J. Applied Membrane Science & Technology, Vol.15, June 2012, 1-6

Investigating Membrane Material and Morphology for Development of Lateral Flow Biosensor

R. Shaimi[1], A. L. Ahmad[2] & S. C. Low[3*]
[1-3]School of Chemical Engineering Campus, Universiti Sains Malaysia, Seri Ampangan, 14300 Nibong Tebal S.P.S. Penang, Malaysia

ABSTRACT

Development of membrane for bio-sensing applications, such as the detection of pathogens in drinking water for epidemics control has a huge global impact especially for public health. Membrane that applied in the bio-sensing devices should fulfill requirements such as high binding ability, fast lateral wicking time and low background staining. This paper explores the selection of membranes, including Nitrocellulose membrane (NC), Cellulose Acetate membrane (CA), Polyvinylidene Fluoride membrane (PVDF) and Nylon membrane for the detection of pathogens in water in the most efficient and rapid way. Membrane modification using glutaraldehyde enable the achieved of higher sensitivity of protein binding. Experimental findings (FTIR, porosity, membrane's binding ability and lateral wicking time) verified the most suitable membrane for bio-sensing application. Among the tested membranes, NC appeared as the most suitable lateral flow membrane as its performances of high protein binding ability and fast lateral wicking time. Throughout this study, we showed the correlation of membrane's material and morphology to its performances for pathogens detection in drinking water.

Keywords: Nitrocellulose, protein binding ability, lateral wicking time, biosensor

1.0 INTRODUCTION

As the demand for point-of-care testing and on-spot detection of pathogens in drinking water increases, there has been much interest in the development of biosensor. Selection of membrane as the support material to bind with bio-molecules oftentimes a protein is not a trivial task. A wide range of porous membranes such as cellulose acetate membrane (CA), polyvinylidene fluoride membrane (PVDF), nylon membrane and nitrocellulose membrane (NC) have found to be compatible in bio-sensing development [1, 2].

NC membrane has long occupied a position of central importance in medical and immunological analysis due to its excellent wetting properties, high binding capacity and low background staining [3]. Nylon membranes, with narrow pore size distribution, hydrophilic and good mechanical rigidity offers good accessibility for potential bio-sensing applications [4]. CA membrane is taken into consideration due to its long elasticity, non-fragile and chemical resistant [5] while PVDF membrane has shown to have high protein binding capacity and chemical stability is suitable for immune-staining procedures [6].

Protein is the most common reagent to be applied onto the membrane surface in an immunoassay. The interaction between protein and membrane is generally affected by the membrane morphology, and eventually reflected the effectiveness of the biosensor. To assure the required sensitivity level of a biosensor, the membrane surface is always modified via cross-linking with glutaraldehyde. Glutaraldehyde served as the

* *Corresponding to:* S. C. Low: (email: chsclow@eng.usm.my)

most suitable cross-linking reagents due to its ability to react with several functional groups of protein that make it found in various fields such as histochemistry, microscopy, biomedical and pharmaceutical sciences [7]. This study was aimed at a quantitative understanding of membrane materials and morphologies, which governs the performance of the lateral flow biosensor. In this report, the interest was focused on determining the protein binding ability and lateral wicking time for NC, Nylon, CA and PVDF membranes. These factors would directly reflect the effectiveness of the biosensor and eventually, develop an in-depth understanding of membrane selection for the development of biosensor.

2.0 RESEARCH METHODOLOGY

2.1 Reagents

Glutaraldehyde 25 % aqueous solutions, methanol, KH_2PO_4, K_2HPO_4 were purchased from Merck (Darmstadt, Germany). Bovine Serum Albumin (BSA) and bicinchoninic acid agent (BCA) protein assay kit were supplied by Sigma-Aldrich (St. Louis, MO). Cellulose Acetate (CA) membrane and Nylon membrane were purchased from Sterlitech Corporation, whereas Polyvinylidene Fluoride (PVDF) membrane and Nitrocellulose (NC- HF 90, HF 135, HF 180) membranes were supplied by Milipores (Bedford, MA). All chemicals were reagent grade and were used without further purification.

2.2 Membrane Characterization

2.2.1 Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR)

The bonding characteristic of NC (HF-90, HF-135 and HF-180), CA, Nylon and PVDF membranes were analyzed using Thermo Scientific fourier transform infrared spectrometer (NICOLET iS10, USA) over the wavenumber range of 4000-600 cm^{-1}. Each spectrum results from 32 scans at 4 cm^{-1} resolution at a 45°C incident angle using a diamond crystal.

2.2.2 Porosity

The porosity of the membranes was calculated according to the equation

$$\varepsilon = \frac{V_A - V_E}{V_A} \times 100\% \quad (1)$$

where V_A was the membrane's apparent volume that calculated based on the film thickness and surface area (1 cm × 1 cm). The membrane sample was then dried in an oven to eliminate any water vapor contaminant in the membrane. The membrane's existent volume (V_E) was then determined via respective polymer density and dry weight of membrane.

2.3 Membrane Modification

Membranes samples (circular with diameter of 15 mm) were first washed with 10 % methanol for 30 minutes and then dried with N_2 gas for 10 min. Dried membranes were then incubated in 3 ml of glutaraldehyde (1 wt. %) and shaken for 1 h at 90 rpm. Subsequently, modified membranes were rinsed with deionised water.

2.4 Membrane Performance Test

2.4.1 Protein Binding Ability

BSA solution (0.5mg/ml) was prepared in 0.05M phosphate buffer (pH 7.4) for protein immobilization. The modified membranes were incubated in the protein solution (1 ml) and shaken for 1 h at 37 °C and 90 rpm. After incubation, the protein immobilized membranes were washed (repeated twice) with phosphate buffer to remove unbound protein on the membrane surface. Each sample (with and without glutaraldehyde) replicate was transferred into the test tube. Subsequently, 2.0 ml of bicinchoninic acid working agent (50 parts BCA solution with 1 part of 4 % Cupric Sulfate) was added and incubated for 30 minutes at 37 °C. Liquid content were then sent for photometric measurement at wavelength of 562 nm.

2.4.2 Lateral Wicking Time

The liquid flow time (wicking time) measurement was conducted on 8 cm long and 1 cm width of membrane strip. Deionised water was used as the medium for the wicking test. Times were measured when the wicking medium was migrated to the height of 1, 2 and 3 cm standardwise after initial contact between the membrane and wetting agent.

3.0 RESULTS AND DISCUSSION

3.1 Effect of Membrane's Material on Biosensor Performances

Membrane appeared to be one of the key elements in a biosensor that significantly affect on protein binding and lateral wicking performances. There are few critical membrane properties that affected both binding and wicking performances, including the membrane's pore size, pores' connectivity and membrane's material. In this study, a total of 6 types of membranes were tested for potential bio-sensing application. Fig. 1 represent the binding ability of all membranes, where nylon membrane shown to achieve highest protein binding level (1171.80 ± 17.65μg/cm^3), followed by NC membrane, PVDF membrane and CA membrane.

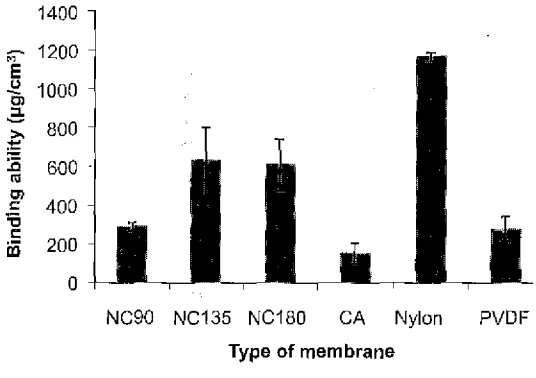

Figure 1 Comparison of pure binding ability (without glutaraldehyde) in NC (HF-90, HF-135, HF-180), CA, nylon and PVDF membranes

The high protein binding ability of NC and nylon membranes could be explained through the physiochemistry properties of membrane. FTIR spectra of NC (Fig. 2b) shown stretching vibration band of hydrogen bonded (-OH) at 3402.56 cm^{-1}. As water molecules played an important role in antigen-antibody interaction, the presence of hydroxyl group in membrane was helped to stabilize the complex [8]. In other words, high OH group contributed to a constant association between antigen and antibody, thus leading to the excellent antigen-antibody complexes and a stable biosensor signal.

Besides to the hydrogen bonding, the affinities of antigen-antibody also involved in a variety of forces, including electrostatic forces between COO- and NH_3^+, hydrophobic interaction as well as Van der waals forces [9]. The strong peaks of NO_2 at 1636.98 cm^{-1} and 1274.73 cm^{-1} as well as NO at 830.48 cm^{-1} and 747.98 cm^{-1} justified the strong binding ability of protein onto NC membrane (Fig. 2b). The strong dipole of the nitrate group in the membrane polymer matrix will retain the high dipole of the peptide bonds of protein molecules by electrostatic force [10]. For nylon membrane (Fig. 2a), the protein binding was confirmed by the sharp and high intensity peaks of N-H and C=O groups at 3299.27 cm^{-1} and 1631.75 cm^{-1}, respectively.

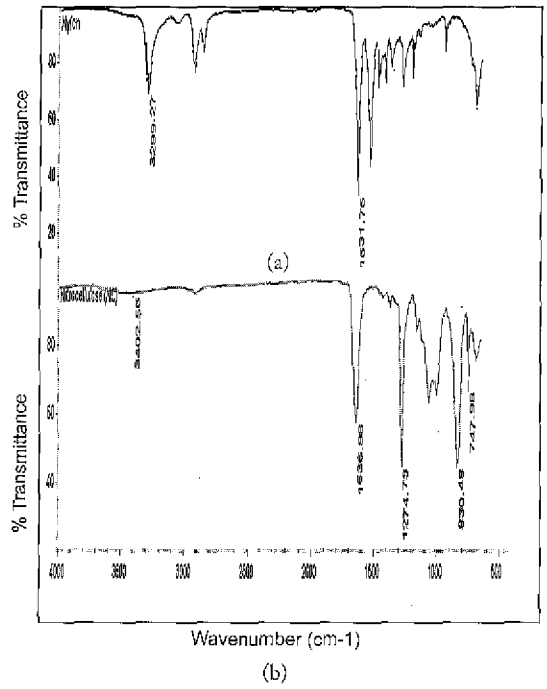

Figure 2 ATR-FTIR spectra of (a) Nylon membrane and (b) NC membrane

Membrane with high protein binding ability will gives sharper and high sensitivity protein lines on the membrane. However, it could not be concluded that membrane with high protein binding capability is better in every diagnostic application. As for biosensor, lateral wicking time or in plane liquid distribution in the membrane strip is relatively important. Lateral wicking time is referring to time required for liquid to migrate to certain length along the membrane strip. If a membrane has a very short lateral wicking time, it means that the target analyte could diffuse faster along the lateral plane of the membrane and eventually creates a rapid detection from the point of application.

Fig. 3 represented the lateral wicking time performance in respective membranes. Among all the membranes, NC membranes (HF-90, HF-135 and HF-180) have generally shorter wicking time compared to others membranes (Nylon, CA and PVDF), with wicking time 0.52 min 1.38 min and 2.32 min, respectively. PVDF membrane took the longest time (13.32 min) to migrate 3 cm height along the membrane strip. In this regards, NC membrane was preferable for diagnostic application for the concerned of rapid detection.

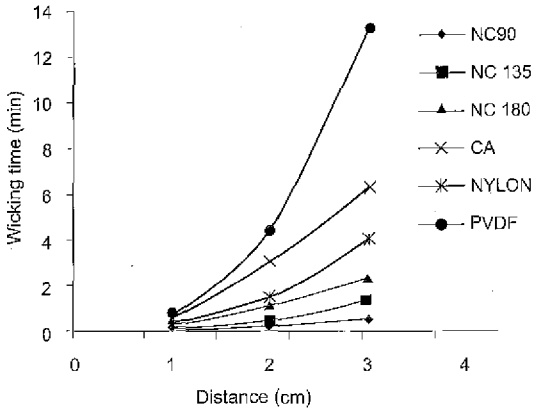

Figure 3 Performance of lateral wicking time (min) in NC (HF-90, HF-135, HF-180), CA, nylon and PVDF membranes

In general, the rapid lateral wicking time of NC membranes were inter-related to the membrane porosity. High porosity of membrane was expected to perform faster lateral wicking or shorter time for the wetting agent to migrate along the membrane strip. NC membranes showed to have the highest porosity, which were 93%, 60% and 69% for HF-90, HF-135 and HF-180, respectively, as shown in Fig. 4. Although the nylon membrane showed to have the highest protein binding ability (1171.80 + 17.65μg/cm^3) in Fig. 1 but the membrane took much longer time (4.02 minutes) to migrate 3 cm distance along the membrane strip compared to all three types of NC membranes. This slower lateral wicking speed was not preferable in a sensing application, as it will defeat the aims of rapid detection yet could generate higher background noise that reduced the reliability of a sensor. In this regards, NC membrane (HF 180) was selected, due to its high binding ability (613.57 + 136.75 μg/cm^3) and fast lateral wicking speed (migrated 3 cm along membrane strip within 2.32 minutes).

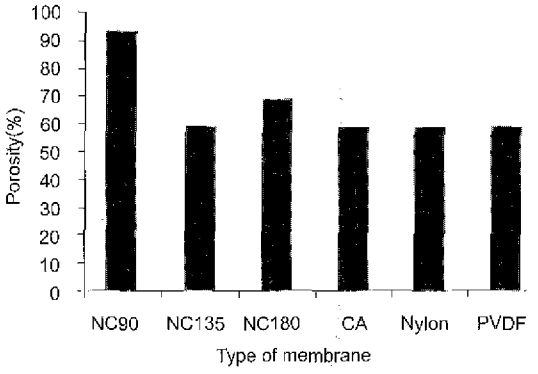

Figure 4 Porosity of NC (HF-90, HF-135, HF-180), CA, nylon and PVDF membranes

3.2 Effect of Crosslinking of Glutaraldehyde on Protein Immobilization

The lack of stability and activity of the protein in the solid-liquid interface is the major obstacle for the widespread use of biosensor [11]. In this regards, membrane modification is needed to improve both stability and sensitivity of protein immobilization. Immobilization of protein through treatment of glutaraldehyde has been found to be a reliable and simple procedure that yielded the high stabilization of immobilized protein [12]. In turns, it improved the sensitivity of a biosensor. Fig. 5 shows the protein binding ability of membranes that treated with glutaraldehyde (1 wt.%).

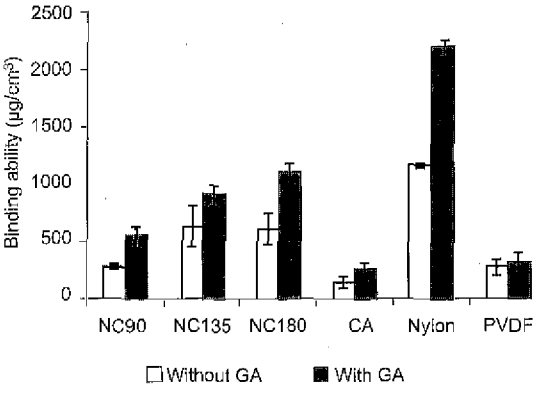

Figure 5 Effect of glutaraldehyde (1 wt.%) on the protein binding ability in varying membrane

Membrane that modified with glutaraldehyde was found have significant improvement of protein binding compared to membrane without modified.

This is the expected result where the cross-linking of glutaraldehyde could generate better interactions with protein molecules and thus, higher density of protein molecules could attached within the membrane polymer matrix. However, the concentration of glutaraldehyde need to be controlled, as the excess concentration of glutaraldehyde could have the possibility to denature the protein molecule and eventually less density of protein molecules that able to bind to the membrane [13].

As for biosensing application is concerned, the protein stability is needed in order to prevent it from lose of the activity [14]. In order to confirm the protein stability, the immobilized membranes (with and without glutaraldehyde) were stored at room temperature and the proteins bound onto the membrane surface was quantified over a week. Each sample was carried out in triplicate. From Fig. 6, the result proved that membrane modified with glutaraldehyde was significant improved in their protein stability. Within a week of immobilization, the modified membrane was recorded protein binding at 688.52 μg/cm^3, compared to 398.91 μg/cm^3 for the unmodified membrane.

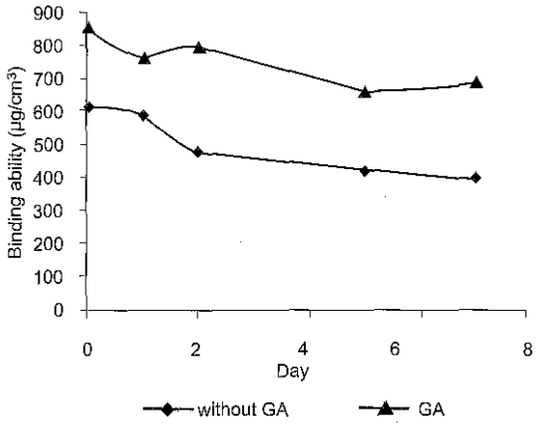

Figure 6 Stability of protein bound on membrane treated with and without glutaraldehyde (0.5 wt.%)

4.0 CONCLUSION

This study has provided fundamental information regarding membrane material and morphology in development of lateral flow biosensor. Of the 6 types of membrane, NC 180 modified with glutaraldehyde offered the best performance combining high binding protein ability (1105.04 ± 80.87μg/cm^3), fast lateral wicking time (2.32 minutes), and high stability. These results were shown to give high sensitivity for the development of lateral flow biosensor in detection of pathogen in drinking water.

ACKNOWLEDGMENT

The authors wish to thank the financial support granted by Universiti Sains Malaysia Short Term Grant (60311008), RU grant (811178) and Membrane Science and Technology Cluster (8610012).

REFERENCES

[1] Singh, N., Wang, J., Ulbricht, M., Wickramasinghe, S. R. & Husson, S. M. 2008. Surface-initiated atom transfer radical polymerization: A new method for preparation of polymeric membrane adsorbers. *Journal of Membrane Science.* 309 : 64-72.

[2] Fang, Z., Ge, C., Zhang, W., Lie, P. & Zeng, L. 2011. A lateral flow biosensor for rapid detection of DNA-binding protein c-jun. *Biosensors and Bioelectronics.*

[3] Morais, S., Maquieira, Á. & Puchades, R. 1999. Selection and characterisation of membranes by means of an immunofiltration assay. Application to the rapid and sensitive determination of the insecticide carbaryl. *Journal of Immunological Methods.* 224 : 101-109.

[4] Narang, J., Chauhan, N., Singh, A. & Pundir, C. 2011. A nylon membrane based amperometric biosensor for polyphenol determination. *Journal of Molecular Catalysis B: Enzymatic.*

[5] Pundir, C., Sandeep Singh, B. & Narang, J. 2010. Construction of an amperometric triglyceride biosensor using PVA membrane bound enzymes. *Clinical Biochemistry.* 43 : 467-472.

[6] Aizawa, K. & Gantt, E. 1998. Rapid method for assay of quantitative binding of soluble proteins and photosynthetic membrane proteins on poly (vinylidene difluoride) membranes. *Analytica Chimica Acta.* 365 : 109-113.

[7] Migneault, I., Dartiguenave, C., Bertrand, M. J. & Waldron, K. C. 2004. Glutaraldehyde:

behavior in aqueous solution, reaction with proteins, and application to enzyme crosslinking. *BioTechniques.* 37 : 790-806.

[8] Pal, S., Ying, W., Alocilja, E. C. & Downes, F. P. 2008. Sensitivity and specificity performance of a direct-charge transfer biosensor for detecting Bacillus cereus in selected food matrices. *Biosystems Engineering.* 99 : 461-468.

[9] Sibai, A., Elamri, K., Barbier, D., Jaffrezic-Renault, N. & Souteyrand, E. 1996. Analysis of the polymer-antibody-antigen interaction in a capacitive immunosensor by FTIR difference spectroscopy. *Sensors and Actuators B: Chemical.* 31 : 125-130.

[10] Van Oss, C., Good, R. & Chaudhury, M. 1987. Mechanism of DNA (Southern) and protein (Western) blotting on cellulose nitrate and other membranes. *Journal of Chromatography A.* 391 : 53-65.

[11] Teles, F. R. R. & Fonseca, L. P. 2008. Applications of polymers for biomolecule immobilization in electrochemical biosensors. *Materials Science and Engineering: C.* 28 : 1530-1543.

[12] López-Gallego, F., Betancor, L., Mateo, C., Hidalgo, A., Alonso-Morales, N., Dellamora-Ortiz, G., Guisán, J. M. & Fernández-Lafuente, R. 2005. Enzyme stabilization by glutaraldehyde crosslinking of adsorbed proteins on aminated supports. *Journal of Biotechnology.* 119 : 70-75.

[13] Zhang, G., Liu, D., Shuang, S. & Choi, M. M. F. 2006. A homocysteine biosensor with eggshell membrane as an enzyme immobilization platform. *Sensors and Actuators B: Chemical.* 114 : 936-942.

[14] Trivedi, U., Lakshminarayana, D., Kothari, I., Patel, N., Kapse, H., Makhija, K., Patel, P. & Panchal, C. 2009. Potentiometric biosensor for urea determination in milk. *Sensors and Actuators B: Chemical.* 140 : 260-266.