_r H^\theta$), entropy change ($\Delta_r S^\theta$) and Gibbs free energy change ($\Delta_r G^\theta$) are as follows [6]:

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

$$\Delta_r G^\theta = -RT \ln(K) \quad (3)$$

$$\ln(K) = -\frac{\Delta_r H^\theta}{R}\left(\frac{1}{T}\right) + \frac{\Delta_r S^\theta}{R} \quad (4)$$

Where the values of $\Delta_r H^\theta$ and $\Delta_r S^\theta$ were obtained from the slope and intercept of the plot of ln(K) versus 1/T and K is an equilibrium constant

RESULTS AND DISCUSSION

Membrane characterization

Porosity

Table 1: Experimental data and results for PVDF membrane porosity

Time (minutes)	Weight (g)		
	Sample 1	Sample 2	Sample 3
0 (Wet)	0.0422	0.0403	0.0385
15	0.0134	0.0139	0.0122
30	0.0135	0.0137	0.0123
45	0.0135	0.0137	0.0123
60 (Dry)	0.0135	0.0137	0.0123
Porosity, ε (%)	82.377	81.013	82.397
Average porosity (%)	81.929		
Standard error (%)	0.79		

From the data obtained in Table 1, the average porosity obtained for PVDF membrane is 81.929% ±0.79%. The protein binding ability on a PVDF membrane will increase if the porosity increases. A membrane should have as high porosity as possible with the intention of increasing the interconnection between membrane pores. A high porosity membrane with small pore size is good for protein binding because it provides a large internal surface area for protein to bind onto the membrane. Higher interconnection between the pores will provide a better protein-binding result as the capturing area for protein increases, therefore explaining the relation between the porosity and pore size for membrane protein binding performance [1].

Water contact angle

Table 2 Water Contact Angle Obtained for PVDF Membrane

Membrane sample	Contact angle (°)
1	97.5
2	97.6
3	106.7
4	107.5
5	101.8
Average angle	102.2
Standard error	4.24

The test was run five times and the average water contact angle that was obtained is 102.2° ± 4.24°.

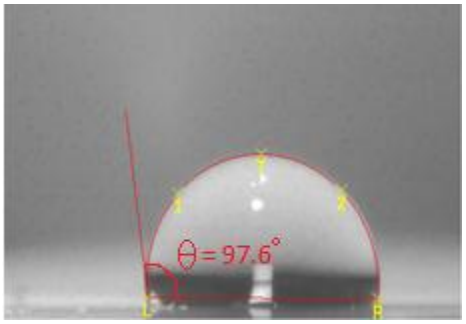

Figure 1: Image of water contact angle on PVDF membrane surface

As shown in figure 1, the contact angle, θ between water droplet and the membrane surface was observed. A contact angle less that 90° means that the surface is hydrophilic and contact angle more than 90° indicates that the surface is hydrophobic. Therefore, PVDF membrane has a hydrophobic surface

Fourier Transform Infrared Spectroscopy

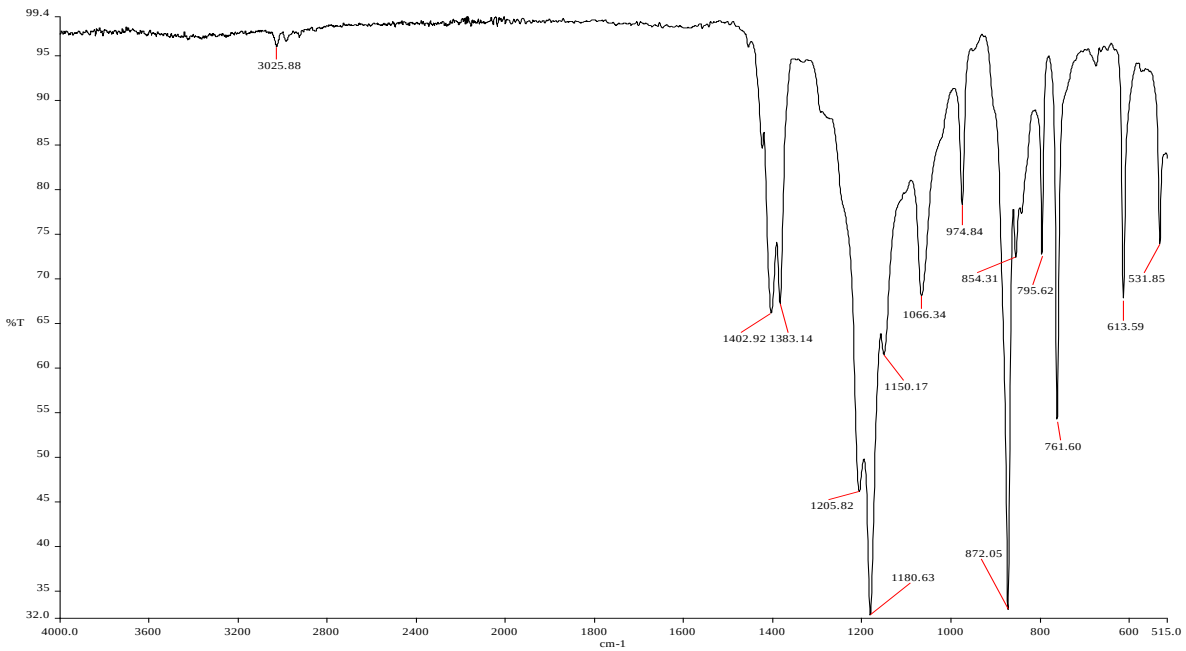

Figure 2 IR Spectra obtained for PVDF membrane

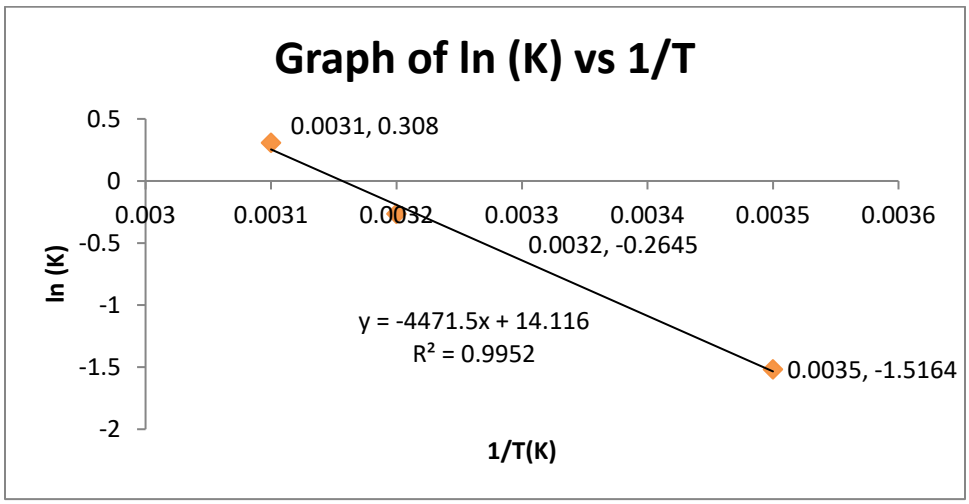

Figure 3 Van't Hoff plots of BSA adsorption onto PVDF membrane for different temperatures

FTIR (Perkin Elmer, FTIR- Spectrum One spectrometer) was used to obtain IR spectra of the prepared PVDF membranes. The α and β forms could be characterized by the absorption peaks at 763 and 840 cm^{-1}, respectively [1]. The crystalline phases were confirmed when peaks of 761.85cm^{-1} and 854.30 cm^{-1} are obtained, corresponding to the α and β phases respectively. The peaks can be observed in the spectra shown Figure 2. Under the correct physicochemical conditions, a higher proportion of β phase is preferable because it could help in improving the membrane protein binding interaction [2].

Field Emission Scanning Electron Microscope (FESEM)

As shown in Figure 4 in the supplementary data section, the pore size for the PVDF membrane is 1.98μm ±0.4μm. the pore size is very small and it can offer good accessibility and large surface areas for protein adsorption [4].

Thermodynamic parameters of adsorption

In order to know the nature of the adsorption process, whether it is exothermic or endothermic, the adsorption of BSA onto PVDF membrane surface was carried out at 298K, 308K and 318K with a constant initial concentration of 0.5mg/mL, pH 7, solution volume of 3mL and contact hour of 3 hours. The adsorption capacities of BSA on PVDF membrane surface decreased as the temperature increased.

The thermodynamic parameters of Gibbs free energy, $\Delta_r G^\theta$ were calculated using Eq. (3) whereas the change in standard enthalpy, $\Delta_r H^\theta$and change in standard entropy $\Delta_r S^\theta$were calculated using Eq.(4) by solving the slope and intercept of the plot of ln(K) versus 1/T in figure 3 and K is calculated using Eq.(2)

The change in standard Gibbs free energy $\Delta_r G^\theta$ was 3.76, 0.677 and -0.816 kJ/mol at, 298 K, 308 K and 318 K respectively. The decreasing trend in $\Delta_r G^\theta$values indicated that increasing the temperature accelerated the adsorption process of BSA on the membrane surface. The $\Delta_r H^\theta$value was 37.176kJ/mol. The positive value showed that the adsorption process was endothermic. The value of $\Delta_r H^\theta$can determine the type of adsorption involved because the value of $\Delta_r H^\theta$that is in between 40 and 120 kJ/mol implied that it is a chemisorption and any values not in the range is physisorption[5]. Therefore, the adsorption of BSA onto PVDF surface is physisorption. The value in $\Delta_r S^\theta$was 117.6 J/mol.K. A positive $\Delta_r S^\theta$value implied that the distribution of BSA adsorbed on PVDF membrane is more chaotic which might be caused by combination of membranes with BSA.

SUPPLEMENTARY DATA

With the courtesy of Dr.Norhidayah Ideris, the image of PVDF membrane observed under 5000x magnification is provided as shown in Figure 4.

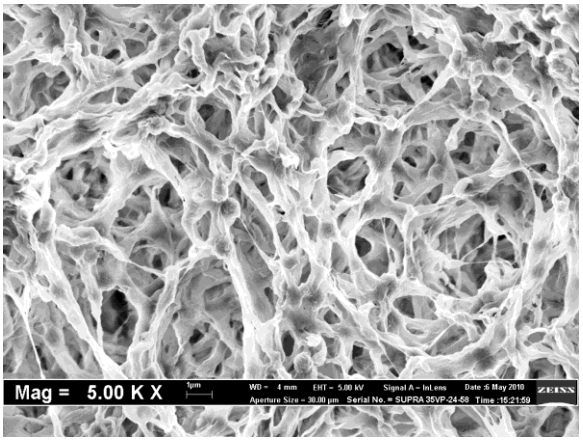

Figure 4 Morphology of PVDF membrane Surface observed using FESEM under 5000x magnification

Table 3 Pore size distribution on PVDF membrane

Scale size (cm)	Actual size (μm)
1.6	2.00
1.2	1.50
2.2	2.75
1.3	1.63
1.5	1.88
1.7	2.13
1.6	2.00
Mean pore size	1.98
Standard Deviation	0.40

CONCLUSION

After performing the characterization PVDF membrane and thermodynamic studies of protein binding onto PVDF membrane, it can be concluded that PVDF membrane is good for protein binding because it high porosity with small pore size that give large internal surface area for capturing area of protein. It is also a hydrophobic type of membrane which can promote hydrophobic interaction between protein and the membrane. For thermodynamic aspects, it was found that protein binding capability decreases as the temperature increase.

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