Journal of Applied Sciences

ISSN 1812-5654

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Applied Sciences 14 (12): 1299-1303, 2014
ISSN 1812-5654 / DOI: 10.3923/jas.2014.1299.1303
© 2014 Asian Network for Scientific Information

Influence of Polymer Concentration on PVDF Membrane Fabrication for Immunoassay Analysis

[1]A.L. Ahmad, [1]N. Ideris, [1]B.S. Ooi, [1]S.C. Low and [2]A. Ismail
[1]School of Chemical Engineering, Engineering Campus, Universiti Sains Malaysia,
14300 Nibong Tebal, Penang, Malaysia
[2]Institute for Research in Molecular Medicine, Health Campus, Universiti Sains Malaysia,
16150 Kubang Kerian, Kelantan, Malaysia

Abstract: This study was aimed to study the influence of different PVDF membrane morphology on the adsorption of single proteins. Porous, symmetric PVDF membranes were prepared from PVDF/NMP solution by the phase inversion process. In this paper, the influence of polymer concentration in the 10.0-22.0 wt.% interval for the batchwise protein adsorption of BSA was investigated. The effect of polymer concentration on pore size distribution and surface morphology has been studied under capillary flow porometer and Scanning Electron Microscopy (SEM) respectively, which were further supported with the porosity test. All the synthesized membranes have a small range of pore size distribution with the decreased in pore size as the polymer concentration increased. It was found the membrane made of semi-crystalline PVDF showed a significant morphology change. For membrane performance, protein binding capacity showed the highest protein being retained at an optimum membrane formulation.

Key words: PVDF polymer, membrane, polymer concentration, protein binding

INTRODUCTION

Polyvinylidenefluoride (PVDF) is a semi-crystalline polymer that is well-known for being thermally stable, possessing a high mechanical strength and low surface energy relative to other polymers. These special features are attributed to fluorine that replacing hydrogen in the hydrocarbon macromolecules, makes the properties of PVDF is enhanced compared to polytetrafluoroethylene, PTFE and polyethylene, PE (Ebnesajjad, 2000). Due to that, PVDF has become a choice in fabrication of porous membranes and widely used in a variety of industrial applications. Controlling the membrane properties to suit the final application is one of the most crucial aspects in membrane technology.

In immunological analysis, specifically in dot blotting, the PVDF membrane is particularly useful because of its intrinsic characteristics. For example, fluorine atoms within the PVDF polymer result in a high enough electro-negativity that they induce an electrostatic force between the membrane and the protein. Furthermore, the hydrophobicity of the PVDF membranes also makes it possible for strong hydrophobic interactions to occur between the membrane and the protein.

However, it is known that the function of the membrane is greatly influenced by its morphology and intrinsic chemical composition. Different type of protein may need different membrane surface properties. For this reason, study on membrane surface properties and internal layers is important in the production of effective and accurate immunological analysis. It is generally known that many factors are contributed to the final morphology of the membrane (Mulder, 1996). Thus, in this study, the formulation and casting conditions were systematically being constant to ensure the final morphology obtained is a direct effect from the different polymer concentration. It is expected that an improved understanding of membrane formation mechanism acquire in this study can be used for future development of higher efficiency membrane performance for immunoblotting application.

MATERIALS AND METHODS

Membrane preparation: PVDF (Solef 6010/1001, Solvay Solexis) was dissolved in anhydrous N-methyl-2-pyrrolidinone (NMP) (99.5%, Sigma-Aldrich) in a sealed water-jacketed flask under continuous agitation with a magnetic stirrer for 6 h at 30°C. The polymer concentration of the PVDF was varied from 10.0 to 22.0 wt.%. Before casting, the PVDF solution was sonicated for 1 h at room temperature to remove all the trapped bubbles. Then, the

Corresponding Author: Norhidayah Ideris, School of Chemical Engineering, Engineering Campus, Universiti Sains Malaysia, 14300 Nibong Tebal, Penang, Malaysia Tel:+604-5996454 Fax: +604-5941013

doped solution was maintained at room temperature for 1 h before beginning the membrane casting. After that, the PVDF solution was cast onto a glass plate with an initial thickness of 500 μm and was then immediately immersed in a coagulation bath consists of 2-propanol (99.8%, ACS, ISO, Reag Ph Eur, Merck) and a deionized-water (75:25 volume ratio) mixture at room temperature for 24 h. The formed membranes were dried in a humidity chamber at 40°C with a relative humidity of 60% for 3 h.

Membrane characterization: Pore size distribution of synthesized membrane was measured by using capillary flow porometry (Porolux 1000, Beneflux Scientific, Belgium). In this method, each membrane sample was cut into 2 cm in diameter and immersed in wetting liquid (surface tension of 12 dynes cm^{-1}) for approximately 3 min. The sample analysis was started by applying nitrogen gas under increasing pressure with maximum flow rate of 200 mL min^{-1}. This method essentially measures the pressure needed to blow inert gas through a liquid-filled membrane, based on the Young-Laplace equation (Kaur *et al*., 2007).

The surface morphology of the prepared membranes was observed using a field emission scanning electron microscopy (FESEM Carl Zeiss, Supra 35VP, Germany), with a magnifications of 1000x. Membrane samples were coated with Au-Pd alloy to enhance the electronic conductivity and observed under 3 kv acceleration voltages with an SE2 detector.

The membrane porosity, ε, is defined as the pores volume divided by the total volume of the porous membrane and is determined from the weight of a liquid, 2-butanol (≥ 99.0%, Merck), that occupies all the pores in the membrane sample. For this analysis, the membrane was immersed in 2-butanol for 2 h, after which the surface of the membrane was dried using filter paper. The membrane was weighed before and after absorbing the 2-butanol and the porosity was calculated using Eq. 1 (Nguyen *et al*., 2010):

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

where, ε is the porosity of the membrane, W_B is the wet membrane weight, W_M is the dry membrane weight, ρ_B is the 2-butanol specific gravity (0.81 g cm^{-3}) and ρ_p is the PVDF specific gravity (1.78 g cm^{-3}) (assuming that the specific gravity of all materials remains unchanged in the wet membranes and there is no air trapped in the membrane pores).

Membrane binding capacity: Membrane protein binding ability is commonly accepted as a universal property and reflects the membrane performance in immunoassay analysis. The detail of this test has been described elsewhere (Ahmad *et al*., 2008). At least three samples from each membrane were used to determine the protein binding to confirm the reproducibility of the experimental data.

RESULTS AND DISCUSSION

The dope solution of 10 wt.% polymer concentration was too dilute, thus, the nascent membrane was cracked during the immersion process in the soft coagulation. Meanwhile, 22 wt.% of polymer concentration caused the solution to agglomerate and hampered the membrane casting. In consequence, only membranes prepared at 13 to 19 wt.% polymer concentration could be analyzed in the form of membrane flat sheets.

Figure 1 represents relative flow rate, RF (%) (Fig. 1a) and pore size distribution (Fig. 1b) for membrane prepared at 13 to 19 wt.%. For percentage of relative flow, it is shown that all the membrane samples reached 100% indicating the gas flow rate of wet and dry samples are equal in a specific pressure. It is also observed that all the samples have a steep slope of a straight line correspond to the narrow pore size distribution. For membrane with polymer concentration of 13.0 wt.%, the pore size distribution is in the range of 0.35-0.48 μm (Fig. 1b).

As the polymer concentration increased, the pore size distribution eventually decreased to approximately 0.28-0.38 and 0.21-0.26 μm, respectively. All single sharp peaks in membrane samples indicating that most of the pore diameters are close to the mean pore diameter. The results were further supported with SEM micrographs of the membrane's surface presented in Fig. 2a-c.

The proposed pore size distribution is due to lower diffusion rate of non-solvent at a high solution viscosity. An increase in the polymer concentration results in significant increase in solution viscosity of PVDF dope solution. This phenomenon will lead to a higher mass transfer resistance between the non-solvent (precipitation bath) and the solvent (NMP) in the system during solidification of the casting solution. Thus, at high polymer concentration, the precipitation process stops after a longer period of time, which leads to the formation of denser membrane, with smaller pore sized distribution (Pereira *et al*., 2002; Saljoughi *et al*., 2009). The mean pore size of every membrane is presented in Fig. 3, which reflects the pore size that mostly dominant in every membrane sample. The data of mean pore size is taken from the capillary flow porometer.

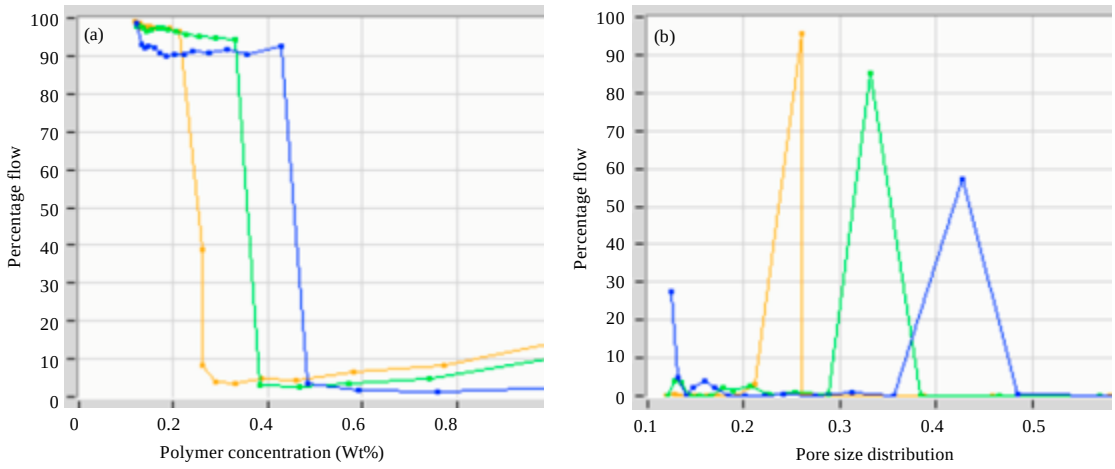

Fig. 1(a-b): (a) Relative flow rate (%) and (b) Pore size distribution. From left is the curve for membrane with polymer concentration 19.0 wt.%, followed by 16.0 wt.% and 13.0 wt.%

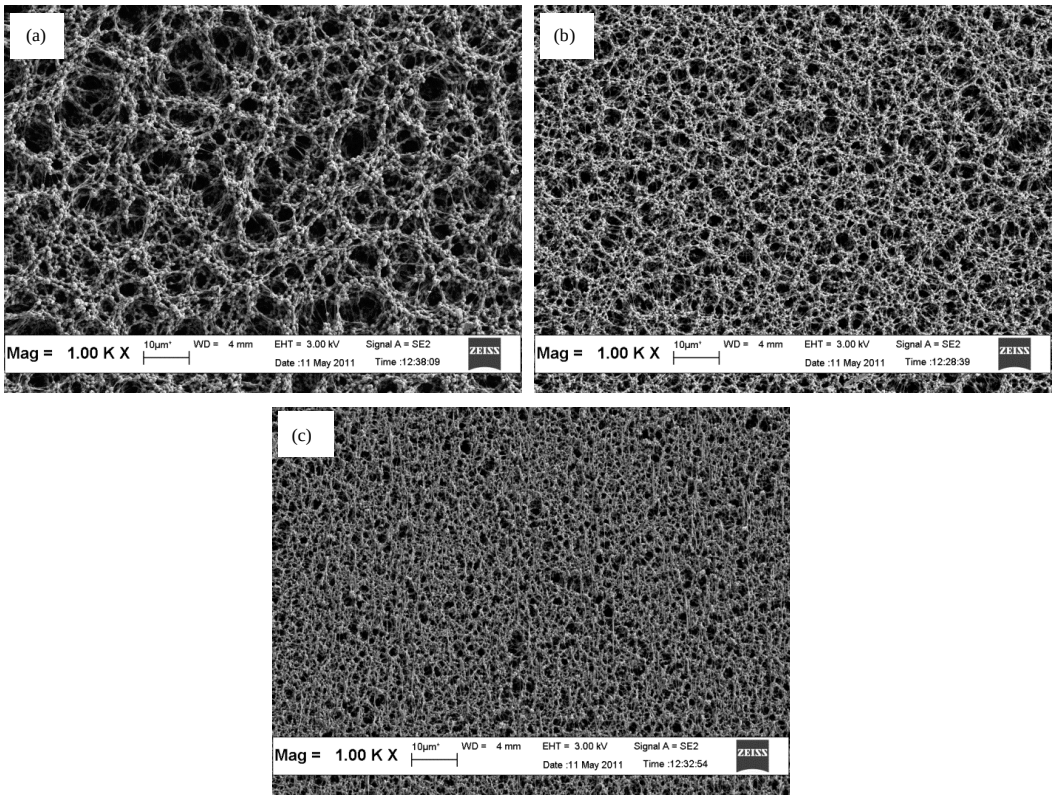

Fig. 2(a-c): Effect of polymer concentration on membrane surface morphology. Dope solutions of PVDF in NMP were prepared at different polymer concentration; (a) 13.0 wt.%, (b) 16.0 wt.% and (c) 19.0 wt.%

Figure 3 also shows that increasing the polymer concentration caused the membrane porosity to decrease significantly from 70.10±0.60 to 60.98±1.09%.

As stated before, higher solution viscosity caused the exchanging process between the solvent and the non-solvent becomes much slower, thus, the membrane

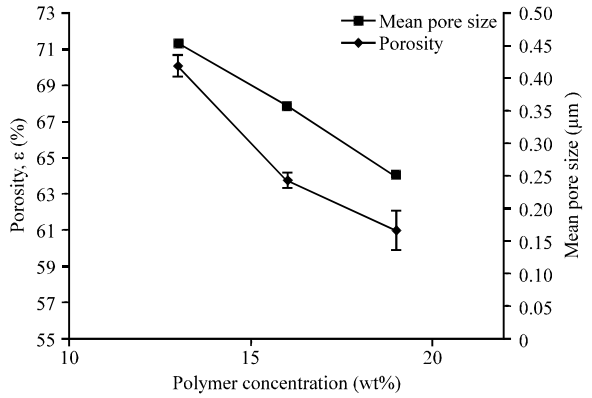

Fig. 3: Porosity values (%) and mean pore size (μm) of the PVDF membrane prepared with different polymer concentration

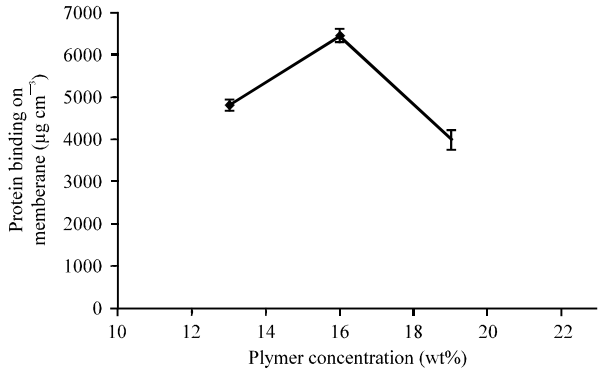

Fig. 4: Effect of PVDF membrane polymer concentration on protein binding performance

structure becomes denser and compact, subsequently lead to lower porosity for a higher polymer concentration. In the same figure, it also shows the mean pore size of the synthesized membrane.

As stated in the literature, the most important driving forces for the protein binding mechanism are electrostatic and hydrophobic interactions (Giacomelli, 2006). However, instead of electrostatic interaction, on the hydrophobic surface such as PVDF membrane, hydrophobic interaction is also dominating the binding and will be contributing for a high protein binding, even under electrostatically adverse conditions (Norde, 1998).

In Fig. 4, it is clearly shown that the performance of protein binding is increased from 4795.20±124.29 to 6425.54±142.30 μg cm^{-3} at the polymer concentration of 13 to 16 wt.%, respectively. However, as the polymer concentration is increased from 16 to 19 wt.%, the performance of membrane-protein binding ability dropped from 6425.54±142.30 to 3982.11±234.12 μg cm^{-3}. This performance result is highly correlated to the morphology of the microporous membrane. In general, a combination of smaller pore size and higher porosity is required to obtain microporous membrane with high interconnection between the pores (Baker, 2003). This morphology is desired since the internal surface area for hydrophobic and electrostatic interaction between the membrane and the protein will increase.

Membranes with 13 wt.% polymer concentration provided the highest porosity, with a percentage value of 70.10%. However, combination of bigger pore size distribution (0.4548 μm) creating a morphology that was not effective in capturing protein. As a polymer concentration is increased to 19 wt.%, the protein binding performance was the lowest, with only 3982.11 μg cm^{-3} being retained. Therefore, even though a smaller pore size is desired, the pore volume (porosity) of the membrane sample must also be considered. It was discussed previously that the 19 wt.% membrane caused the structure to become denser and as a result, the capturing area for protein decreased. Based on Fig. 4, it is clear that the highest protein binding performance recorded was achieved at a polymer concentration of 16 wt.% with retention of 6425.10±142.301 μg cm^{-3} of BSA.

CONCLUSION

The results show the PVDF membrane morphology is greatly affected by the polymer concentration during membrane fabrication. It was found that the mean pore size and porosity decreased with the polymer concentration. Polymer concentration of 16 wt.% apparently possessed a balanced combination of pore size distribution and porosity and is therefore provided a large internal surface area for protein binding. In this study, a membrane with polymer concentration of 16 wt.% is preferred among the three different polymer concentration with the highest amount of protein being retained (6425.54±142.30 μg cm^{-3}). The performance test indicates the structural properties are a great importance for the membrane in an immunoassay application.

ACKNOWLEDGMENTS

The authors would like to acknowledge Universiti Sains Malaysia, USM (Vice-Chancellor Award 2010), USM-PGRS Grant (8034059), USM Membrane Cluster (8610012) and ERGS Grant (6730004) for financial support.

REFERENCES

Ahmad, A.L., S.C. Low, S.R.A. Shukor and A. Ismail, 2008. Synthesis and characterization of polymeric nitrocellulose membranes: Influence of additives and pore formers on the membrane morphology. J. Applied Poly. Sci., 108: 2550-2557.

Baker, R.W., 2003. Membrane Technology. In: Encyclopedia of Polymer Science and Technology, H.F. Mark (Ed.). A John Wiley and Sons Publication, New Jersey, pp: 184-249.

Ebnesajjad, S., 2000. Fluoroplastics. Volume 1: Non-Melt Processible Fluoroplastics the Definitive User's Guide and Databook. Plastics Design Library, New York.

Giacomelli, C.E., 2006. Adsorption of Immunoglobulins at Solid-Liquid Interfaces. In: Encyclopedia of Surface and Colloid Science, Hubbart, A.T. (Ed.). Boca Raton, Fla. Taylor and Francis.

Kaur, S., Z. Ma, R. Gopal, G. Singh, S. Ramakrishna and T. Matsuura, 2007. Plasma-induced graft copolymerization of poly (methacrylic acid) on electrospun poly (vinylidene fluoride) nanofiber membrane. Langmuir, 23: 13085-13092.

Mulder, M., 1996. Basic Principles of Membrane Technology. 2nd Edn., Kluwer Academic Publishers, Netherlands.

Nguyen, Q.T., O.T. Alaoui, H. Yang and C. Mbareck, 2010. Dry-cast process for synthetic microporous membranes: Physico-chemical analyses through morphological studies. J. Memb. Sci., 358: 13-25.

Norde, W., 1998. Driving Forces for Protein Adsorption at Solid Surfaces. M. Dekker, New York.

Pereira, C.C., R. Nobrega and C.P. Borges, 2002. Membrane formation with presence of lewis acid-base complexes in polymer solution. J. Applied Poly Sci., 83: 2022-2034.

Saljoughi, E., M. Sadrzadeh and T. Mohammadi, 2009. Effect of preparation variables on morphology and pure water permeation flux through asymmetric cellulose acetate membranes. J. Membr. Sci., 326: 627-634.