Equilibrium Adsorption Isotherm of Bovine Serum Albumin (BSA) onto Nitrocellulose Membrane

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Rapid diagnosis of infectious diseases and timely initiation of appropriate treatment are critical determinants that endorse optimal clinical outcomes and public health. Therefore, nitrocellulose membrane is used as a material on diagnosis kit to overcome this problem. The objectives of this research are to characterize NC membrane in term of its morphology and polymorph and investigate the equilibrium aspects of protein adsorption. Water contact angle is analyzed as indication for hydrophobicity of the membrane and it is found NC is hydrophilic with zero angle**.** The porosity of membrane is found to be 12.75% which indicates NC membrane has a non-porous structure. From the FESEM image, the pore size distribution is highest within range of 0.5µm to 1.0µm with frequency of 139. FT-IR analysis showed at the peak of 3429.19 cm^{-1} strongly O-H that proved NC membrane able to absorb water and at the peak of and at the peak of 1094.98 cm^{-1} of C-N bond and proved of present of nitrile grou[. BSA is used as standard protein and BCA assay kit is used as indicator for protein blotting. Langmuir and Freundlich are common isotherms applied in adsorption. BSA standard curve is used to detect the final concentration of protein based on the absorbance value obtained from UV-VIS spectroscopy. The results showed that Langmuir Type 1 and Type 2 are best fitted for quantify protein adsorption with R^2 value 0.9997 and 0.9994 respectively. It is showed that the membrane characteristics influenced binding of protein. A proper storage of the membrane also important to avoid destruction on sample surface and error on obtaining desired results.

I. INTRODUCTION

Biosensor is one of technology that widely used in immunological analysis. It provides simplicity in detection of pathogens, antigen or viruses. Development of diagnosis kit is essential as it can be carry at any place besides easy to handle. Protein blotting on polymer particles has substantial prominence in biomedical applications. The idea of diagnosis kit is coming out because infectious diseases have remained as the foremost global health problem. Current diagnosis of pathogenic bacteria mostly relies on laboratories-based tests including microscopy. As for that, some of the weaknesses of the conventional diagnostic for infectious diseases have discovered; time consuming, required centralized laboratories, experiences personnel to handle and huge equipment. Portable standalone biosensors can facilitate detection of infectious disease without the present of any expert to handle it. The studies of protein blotting have been carried out from all view points for the last two decades, but the results are inconsistent due to its complexity. In this research, the study of equilibrium adsorption of BSA onto nitrocellulose membrane is carried out to identify the ability of membrane to binds protein besides to analyze the best fit linear isotherm for the analysis. In the past decades, nitrocellulose membrane is a standard used in Southern, Western and Northern Blotting. The ability of physical and chemical properties of nitrocellulose membrane will affect the capability for protein binding. In membrane characterization, pore distribution and pore of the membrane is identified to analyze the porosity of the membrane. Water contact angle is taking into account to analyze the properties of the membrane. This indication is very important as it will classify type of substances that can be analyze by nitrocellulose membrane as hydrophobicity and hydrophilic influenced the substances that can bind on membrane.

Proteins are the most common reagent applied onto membrane surface whereby the loading capacity is depending on the membrane structure itself including surface morphology and available surface area for immobilization. Generally, BSA is used as standard protein for analyze membrane structure. BCA kit assay is used to detect the amount of protein bind onto membrane surface.

In order quantify the protein bound on membrane, adsorption kinetics and adsorption isotherms need to be emphasized. Linear, Langmuir and Freundlich are the common isotherm used to quantify the amount of protein adsorbed. The linearization is applied for the isotherm to get the best fit line on protein adsorption.

II. METHODOLOGY

A. Materials

Nitrocellulose membranes is used . Bovine Serum Albumin is used as standard model protein. The BSA solution is prepared using 0.05M phosphate buffer solution. BCA assay kit is used to identify the amount of protein adsorbed on membrane surface.

B. Porosity of membrane

The porosity,ε of nitrocellulose membrane is defined by pore volume over total volume of the membrane.

Nitrocellulose membrane is dried using filter paper before immersed in distilled water for 2 hours. Porosity of nitrocellulose membrane is determined from weight of liquid distilled water that occupy nitrocellulose membrane pores within 2h. Thus, weight of nitrocellulose membrane is recorded before and after immersion process in distilled water. The porosity of the liquid is calculated using this formula:

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

ε = Porosity of the membrane
W_B = Mass of wet membrane
W_m = Mass of dry membrane
ρ_B = Distilled water specific gravity (1 g/cm^3)
ρ_p = Nitrocellulose specific gravity (1.62 g/cm^3)

The flat sheet membrane was cut 2cm x 2cm before immersed in the distilled water. Next, the membrane is dried in an oven to eliminate the water vapor contained in the membrane. The membrane is dried until a constant mass is achieved and the porosity is determined using equation 1.

B. Water contact measurement

A sessile drop method is applied to measure static contact angle of water of the membranes by using KYOWA KAIMEN KAGAKU CA-D. The angles reported should be reliable to ± 1°. The angle is calculated automatically by using the software.

C. FT-IR Analysis

For study of surface chemical composition changes of the nitrocellulose membrane, investigation is carried out by using an attenuated total reflection (ATR) Fourier transform infrared (FT-IR) spectroscopic with a Nicolet MAGNA IR560 spectrometer using Ge crystal. The wave number is set within range number of 1000-4000 cm^{-1}. The spectra are collected by cumulating 64 scans at resolution of 4 cm^{-1}. The data of all ATR-FTIR spectra are recorded at ambient temperature (N.Akashi, 2004).

D. Membrane binding ability

The experiment is carried out at room temperature (27°C) and ambient pressure.. A sample 12 mm of nitrocellulose membrane is used and volume of the membrane is calculated. Sample of nitrocellulose membrane is incubated in 3 mL of each dilution series of BSA which is 0.5, 1.0, 1.5, and 2.0 g/La with a 0.05M phosphate (pH 7.0, 3 mg/mL) which acts as a buffer solution. The sample is shake for 3h at 25□C. Next, the sample membrane is washed two times using buffer solution to remove the unbound protein. The sample of nitrocellulose membrane is transferred in test tube. Subsequently, 2-mL of BCA is added that acts as a working reagent. The sample of membrane is next incubated at 37□C for 30 min. The BSA concentration is measured using spectrophotometer (Spectronic Genesys, USA) at 562-nm wavelength. The absorbance value is used to construct best fit standard curve by comparing with different isotherms. Amount of BSA adsorbed to the unit mass of nitrocellulose membrane, Qe can be calculated as followed:

$$Ce = Ci - Cm \quad (2)$$

Ci : Initial Concentration of BSA
Cm : Concentration of BSA bound on membrane
Ce : Equilibrium Concentration of BSA

$$Qe = \frac{(Ci - Cm)\ x\ V}{W} \quad (3)$$

E. Adsorption isotherms

1. Langmuir (Type 1):

$$\frac{C_e}{q_e} = \frac{1}{q_{0b}} + \frac{C_e}{q_0} \quad (4)$$

2. Langmuir (Type 2):

$$\frac{1}{q_e} = \frac{1}{Q^0} + \frac{1}{K.Q^0}.\frac{1}{C_e} \quad (5)$$

q_e = Amount of proteins adsorbed at equilibrium (mg g^{-1})
q_o = Maximum adsorption capacity (mg g^{-1})
C_e = Equilibrium concentration of the adsorbate (mg L^{-1})
b = Langmuir constant (L mg^{-1})

3. Freundlich:

$$log\ q_e = log\ K_F + \frac{1}{n} log\ C_e \quad (6)$$

q_e = Amount of proteins adsorbed at equilibrium (mg g^{-1})
C_e = Equilibrium concentration of the adsorbate (mg L^{-1})
K_F = Empirical constants (L mg^{-1})

RESULTS AND DISCUSSION

A. Porosity, pore sizes and pore distribution of the membrane

In this experiment, the membrane is firstly cut and immersed in distilled water for 2 hours. After the immersion process, the membrane is dried using oven for one hour until constant reading is obtained which is 0.029g. Next, the porosity of the membrane is calculated and the result obtained is 12.75 % and indicates nitrocellulose membrane has a non-porous structure. Supposed the NC membrane is porous membrane and advantage on selectivity, however the results obtained is not as expected. Therefore, it might have any error during conducting the experiment or destruction on membrane sample that lead to non-porous results. The advantage of porous membrane is in selectivity.

From the FESEM image analyzed using ImageJ software, the NC membrane has a broad distribution from 0.4µm to 7.8 µm. The smallest pore size found on NC membrane is 0.433792 µm and the largest pore size is 7.758042µm. The highest frequency count of pore size is within range of 0.5µm-1.0µm with the frequency of 139.

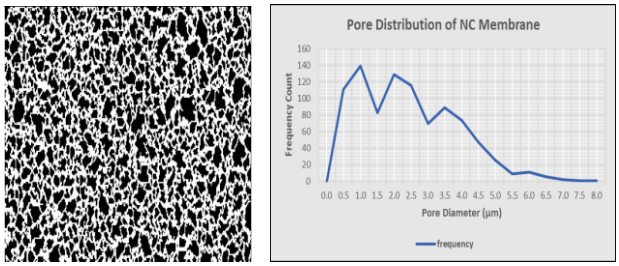

Figure 1: Pore distribution and surface morphology of the membrane

B. Water contact angle

In this experiment, sessile liquid is dropped onto the nitrocellulose membrane. It has found that, the sessile liquid totally immersed onto the membrane giving 0° contact angle. This has indicated nitrocellulose membrane has high wetting properties and can be characterized as hydrophilic membrane. Nitrocellulose membrane being hydrophilic means it has tendency to allow water enter the pores. This condition reflects the statement of better wetting properties, better adhesiveness and higher surface energy.

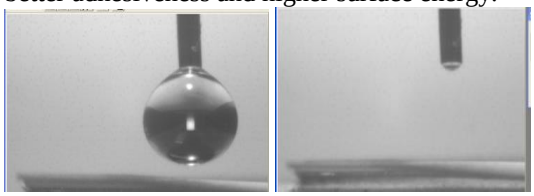

Figure 2: Water contact angle on NC membrane

C. FT-IR Analysis

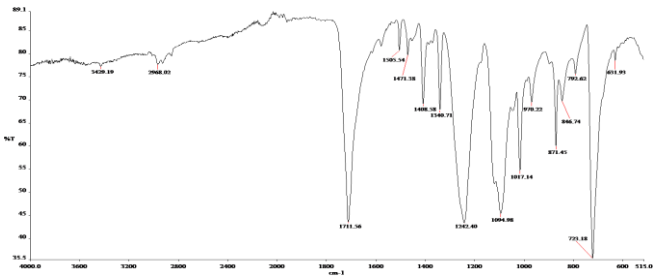

Figure 3: FTIR spectrum of NC membrane

At the peak of 3429.19 cm^{-1}, it is strongly shows of H-bonded O-H of alcohol group where the absorbance is fall in range of 3200-3600 cm^{-1}. Strong bond of O-H group shows NC membrane is favor water and considered as hydrophilic membrane.At the peak of 2968.02 cm^{-1}, it is strongly shows of C-H bonded of alkane group where the absorbance is falls in range of 2850-3000 cm^{-1}. At the peaks of 1340.71 cm^{-1} and 1017.14 cm^{-1}, it is strongly shows of C-O bonded of ether group where the absorbance is fall in range of 1000-1350 cm^{-1}. At the peaks of 1505.54 cm^{-1}, 1471.38 cm^{-1} and 148.58 cm^{-1}, the peaks show C=C bonded of aromatic group where the absorbance is fall in range of 1400-1600 cm^{-1}. At the peaks of 871.45 cm^{-1}, 846.74 cm^{-1}, 796.62 cm^{-1} and 723.18 cm^{-1}, it strongly shows C-H bonded of aromatic group where the absorbance is fall in range of 680-860 cm^{-1}. From the above analysis, it can be concluded that nitrocellulose membrane can be classified as cyclic nitrate where most of the peaks showed of cyclic characteristic.

D. Membrane binding and adsorption isotherms

Series of BSA solution is prepared starting from range of 0.5 g/L to 3.0 g/L. For each dilution, a sample of membrane is being immersed and shaken for 3 hours and 30 minutes at the temperature of 37.6 °C with speed of 170 rpm. Next, all the membrane samples are washed two times using buffer solution before transferred to a new sample bottle. The BCA assay reagent is added in every membrane samples and shaken or another 30 minutes at the same speed and temperature respectively. The color changes are from green to purple are recorded. Lastly, the final concentration of protein bonded on the membrane is identified and measured using ultra-violet spectroscopy.

Sample	Mass, (g)	Initial Conc. of BSA (µg/mL), Ci	Absorbance Value	Final Conc. of BSA (µg/mL), Cm	Equilibrium Conc. BSA Bound to Membrane, Ce	Amount of BSA Adsorbed onto NC membrane
1	0.0219	500	0.446	410	90	0.0082
2	0.0221	1000	0.893	860	340	0.0309
3	0.0249	1500	0.972	875	625	0.0503
4	0.0292	2000	1.027	890	1110	0.0761

Table 1: Results of protein bound onto membrane

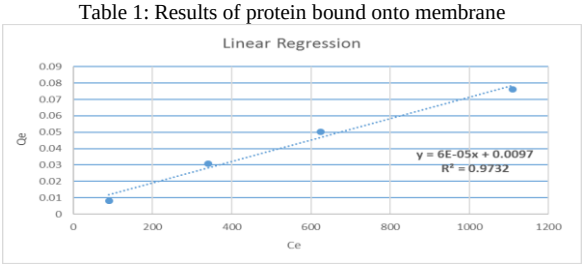

Figure 4: Graph of Linear Regression

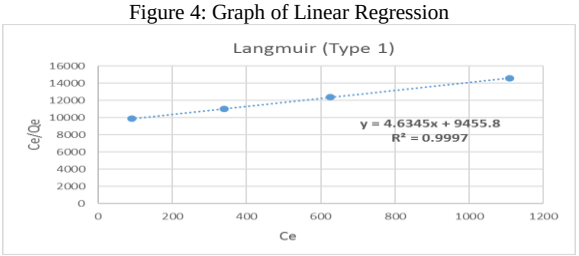

Figure 5: Graph of Langmuir Type 1

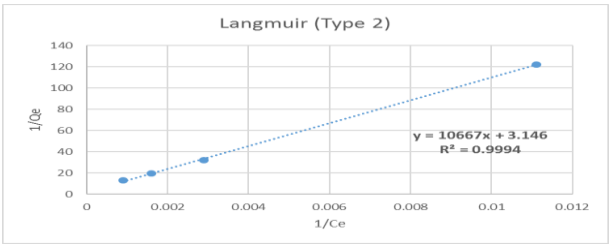

Figure 6: Graph of Langmuir Type 2

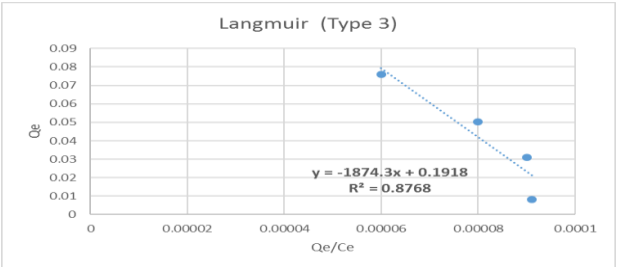

Figure 7: Graph of Langmuir Type 3

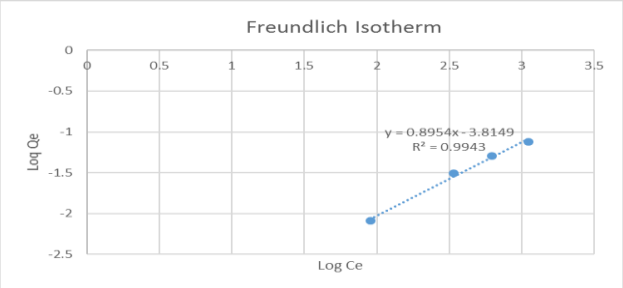

Figure 8: Graph of Freundlich

The graphs above are plotted to know which isotherms are fit for linearization. In this case, pH of BSA solution, experiment at room temperature, thickness of nitrocellulose membrane and volume of the membrane are constant variables. BSA standard curve is used to identified *Cm*. Linearization of each isotherm have been identified (Vidya Rajesh, 2014).

The experiment is carried out from a range of 5 gL^{-1} to 2.0 gL^{-1} because the BCA kit assay can detect protein adsorbed at the maximum concentration 2.0 gL^{-1} (Yoon, 2002). For linear isotherm, the graph of *Qe versus Ce* is plotted directly and the straight line is obtained $y = 6E\text{-}05x+0.0097$ *and* $R^2= 0.9732$. For Langmuir isotherms there are three types. For the first type the graph plotted is *Ce/Qe versus Ce* and the straight line is obtained with $y= 4.63452x + 9455.8$ *and* $R^2 = 0.9997$. For the second type of isotherm, the graph plotted is *1/Qe versus 1/Ce* and the straight line is obtained with $y = 10667x + 3.146$ *and* $R^2 = 0.9994$. For the third type of Langmuir isotherm, the graph plotted is *Qe versus Qe/Ce* and the straight line obtained is $y = -1874.32 + 0.1918$ *and* $R^2 = 0.8768$. Lastly for Freundlich, graph plotted is *loq Qe versus log Ce* and the straight line obtained is $y = 0.8954x – 3.5149$ *and* $R^2 = 0.9943$.

From the above results, linear regression, Langmuir Type 1 and Langmuir Type 2 show the best fit line. Even though other isotherms obtained the straight line, it is not best fit as the three above. As can see in Langmuir Type 3, the straight line obtained elevated downward. For the Freundlich, even though the line is elevated upward, but it constructed at negative axes of y-direction.

III. Conclusion

This study systematically investigated the protein-protein and protein-membrane interaction under the effect of surface morphology. This study also investigated the best adsorption isotherm that fit with protein bound on the membrane surface. The study shows that protein binding is totally related with the surface morphology of the membrane. The physical and chemical characteristics of the membrane itself influenced the protein accessibility to bind on the membrane. The isotherms studied shows that Langmuir type 1 and type 2 are best fitted for quantify the protein adsorption.

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Supplementary Data

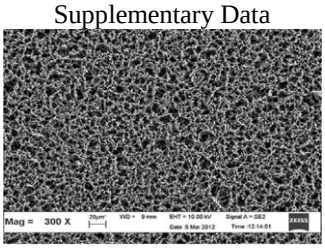

Figure 9: FESEM Image

References

[1] (1990). In M. C.Potter, *Handbook of Industrial Membrane Technology.* NOYES Publication.

[2] Asif Ahmed, J. V. (2014). *Biosensors for Whole-Cell Bacterial Detection*.

[3] Asif Ahmed, Jo V. Rushworth,*,* Natalie A. Hirst,* and Paul A. Millner*. (2014). Biosensors for Whole-Cell Bacterial Detection.

[4] Bio Rad Laboratories. (2015). Protein Blotting Guide. *Westen Blotting* .

[5] Brown, B. S. (1996). Biological Membranes.

[6] Fei Yan a, ⇑. Y. (2015). Determination of adsorption isotherm parameters with correlated errors. 1-10.

[7] GENERAL CHARACTERISTICS OF INORGANIC MEMBRANES. (1991). In H. HSEISH, *INORGANIC MEMBRANES SYNTHESIS, CHARACTERISTICS AND APPLICATIONS* (p. 64).

[8] K. C. Khulbe, C. Y. (2008). Synthetic Polymeric Membranes: Characterization by Atomic Force Microscopy. In *Synthetic Polymeric Membranes: Characterization by Atomic Force Microscopy* (p. 19). Springer.

[9] Mandy LY Sin, K. E. (2014). *Advances and challenges in biosensor-based diagnosis of infectious diseases*, 2.

[10] Minghua Wanga, b. B. (2017). Electrochemical biosensing based on protein-directed carbon nanospheres. *Biosensors and Bioelectronics*, 10.

[11] Nanayakkara YS1, P. S. (2010). *The effect of AC frequency on the electrowetting behavior of ionic liquids*, 12.

[12] Nimmo J.R, N. (2013, September 13). Porosity and Pore Size Distribution. p. 11.

[13] S.Brandon. (2003). Colloid Interface Science. *Colloid Interface Science*, 8.

[14] S.C. Low, R.Shaimi, J.K. Lim. (2013). Colloids and Surface. *Electrophoretic Interactions between Nitrocellulose Membranes and Proteins*.

[15] Springer. (2007). Synthetic Membranes for Membrane Processes.

[16] Vidya Rajesh, N. R. (2014). *Adsorption isotherms, kinetics and thermodynamic studies towards understanding the interaction between a microbe immobilized polysaccharide matrix and lead* , 5.

[17] Whatman. (2015). Protein Research. *Nitrocellulose Membranes an Excellent Surface for Advanced Protein Research*, 6.

[18] Yoon, J. H. (2002). Protein Adsorption on Polymer Particles. *Encyclopedia of Surface and Colloid Science*.