

Correlation Study of PVDF Membrane Morphology with Protein Adsorption: Quantitative Analysis by FTIR/ATR Technique

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Abstract. Microporous PVDF membranes were used as protein capture matrices in immunoassays. Because the most common labels in immunoassays were detected based on the colour change, an understanding of how protein concentration varies on different PVDF surfaces was needed. Herein, the correlation between the membrane pore size and protein adsorption was systematically investigated. Five different PVDF membrane morphologies were prepared and FTIR/ATR was employed to accurately quantify the surface protein concentration on membranes with small pore sizes. SigmaPlot® was used to find a suitable curve fit for protein adsorption and membrane pore size, with a high correlation coefficient, R^2 , of 0.9971.

1. Introduction

Adsorption of protein molecules on a sorbent surface is an important phenomenon in many fields of technology, particularly in biomedical and pharmaceutical fields. Many studies have shown how this process is greatly influenced by the characteristics of the specific protein, sorbent surface, and physicochemical environment [1]. Several reviews on protein adsorption on artificial membrane surfaces have been published, by focussing either on protein-repellent [2, 3] or protein-adsorptive [4, 5] membranes for applications such as ultrafiltration and anion-exchange chromatographic separation processes. One such application is the biochemical immunoassay, where a higher amount of protein adsorption with strong binding is necessary for achieving accurate results. These assays are generally classified as homogeneous or heterogeneous. In the latter, one protein constituent is immobilized on a solid sorbent surface, while the other components are delivered via the solution phase. The output signal of this type of assay usually consists of an observable colour change such as that observed in the widely used enzyme-linked immunosorbent assay (ELISA) [6].

A sorbent surface used for protein adsorption in immunodetection is polymeric membrane, commonly made of nitrocellulose or polyvinylidene fluoride (PVDF) membrane [7]. In the present study, microporous flat PVDF sheets were synthesized with micropores in the range 0.2–1.12 μm . An accurate analysis of the dependence of protein concentration adsorbed onto these membrane surfaces on the size of the micropores was necessary. Previous studies to quantify protein adsorption have been mainly based on the concept of a linear relationship between protein concentration and colour intensity [8]. However, such methods do not provide an actual quantity of protein, they only allow for a comparative analysis [9].



Other approaches have also been employed for measuring protein adsorption, including evanescent wave techniques such as Fourier transform infrared/attenuated total reflectance (FTIR/ATR) [9-11], surface plasmon resonance (SPR) [12, 13], and total internal reflection fluorescence (TIRF) [14, 15]. Among these techniques, TIRF provides a higher surface sensitivity and can measure competitive protein adsorption. However, the proteins need to be labelled with a fluorophore such as fluorescein isothiocyanate (FITC), which increases the number of steps and cost to the process. In addition, the difficulty of waste disposal is often complicated and expensive. FTIR/ATR and SPR techniques have the capability to measure protein surface concentration. Furthermore, FTIR/ATR provides additional information about the structure and conformation of protein molecules [1].

In the present study, FTIR/ATR was employed for its advantages in the analysis of thin films and homogenous solutions. This technique was first proposed by Harrick and de Pru in 1966 [16]. It is particularly sensitive to light-absorbent species near the internal reflection element (IRE), making it a potential method for quantitative measurement of adsorbed species. The evanescent wave, which picks up information from a surface in ATR spectrometry, penetrates the surface to a depth of a few micrometers; this depth is known as the depth of penetration, d_p . The ATR measurement has a constant d_p as long as the wavelength, incident angle, and refractive index of the IRE and sample remain the same [17]. Because the thickness of the adsorbed protein layer is considered to be much smaller than d_p , it is expected that it will experience an almost identical evanescent wave intensity, regardless of the pore size on the membrane surface [18, 19]. Multi-bounce FTIR/ATR spectroscopy has better sensitivity compared to the single-bounce spectroscopy; however, for highly absorbent materials such as polymers, the single-bounce spectroscopy is the most suitable setup for the ATR measurement.

Because the output signal of the immunoassay analysis is identified by an observable colour change of the sorbent surface, this study focussed on achieving a greater understanding of the correlation between the PVDF membrane (sorbent surface) characteristics and protein adsorption. The knowledge gained from this study will contribute to the development of higher efficiency PVDF membranes for biomedical applications.

2. Materials and methods

2.1 Materials

Bovine serum albumin (BSA) and deuterium oxide, D_2O , with an isotopic purity of 99.9 atom %D was obtained from Sigma-Aldrich (St Louis, MO, USA). Potassium phosphate (monobasic and dibasic anhydrous) were obtained from Merck (Germany) and were deuterated in D_2O for the preparation of a phosphate buffer solution. The buffer (0.05 M) was maintained at pH 7 in order to replicate the physiological conditions for immunoassays. All chemicals were used without further purification.

2.2 Protein Adsorption on PVDF Membrane

A symmetrical, microporous PVDF membrane was prepared via non-solvent-induced phase inversion in accordance with a previous published work [20]. The different PVDF membranes were cut into 1cm \times 1cm sections, and the upper surface was marked with a pencil. The samples were then soaked in ethanol for 15 s at room temperature, and placed on pre-wetted filter paper. A 3 μ L aliquot of protein (BSA, bulk concentration, $C_b = 3$ mg/mL) in the phosphate buffer (pD 7 in D_2O) was applied to the membrane surface and exposed to air for 30 minutes to allow the adsorption process to take place. Then, the samples were washed with the phosphate buffer to remove any unbound protein molecules within the PVDF membrane matrix. Finally, the membrane was left for air-dried for another 30 min before carried out the adsorption measurement using ATR-FTIR [21]. At least three samples from each membrane were used to determine the protein adsorption in order to confirm the reproducibility of the experiment.

2.3 Membrane-Protein Adsorption Correlation

The obtained ATR spectra were quantitatively analysed using the theory of Harrick and de Pru [16, 22]. An FTIR spectrometer (OMNIC iS10 Nicolet, Thermo Scientific, USA) was equipped with a Smart iTR diamond ATR accessory, which was used with an incident angle of 42° and single-bounce optics. The final protein adsorption (Γ) is expressed as followed, in the unit of mol/cm²,

$$\Gamma = \frac{\frac{A}{N} - \varepsilon C_b d_e}{1000 \varepsilon \left(2 \frac{d_e}{d_p} \right)} \quad (1)$$

where, Γ is the protein adsorption, A is the area of absorbance, N is the total number of internal reflections, ε is the absorption coefficient, C_b is the bulk concentration, d_e is the effective thickness and d_p is the depth of penetration. The absorption coefficient (ε) of the protein solution was found to be 106.342×10^3 cm/g. The ε value was subsequently used in the analysis of the ATR spectra, as described above.

In this study, PVDF membranes with mean pore sizes; 0.28 μm , 0.37 μm , 0.55 μm , 0.72 μm and 1.12 μm were used to determine membrane–protein adsorption correlation. All the membrane samples possessed a high porous structure with porosity value of more than ~60%. Based on the morphology characterisation, five highly porous membranes with different pore structure were selected and the protein measurement adsorption was carried out as described previously. Then, the correlation between protein adsorption and membrane pore structure was further explained and evaluated using the SigmaPlot® software, version 12.3.

3. Results and discussion

The membrane morphology must be able to capture and retain various proteins for its application in the biomedical field, such as in protein assay tests. Generally, the quantity of protein adsorbed onto the membrane surface is directly related to the surface pore structure and porosity of the membrane. In the present study, five different membrane morphologies comprised of different surface pores, D_p were selected and examined to develop a correlation between membrane morphologies and adsorbed protein.

The quantitation of the protein adsorbed on PVDF membranes with different morphology was analysed by the FTIR/ATR approach. In this method, the amount of the protein adsorbed was calculated based on the area of the absorbance region of interest. The spectral range selected was that corresponding to the amide I' region (1705–1595 cm^{-1}) because of the structure of the protein backbone [22]. Thus, the protein was prepared in deuterated water to avoid the strong peaks at 3360 cm^{-1} (H–O stretching band) and 1640 cm^{-1} (H–O–H bending vibration), which would interfere with the peak of interest. Figure 1 (left) shows the ATR spectra of all the membrane samples before the adsorption of the protein. As can be seen, the spectra exhibit almost identical trends across the entire wavelength range, with no peaks evident at from 1500 to 1700 cm^{-1} . After protein adsorption measurement, it was proved that all the membranes were able to retain certain amount of protein on membrane surfaces as the existence of peak at ~1653 cm^{-1} . It can be seen that the highest peak intensity was observed for the membrane with the smallest pore size with average peak intensity of 0.0140.

On increasing the pore size, the peak intensity reduced to 0.0136 and 0.0120 at 0.37 μm and 0.55 μm respectively, and becoming almost the same at 0.75 μm and 1.123 μm with peak intensity of 0.0116 and 0.0114 respectively. The area of absorbance or area under the curve within the range 1705–1595 cm^{-1} (amide region) was then used in Equation 1 for protein surface concentration measurement using the C_b value of 3.0 mg/mL. The ε value was obtained from the absorbance measurements using the Beer's law. As the peak of interest was at approximately 1653 cm^{-1} , the value of d_p was calculated at $\lambda = 1/1653$ cm^{-1} , $\theta = 42^\circ$, $n_1 = 2.4$ (IRE surface), and $n_2 = 1.42$, with the assumption that protein adsorption had a negligible effect on the refractive index of the PVDF membrane (n_2). The refractive index of the IRE surface (diamond) and PVDF membrane polymer were obtained from Thermo Scientific and Solvay Solexis, respectively. A value of 1.284 μm was obtained for d_p , which was then used to calculate d_e .

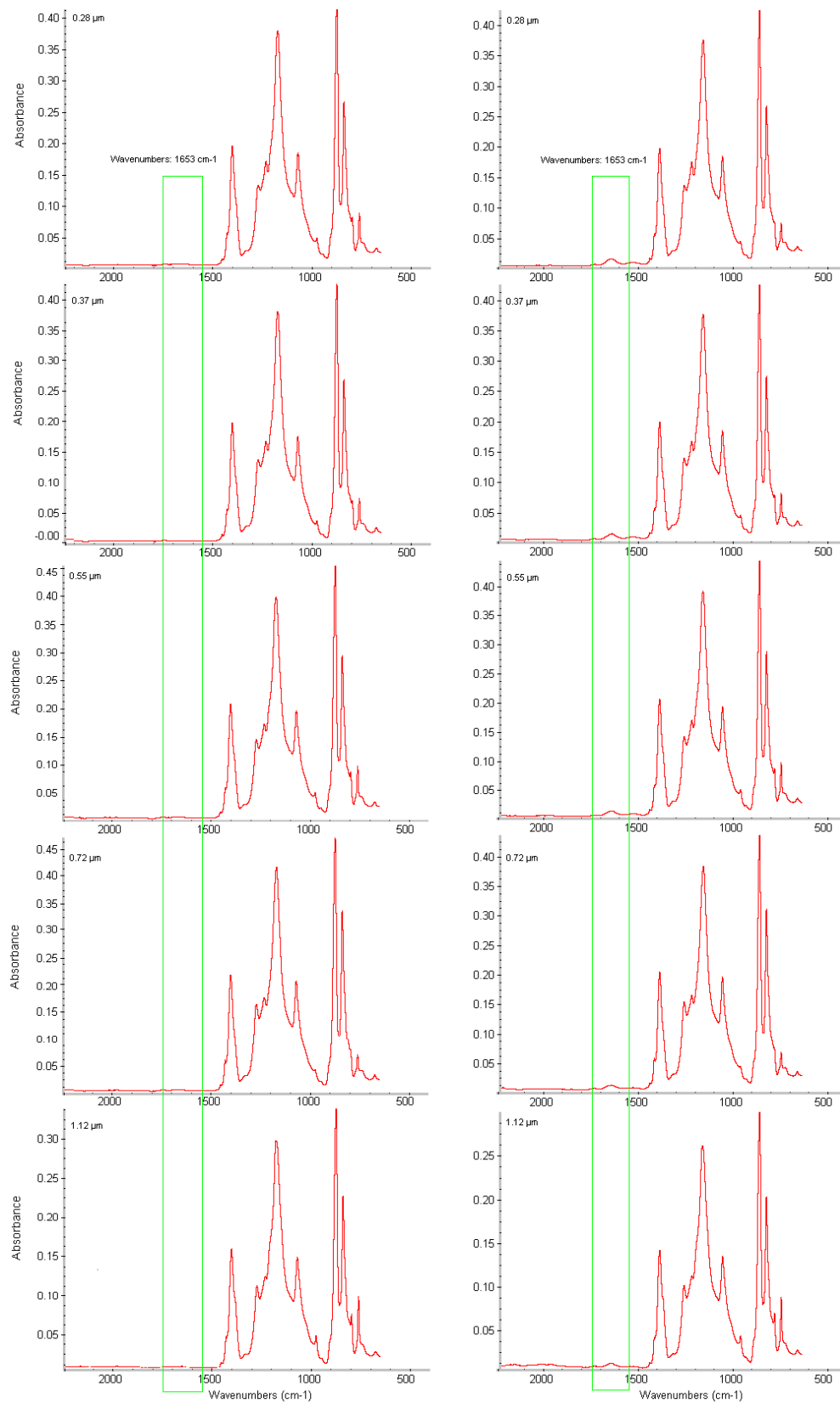


Figure 1. ATR peak intensity for PVDF membrane samples before (left) and after (right) protein adsorption

The correlation between the proteins adsorbed on different membrane surfaces with various pore sizes was next evaluated. As shown in Figure 2, the membranes with lower pore sizes retained more protein at an initial applied protein concentration of 3.0 mg/ml and the amount of protein was gradually

decreased as the pore size is increased. It also shows that the correlation between pore size and protein adsorption was non-linear. Variation of protein adsorption was observed to be less significant for smaller values of pore size which is from 0.28 μm to 0.37 μm . However, as the pore size increased to 0.55 μm , the protein adsorption decreased until it reached almost a plateau at 1.123 μm . Thus, in order to develop a correlation between these two factors, various expressions were examined in order to find a suitable curve fit for the values of protein adsorption (Γ) and pore size diameter (D_p). SigmaPlot® version 12.3 was used to develop a curve fit for the function of pore size diameter (D_p) and protein adsorption (Γ). Equation 2 showed an acceptable correlation coefficient with a high value of R^2 (0.9971)

$$\Gamma = 2.5995 \times 10^{-9} + (6.452 \times 10^{-10}) / [1 + 10^{(0.4622 - D_p)(-6.1141)}] \quad (2)$$

Because of the same protein adsorption method was used, these findings were highly dependent on the membrane pore size. As the amount of the protein adsorbed was found to be related to surface area availability, an appropriate combination of small pores and high porosity is required for obtaining microporous membranes with a high degree of interconnectivity. Generally, the membranes with smaller pore size at equal porosity would maximise the internal surface area for interactions between the membrane surface and the protein. On smaller D_p , the immobilised protein was able to bind and diffuse firmly and horizontally within the smaller pore matrix because more interconnecting polymer structures were formed within the membrane. In addition, smaller D_p allowed a longer time for the membrane–protein interaction to take place as the diffusion resistance of the protein solution is much higher in smaller pore size [8].

As previously mentioned before, the protein adsorption was decreased notably at 0.55 μm and becomes lowest at D_p of 1.123 μm . This is mainly due to the incapability of the protein to tightly immobilize onto the membrane. Bigger pore size with lower interconnecting polymer matrix will reduce affinity forces of membrane-protein interaction and cause the protein to detach during the washing process. Besides, lower interconnecting matrix will cause the deposited protein solution to diffuse vertically due to the gravity effect [23]. For this reason, the resulting amount of protein adsorption was low as compared to the membrane with smaller pore size and not much difference in amount of protein adsorption, Γ can be observed even when membrane pore size, D_p was further increased.

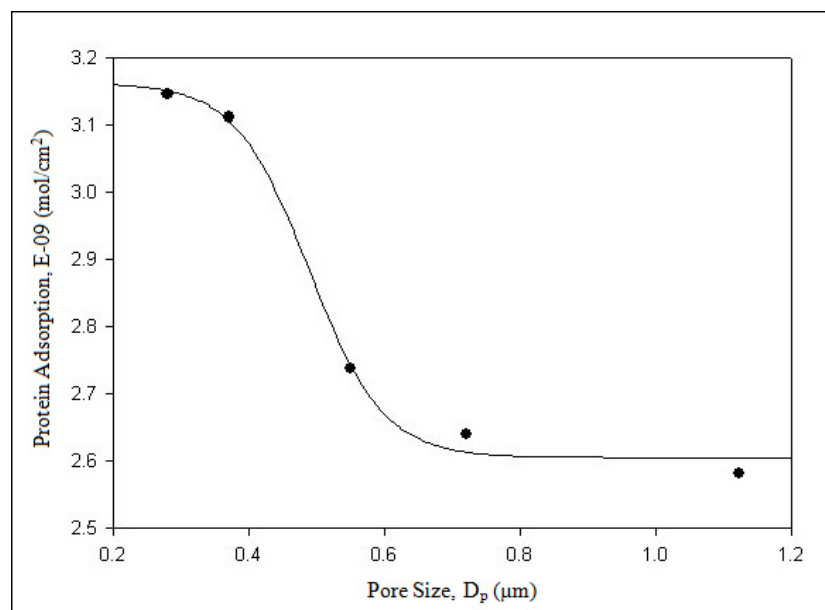


Figure 2. Curve fit of protein adsorption, Γ (mol/cm^2) with respect to various pore size, D_p (μm). Protein conditions: BSA, pD 7, 3 mg/mL

4. Conclusion

In summary, this study systematically investigated the relationship between the protein adsorption and the membrane pore size. Measurement of protein adsorption by FTIR/ATR demonstrated that it was possible to obtain accurate data within a small range of membrane pore size. The results indicated that protein adsorption on a symmetrical, highly microporous membrane was significantly governed by pore size. A good correlation between the adsorption and the pore size was observed, with a high R^2 value obtained for the fitted data. It is expected that the estimation of protein adsorption on different PVDF membranes pore size will be useful for a wide range of applications that involve proteins, particularly quantitative immunoassays.

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