Equilibrium Studies on Adsorption of Bovine Serum Albumin (BSA) Using PVDF Membrane (Immobilon-PSQ)

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***Abstract*— Polyvinylidene fluoride (PVDF) is a semi-crystalline structure that is an outstanding and distinctive polymer that widely used as a membrane material [1]. A study on the capability and characteristic of PVDF membrane [2] to adsorpt protein is done due to the its outstanding uses in development of biosensor. Studies on the characteristic of PVDF membrane in term of morphology and polymorph as well as equilibrium adsorption of BSA on PVDF membrane is carried out. In this study, the initial cocentration of protein is varied in the range of 0.25 mg/L , 0.5 mg/L , 0.75 mg/L , 1.0 mg/L and 1.25 mg/L. Characteristic of PVDF membrane such as the pore size, porosity, water contact angle and functional group by using Field emission scanning electron microscopy (FESEM), VCA-3000S water surface analysis (AST, USA), Fourier transformation infrared spectroscopy (FTIR) respectively and also weighing scale. The concentration of BSA adsorbed is measured at λ = 562 nm by using UV-vis spectrophotometer. The linear equllibrium isotherm model used to obtain the amount of BSA bind to the membrane are Langmuir and Freundlich Isoterm.**

Keywords*— *equilibrium concentration, PVDF membrane, protein binding

I. INTRODUCTION

Membrane can be classified into two types which are biological membrane and synthetic membrane. As for the biological membrane, it is a type of membrane that can be found within living things including humans, animals and plants. Biological membrane is a selective permeable barrier that allow specific molecules or ions to pass through it. In the other hand, synthetic membrane is made by human to be applied in laboratory or industry. Throughout the years, human has come out with various type of synthetic membranes which can either be in the form of liquid or solid. Synthetic membrane can be organic and also inorganic. Polymeric and liquid are examples of organic membranes while ceramic and metal are examples of inorganic membranes. Polymeric membrane has been widely used in the industry because of it is much more cost effective as compare to ceramic membrane. Polyvinylidene fluoride (PVDF) is a semi-crystalline structure that is an outstanding and distinctive polymer that widely used as a membrane material [6]. PVDF is produce by the polymerization process of vinylidene difluoride. PVDF offers many unique and prominent advantages including good membrane forming ability, thermal stability, excellent chemical resistance [1], high mechanical strength and antioxidation activity which led to attract tremendous attentions in the membrane science [6, 8]. Example of applications of PVDF membranes are gas separation, waste water treatment and biomedical. The specialty of PVDF membrane to obtain high purity of product, low density, well resistant to solvents, acid and bases are the advantages of it. These advantages are made used in biomedical applied for hemolysis process, diagnostic kit and wound dressing. As for diagnostic kit, PVDF membrane is used to adsorpt protein for process such as biosensors, western blots and dot-blots. These three process apply PVDF membrane to immobilize protein. PVDF membrane (Immobilon PSQ), PVDF membrane (Immobilon FL) and nitrocellulose membrane are examples of PVDF membrane that are commonly used in the biosensor process. PVDF membrane (Immobilon PSQ) has the highest protein binding capacity and binding among the all type of PVDF membrane. The diversity of protein that has their own unique characteristics required control in PVDF membrane properties so that it is suitable for the final application.

II. METHODOLOGY

A. Chemical and Material

PVDF (Immobilon-PSQ, 1-butanol (99.5%, Sigma–Aldrich), deionized water, Novagen® BCA (MERCK Protein Assay Kit) which including Bovine Serum Albumin, bicinchoninic acid (BCA) reagent and phosphate buffer.

B. Membrane Characterization

i. Polymorph analysis

The membrane samples will be analyzed using a Perkin-Elmer FTIR spectrometer where α and β crystal form is observed by FTIR mapping procedure (peak at 763/cm and 840cm/cm respectively) [11]. The crystalline structure which comprised by α and β crystal form is observed by FTIR mapping procedure. The absorbant is calculated using this formula,

$$\text{Absorbant} = 2 - \log_{10} \%T.$$

ii. Water Contact Angle analysis.

Water contact angle of PVDF membrane is determine by dropping 5μL of deionized water slowly on the membrane. The water contact angle can be observed directly by VCA-3000S water surface analysis (AST, USA). Four water contact angles at different locations on any given surface will be averaged to evaluate the the surface wetting ability [14]. AutoFAST software will be used for the calculation of contact angle

iii. Morphology analysis

1) Analysis of Porosity

The PVDF membrane porosity, ε, can determined by weight of wetting liquid that occupies overall membrane pore sample. Membrane sample is weighed before immersed in butanol solution for about 3 hour. The membrane is let to dry by using filter paper before the final weight of the membrane sample is taken. This formula is used to calculate the porosity:

$$\varepsilon = \frac{(W_B - W_M)/\rho_B}{\frac{(W_B - W_M)}{\rho_B} + \frac{W_M}{\rho_p}} \times 100\% \quad [11]$$

Where, ε is the porosity, W_B is the wet membrane weight, W_M the dry membrane weight, ρ_B the butanol specific gravity (0.81 g/cm3) and ρ_P is the PVDF specific gravity (1.78 g/cm3) [4].

2) Pore size distribution

The image for FESEM analysis is provided under supplementary data. Then, Image J processing program is used to indicate the pore size distribution.

C. *Membrane binding ability*

The ability of PVDF membrane (Immobilon PSQ) to bind BSA solution is measured using on 12-mm diameter sample coupon. After that the total volume of the membrane samples will be calculated. All the membranes are then incubated in a 3-mL BSA solution with a 0.05-M phosphate buffer and will be shook for 3 h at 25°C. Unbound BSA on the membrane surfaces is washed for twice with a phosphate buffer [6]. After that, all of the membranes are transferred into test tubes and 2-mL of bicinchoninic acid (BCA) assay reagent will added into it. Later, the test tubes are incubated at 37°C for 30 min. A UV-vis spectrophotometer at a 562-nm wavelength is used to detect the BSA concentration. The corrected reading absorbance reading for the samples is interpolated using preliminary standard curve ranged from 0.25-1.25mg/L[6]. Amount of BSA bind to the membrane, Qe to the unit mass of PVDF at equilibrium can be calculated by;

Qe=[(Ci-Ce).V]/m [11]

Where, Ci and Ce is initial and final concentration respectively and V is the volume of water solution and m is the mass of the film.

D. *Adsorption Isotherm*

Adsorption isotherm study is done to study the amount of BSA protein adsorbed on the PVDF membrane. Linear regression isotherm model is choose in order to obtain the amount of protein bind to membrane. The isotherm model are Langmuir-1, Langmuir-2, Langmuir-3, Langmuir-4 and Freundlich isotherm.

Table 1. Interest isotherm with linear form and plot parameter [12].

Isotherm	Non-linear form	Linear form	Plot
Langmuir-1	$q_e = \frac{q_m K_L C_e}{1 + K_L C_e}$	$\frac{C_e}{q_e} = \frac{1}{q_m} C_e + \frac{1}{K_L q_m}$	C_e/q_e vs C_e
Langmuir-2		$\frac{1}{q_e} = \left(\frac{1}{K_L q_m}\right)\frac{1}{C_e} + \frac{1}{q_m}$	$1/q_e$ vs $1/C_e$
Langmuir-3		$q_e = q_m - \left(\frac{1}{K_L}\right)\frac{q_e}{C_e}$	q_e vs q_e/C_e
Langmuir-4		$\frac{q_e}{C_e} = K_L q_m - K_L q_e$	q_e/C_e vs q_e
Freundlich	$q_e = K_F C_e^{1/n}$	$\log(q_e) = \log(K_F) + \frac{1}{n}\log(c)$	$\log q_e$ vs $\log C_e$

Ce (g/L) is the equilibrium concentration for the linear equilibrium isotherm is considered as an 'independent' variable. Qe (g/g) is the amount of protein adsorbed by the PVDF at equilibrium is assume as 'dependent variable'

The maximum capacity of monolayer adsorption, Qm and Langmuir constant K(L/g) is assume as predictable by examined the best fitted condition of Equilibrium model.

III. RESULTS AND DISCUSSION

Polymorph

Polymorph of the PVDF membrane (Immobilon PSQ) has four significant crystalline forms which are the nonpolar α form and the polar β, γ and δ forms [6]. However, the primary form are α and β. The interest wavenumber peak for the PVDF membrane is 761.67cm^{-1} to characterize α form and 854.19cm^{-1} for β form.

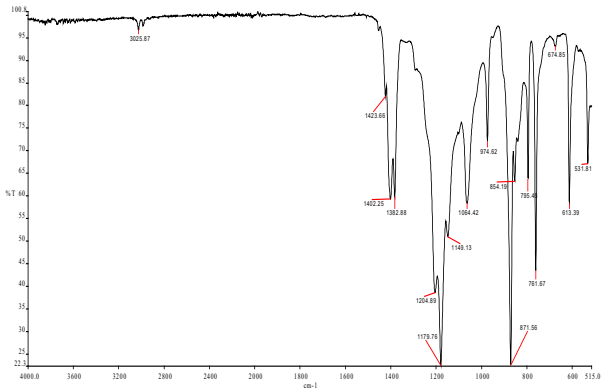

Fig 1: FTIR result for the PVDF-Immobilon PSQ

FTIR is used to study the polymorph of PVDF membrane. From figure 1, the transmittance value is 43% for wavenumber at 761.67cm^{-1}. The calculated value for absorbent at this peak is 0.366. as for the wavenumber at 854.19cm^{-1}, the value of transmittance obtain from the graph is 63%. The calculated value of absorbent is 0.200. The small value of absorbance indicate that the PVDF can retain high amount of protein to the membrane.

Surface wetting ability

Surface wetting ability of the membrane is one of the factor affecting the amount of protein bind to the PVDF membrane. This is due to unique characteristic of protein that only bind its hydrophobic part to hydrophobic part of the membrane and vice versa. The degree of contact angle indicates the membrane sample whether it is hydrophobic or hydrophilic. Membrane sample that have contact angle higher than 90° is classified as hydrophobic while contact angle lower than 90° is hydrophilic samples the [10].

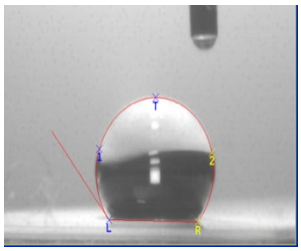

Fig 2: Water contact angle on PVDF membrane

From figure 1, the water contact angle of water on PVDF membrane. From the experiment, it can be seen clearly that PVDF membrane is highly hydrophobic. This results is supported with the calculation of average results from the analysis.

Table 2. Water contact angle result analysis

No.	Water contact angle
1	130.50
2	131.30
3	129.30
4	134.00
	Average = 131.275 (Hydrophobic) SD= 1.213
	Surface tension = 5.61 Surface Energy = 72.8 dyne/cm

Table 2 shows the data obtained from the experiment to study the wetting ability of PVDF membrane. PVDF PSQ sample shows a high water contact angle, which is 131.275 $\pm$ 1.213°. This shows that PVDF membrane has strong hydrophobic character and strong bond capacity as a protein adsorbent.

Porosity characterization

Porosity of membrane structure does affect the protein binding capacity of protein toward membrane surface. High porosity shows that the membrane has strong interconnection between the pores. This is an advantage in protein binding where more BSA molecule will be saturated per surface area by the increase of surface area of membrane available for protein adsorption. Porosity of PVDF membrane can be determined by wetting and weighting technique. In this experiment, 1-butanol is used as wetting liquid for weighting method to obtain the porosity of PVDF membrane. The porosity is calculated using this formula:

$$\varepsilon = \frac{(W_B - W_M)/\rho_B}{\frac{(W_B - W_M)}{\rho_B} + \frac{W_M}{\rho_p}} \times 100\% \qquad [11]$$

Where, ε is the porosity, W_B is the wet membrane weight, W_M the dry membrane weight, ρ_B the 1-butanol specific gravity (0.81 g/cm3) and ρ_P is the PVDF specific gravity (1.78 g/cm3) [22].

Table 3. Weighing method porosity calculation data

Wet membrane weight (g)	Dry membrane weight (g)	Porosity, ε (%)
0.036	0.014	77.545
0.034	0.013	78.021
0.032	0.013	76.257
	Average	77.274

Based on the experiment, the calculated porosity of the PVDF membrane is 77.274 % by average. PVDF-Immobilon PSQ has good porosity value for the protein adsorption on the surface. High posrosity will increase the interconnection between the membrane pores. Higher interconnection between the pores will give a better protein-binding result as the capturing area for protein increases, thus explains the relation between the porosity and membrane protein binding performance.

Morphology

Surface morphology of the membrane will determine the optimum amount of BSA that can be bind by the PVDF membrane. Due to the unique characteristic of protein that bind only to the specific part of the membrane. It is important to study the minimum microstructure pore size of the membrane that can bind the protein. Surface morphology of PVDF membrane can be observed by Field Emission Scanning Electron Microscopy (FESEM) used to analyze the pore diameter or pore size distribution by software tool such as Imaje J Conduction layer coating is used to get clear image [11].

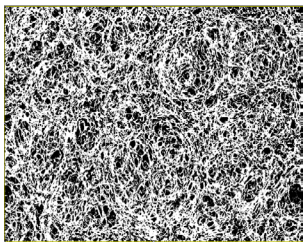

Fig 3: Threashold image of respective sample calculating the pore size diameter.

The figure 10 from supplementary data shows the FESEM result of the PVDF-Immobilon PSQ membrane. From the figure, PVDF membrane do have porous structure where the darken circular regeion shows the void area of the PVDF membrane. The PVDF molecule nodule is observe to has white circular structure where it conected the neigbouring nodule is a visible brighter cyclyndrical structure. As for figure 3, the darken shape is resemble as a pore area. This area is calculated by the Image J tool and the frequency of the area is also calculated. By obtaining the area of pore, diameter of the PVDF membrane is calculated. The calculated area, diameter and their frequency is shown in the table below.

Table 4. Pore size diameter and frequency result analysis.

Area(um^2)	Diameter (um)	Frequency	Number (%)
0.012	0.123	1369	27.645
0.025	0.178	614	12.399
0.037	0.217	360	7.269
0.049	0.249	246	4.967
0.062	0.280	177	3.574
0.074	0.306	136	2.746
0.086	0.330	127	2.564
0.099	0.355	105	2.120
0.111	0.375	77	1.554
0.123	0.395	99	1.999
0.148	0.434	135	2.726
0.185	0.451	168	3.392
0.198	0.502	48	0.969
0.21	0.517	78	1.575
0.235	0.547	486	9.814
0.753	0.979	317	6.401
1.259	1.266	153	3.089
1.802	1.514	72	1.453
2.272	1.600	57	1.151
2.716	1.859	33	0.666
3.296	1.966	21	0.424
3.667	2.116	36	0.726
4.272	2.116	24	0.484
4.79	2.116	14	0.282
	Total	4952	

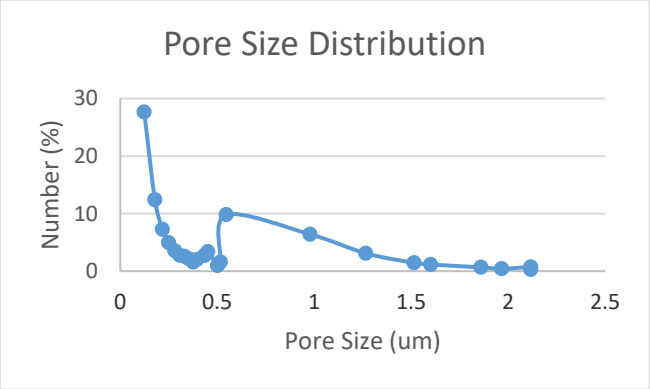

Fig 4. Pore size distribution result analysis by Image J tools

From Figure 3, the pore with diameter 0.123607797um is found to be the most pore size on the PVDF membrane. The second highest amount of pore diameter calculated from image J is diameter at range of 0.5um. This shows that the PVDF membrane has a very small pore size which can improve the amount of protein bind to the membrane. The good morphology characteristic of PVDF-Immobilon PSQ membrane that provide a solid platform for the protein to bind protein is an absolute advantage for the membrane to bind the most protein as possible.

Equilibrium Studies

Studies on adsorption isotherm is important in order to quantify the amount of the BSA protein absorbed once equilibrium state is achieved in the adsorption process. Equilibrium adsorption studies between PVDF membrane and BSA can be carried out by either linear or linearization isotherm or nonlinear correlation isotherm. Linear regression isotherm correlation is used to fit the isotherm describing the BSA adsorption mechanism due to utility constraint.

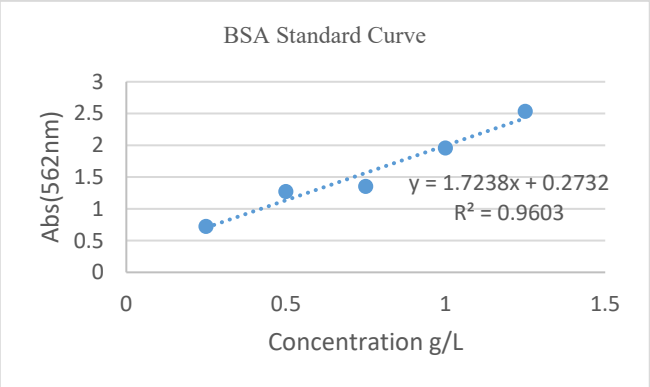

Fig 5. BSA Standard Curve

Based on the standard curve, amount of the absorbance influence the concentration of the adsorbed protein, Cm. The value of Qe for the adsorption at initial concentration protein range from 0.25g/L to 1.25g/l to the unit mass of PVDF membrane is calculated by using equation listed below. Mass of the membrane is taken before the membrane is immersed in the BSA. Table below shows the list of absorbance value for the adsorption studies samples and the calculated value of Qe and Ce, the equilibrium concentration in the system. The value of calculated Cm in the table shows the amount of BSA absorbed by the membrane. Ce value is obtained from the result of UV-vis analysis based on the reading of concentration of BSA after PVDF membrane is immersed into the BSA protein for about 3 hours to achieved equilibrium state.

Total equilibrium Volume = 3ml

$Qe = (Cm).V/m$
$Ce = Ci - Cm$

Table 5. Calculated Ce and Qe for each equilibrium sample model.

Ci (g/L)	Cm (g/L)	Abs (562nm)	Ce(g/L)	Qe (mg/g)	mass(g)
0.25	0.244	0.694	0.005	0.052	0.014
0.5	0.090	1.215	0.409	0.020	0.013
0.75	0.095	1.265	0.654	0.020	0.014
1	0.154	1.838	0.845	0.033	0.014
1.25	0.196	2.248	1.053	0.045	0.013

By using the value of Ce and Qe calculated shown in the table above, adsorption isotherm analysis can be done. Firstly, analysis on Langmuir-1 is done based on the equation below.

$$\frac{Ce}{Qe} = \frac{1}{Qm}(Ce) + \frac{1}{K(Qm)}$$

Ce (g/L) is the equilibrium concentration for the linear equilibrium isotherm is considered as an 'independent' variable. Qe (g/g) is the amount of protein adsorbed by the PVDF at equilibrium is assume as 'dependent variable'. The maximum capacity of monolayer adsorption, Qm and Langmuir constant K(L/g) is assume as predictable by examined the best fitted condition of equilibrium model.

Table 6. Langmuir isotherm calculated variable.

Langmuir 1	
Ce	Ce/Qe
0.005	0.102
0.409	19.521
0.654	31.817
0.845	25.488
1.053	23.171

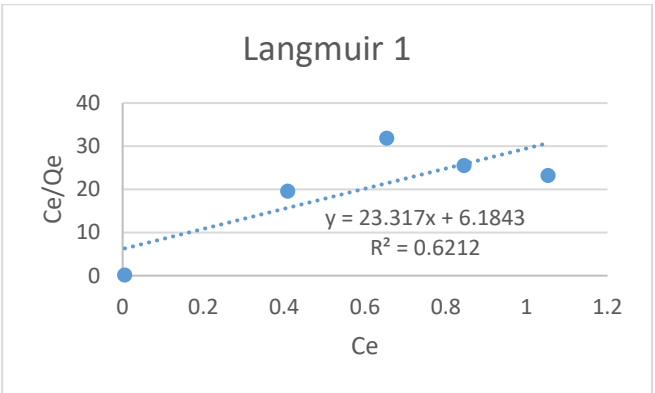

Fig 6. Langmuir-I linearize isotherm mode

The calculated maximum capacity of monolayer adsorption,Qm for Langmuir 1 is 0.04288716g/g. The value of Langmuir constant, K is 3.77035L/g.

Secondly, analysis on Langmuir-2 is done based on the equation below.

$$\frac{1}{Qe} = \frac{1}{K(Qm)}\frac{1}{Ce} + \frac{1}{Qm}$$

Ce (g/L) is the equilibrium concentration for the linear equilibrium isotherm is considered as an 'independent' variable. Qe (g/g) is the amount of protein adsorbed by the PVDF at equilibrium is assume as 'dependent variable'. The maximum capacity of monolayer adsorption, Qm and Langmuir constant K(L/g) is assume as predictable by examined the best fitted condition of equilibrium model.

Table 7. Langmuir isotherm calculated variable.

Langmuir 2	
1/Ce	1/Qe
186.3568174	19.07612059
2.443947872	47.71028786
1.528892529	48.64567995
1.183091054	30.15489666
0.949612218	22.00347347

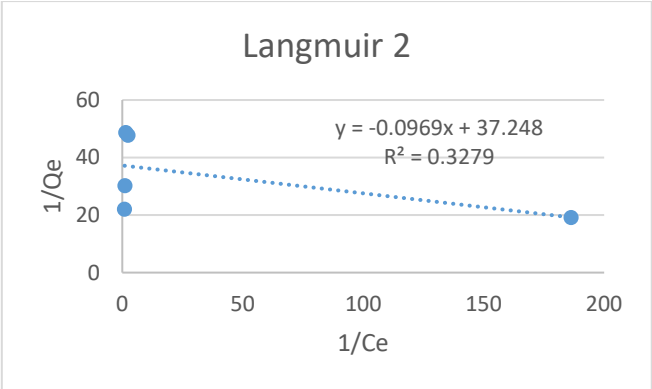

Fig 7. Langmuir-I linearize isotherm model.

The calculated maximum capacity of monolayer adsorption,Qm for Langmuir 2 is 0.02684708g/g. The value of Langmuir constant, K is 384.396L/g.

Thirdly, analysis on Langmuir-3 is done based on the equation below.

$$Qe = Qm - \frac{Qe}{K(Ce)}$$

Ce (g/L) is the equilibrium concentration for the linear equilibrium isotherm is considered as an 'independent' variable. Qe (g/g) is the amount of protein adsorbed by the PVDF at equilibrium is assume as 'dependent variable'. The maximum capacity of monolayer adsorption, Qm and Langmuir constant K(L/g) is assume as predictable by examined the best fitted condition of equilibrium model.

Table 8. Langmuir isotherm calculated variable.

Langmuir 3	
Qe	Qe/Ce
0.05242156	9.769115085
0.02095984	0.051224756
0.02055681	0.031429153
0.03316211	0.039233796
0.04544737	0.043157378

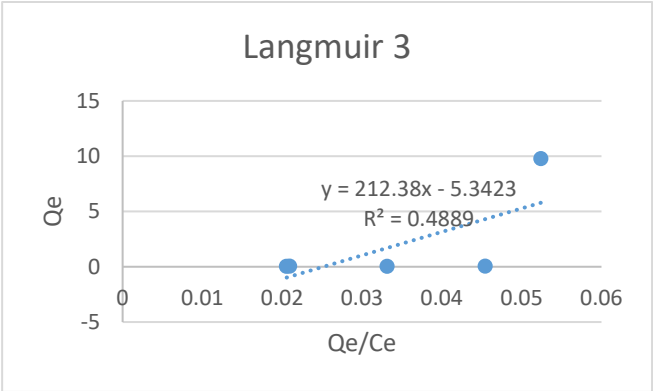

Fig 8. Langmuir-I linearize isotherm model.

The calculated maximum capacity of monolayer adsorption,Qm for Langmuir 3 is 5.3423g/g. The value of Langmuir constant, K is 212.38L/g.

Then, analysis on Langmuir-4 is done based on the equation below.

$$\frac{Qe}{Ce} = K(Qm) - K(Qe)$$

Ce (g/L) is the equilibrium concentration for the linear equilibrium isotherm is considered as an 'independent' variable. Qe (g/g) is the amount of protein adsorbed by the PVDF at equilibrium is assume as 'dependent variable'. The maximum capacity of monolayer adsorption, Qm and Langmuir constant K(L/g) is assume as predictable by examined the best fitted condition of equilibrium model.

Table 9. Langmuir isotherm calculated variable.

Langmuir 4	
Qe	Qe/Ce
0.05242156	9.769115085
0.02095984	0.051224756
0.02055681	0.031429153
0.03316211	0.039233796
0.04544737	0.043157378

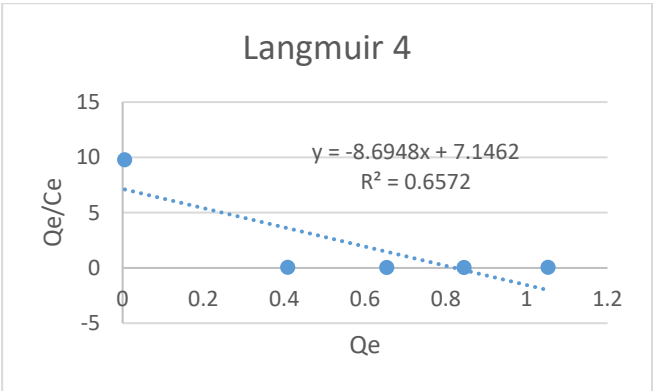

Fig 9. Langmuir-I linearize isotherm model.

The calculated maximum capacity of monolayer adsorption, Qm for Langmuir 4 is 0.82189g/g. The value of Langmuir constant, K is 8.6948L/g

After that, analysis on Freundlich isotherm is done based on the equation below.

$$\log (Qe) = \log(K) + \frac{1}{n} \log (Ce)$$

Ce (g/L) is the equilibrium concentration for the linear equilibrium isotherm is considered as an 'independent' variable. Qe (g/g) is the amount of protein adsorbed by the PVDF at equilibrium is assume as 'dependent variable'. The maximum capacity of monolayer adsorption, Qm and Langmuir constant K(L/g) is assume as predictable by examined the best fitted condition of equilibrium model. The value of n>1 is an indication of the favorability adsorption.

Table 10. Freundlich isotherm calculated variable.

Freundlich	
loqCe	logQe
-2.270345285	-1.280490059
-0.388091938	-1.678612037
-0.184376959	-1.687044278
-0.073018171	-1.479357844
0.022453706	-1.342491244

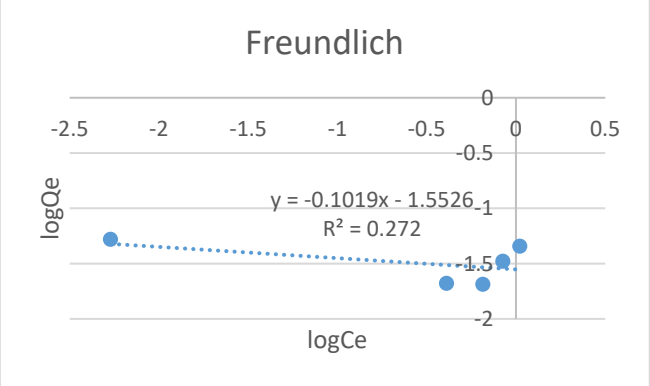

Fig 10. Freundlich linearize isotherm model.

Based on the equation, the value of n calculated is 9.813. this represent that PVDF-Immobilon PSQ membrane favor BSA absorption. The value of Freundlich constant, K is 1.5526L/g.

IV. CONCLUSION

As for conclusion, the objective of this study to obtain the functional group of PVDF membrane is achieved. The functional group indicate the relationship between C=C and also C=C-H of the PVDF membrane. The other peaks fall below 1423.66 cm^{-1}. The absorption below from this wavenumber are normally N-H, O-H or alkanyl C-H stretches. It is called fingerprint region and the C-H stretching absorptions. The transmittance value is 43% for wavenumber at 761.67cm^{-1}. The calculated value for absorbent at this peak is 0.366. as for the wavenumber at 854.19cm^{-1}, the value of transmittance obtain from the graph is 63%. The calculated value of absorbent is 0.200. The small value of absorbance indicate that the PVDF can retain high amount of protein to the membrane.From this study, it is clearly shows that PVDF membrane has high surface wetting ability with contact angle of 131.275^0. The porosity of the PVDF membrane which is 77.274 % by average indicate that this membrane has good protein adsorption on the surface. The pore with diameter 0.123um is found to be the most pore size on the PVDF membrane show that it has good morphology characteristic for the protein binding. Langmuir 3 shows the highest maximum capacity of monolayer adsorption, Qm with the calculated value of 5.3423g/g. The best fit graph with the R^2 value of 0.6572 is by using Langmuir 4 isotherm.

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Supplementary Data

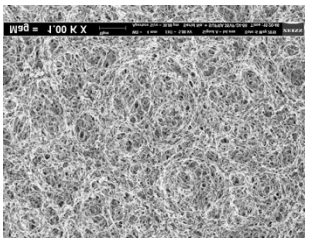

Fig 10: PVDF-Immobilon PSQ image morphology.

References

[1] Xing, J., et al., *Morphology and properties of polyphenylene sulfide (PPS)/polyvinylidene fluoride (PVDF) polymer alloys by melt blending.* Composites Science and Technology, 2016. **134**: p. 184-190.

[2] Nakamura, K. and K. Matsumoto, *Protein adsorption properties on a microfiltration membrane: A comparison between static and dynamic adsorption methods.* Journal of Membrane Science, 2006. **285**(1-2): p. 126-136.

[3] Munagapati, V.S. and D.S. Kim, *Equilibrium isotherms, kinetics, and thermodynamics studies for congo red adsorption using calcium alginate beads impregnated with nano-goethite.* Ecotoxicol Environ Saf, 2017. **141**: p. 226-234.

[4] Ahmad, A.L., et al., *Morphology and polymorph study of a polyvinylidene fluoride (PVDF) membrane for protein binding: Effect of the dissolving temperature.* Desalination, 2011. **278**(1-3): p. 318-324.

[5] Zhou, Y., et al., *Equilibrium and thermodynamic studies on adsorption of BSA using PVDF microfiltration membrane.* Desalination, 2012. **307**: p. 61-67.

[6] Vitola, G., et al., *PVDF membrane biofunctionalization by chemical grafting.* Journal of Membrane Science, 2015. **476**: p. 483-489.

[7] Cho, E., et al., *Fabrication of electrospun PVDF nanofiber membrane for Western blot with high sensitivity.* Journal of Membrane Science, 2012. **389**: p. 349-354.

[8] Sassolas, A., L.J. Blum, and B.D. Leca-Bouvier, *Immobilization strategies to develop enzymatic biosensors.* Biotechnol Adv, 2012. **30**(3): p. 489-511.

[9] Orr, V., et al., *Recent advances in bioprocessing application of membrane chromatography.* Biotechnol Adv, 2013. **31**(4): p. 450-65.

[10] Ghosh, R., *Purification of lysozyme by microporous PVDF membrane-based chromatographic process.* Biochemical Engineering Journal, 2003. **14**(2): p. 109-116.

[11] Marques, S.M., et al., *PVD-grown antibacterial Ag-TiN films on piezoelectric PVDF substrates for sensor applications.* Surface and Coatings Technology, 2015. **281**: p. 117-124.

[12] Zhao, X., et al., *Preparation and characterization of superior antifouling PVDF membrane with extremely ordered and hydrophilic surface layer.* Journal of Membrane Science, 2015. **494**: p. 48-56.

[13] Asuquo, E., et al., *Adsorption of Cd(II) and Pb(II) ions from aqueous solutions using mesoporous activated carbon adsorbent: Equilibrium, kinetics and characterisation studies.* Journal of Environmental Chemical Engineering, 2017. **5**(1): p. 679-698. 25. Umar, I., Bala, N., Danlami, N., & Abdulfatah, A. Y. (2017). Comparison of Linear and Non-Linear Methods of Freundlich and Langmuir Isotherm Models for the Adsorption of Lead Using Sugarcane Bagasse, *1*(5), 69–72.