Synthesis and Characterization of Polymeric Nitrocellulose Membranes: Influence of Additives and Pore Formers on the Membrane Morphology

A. L. Ahmad,[1] S. C. Low,[1] S. R. Abd. Shukor,[1] A. Ismail[2]

[1]*School of Chemical Engineering, Engineering Campus, Universiti Sains Malaysia, Seri Ampangan, 14300 Nibong Tebal, Seberang Perai Selatan (SPS), Penang, Malaysia*
[2]*Institute for Research in Molecular Medicine, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia*

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ABSTRACT: The control of the membrane surface and cross-section morphology is extremely important in the enhancement of the wicking and binding ability of the lateral flow membrane, which is one of the processing materials in medicine and health care analysis devices. The lateral flow rate and protein-binding performance is based on the thin layers of the membrane. The challenge of this study was to combine the influences of additives and pore-former materials to obtain a thin lateral flow nitrocellulose membrane with controlled membrane morphologies. Water was found to be an effective pore former for enhancing the porosity and pore size of the membrane. However, too high of a water content increased the surface roughness and decreased the membrane protein-binding ability. Different properties of the individual plasticizers/additives contributed to the disparity of membrane performance in binding and solute lateral wicking time. The correlations between the effects of additives and pore former toward the final membrane structure and performance of the membrane-forming system are discussed extensively. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 108: 2550–2557, 2008

Key words: membranes; microstructure; morphology; synthesis

INTRODUCTION

The membrane is a key element in the production of rapid diagnostic test strips. The study of the behavior of the surface and internal layers of membrane is fundamental in the development of the lateral flow membrane as one of the processing materials in medicine and health care analysis devices. Controlled membrane surfaces and cross-section structures are important in all types of membrane applications. At present, thin-film nitrocellulose (NC) membranes with high mechanical stability and tensile strengths are often described as universal blotting surfaces for protein research,[1,2] lateral flow immunochromatography testing,[3] and immobilization of proteins.[4] If the membrane surface and internal layer structure could be controlled precisely, various kinds of immunological analysis could be performed effectively and accurately.

Membrane production is, nonetheless, a very sensitive process. The most commonly used and important class of membrane preparation method is the phase-inversion technique.[5–7] *Phase inversion* refers to the process in which a polymer solution (liquid phase) inverts into a swollen, three-dimensional macromolecular network (solid state).[7,8] At a particular stage during solvent evaporation, the high-polymer-concentration phase solidifies and forms a solid matrix.[7] In the synthesis of NC membranes, the phase-inversion technique is deemed to be the most suitable method for modifying the surface and internal layer morphology of the membrane.[9] In this process, NC membranes are made from solutions to form a porous solid film.

The microstructure of a membrane is known to depend on various rheological factors, such as the composition of the casting materials; the choice of polymer, solvent, nonsolvent, and additives; and the gelation and crystallization behaviors of the polymer.[6] The addition of organic or inorganic additives, such as glycerol and poly(ethylene glycol) (PEG), as third components to a casting solution has been proven capable of enhancing the permeation properties of membranes.[10,11] Hence, by the manipulation of the initial stage of phase transition and rheological factors, the membrane morphology can be controlled, and porous membranes can be prepared at the desired pore size, porosity, thickness, and surface roughness.[12–14]

Technologically speaking, the solute-spreading trend or liquid-wicking trend reflects the surface

Correspondence to: A. L. Ahmad (chlatif@eng.usm.my).

TABLE I
Composition of the Casting Dope

Component	Casting dope composition (%)								
	M1	M2	M3	M4	M5	M6	M7	M8	M9
NC	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
MA	74.5	76.0	76.5	74.5	74.5	74.5	74.5	76.5	72.5
IP	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Water	3.5	3.5	3.5	3.5	3.5	3.5	3.5	1.5	5.5
Glycerol	—	—	—	—	—	—	2.0	2.0	2.0
PEG 200	2.0	—	—	—	—	—	—	—	—
PEG 600	—	—	—	2.0	—	—	—	—	—
PEG 3000	—	—	—	—	—	2.0	—	—	—
SHS 0.5	—	0.5	—	—	—	—	—	—	—
SHS 2.0	—	—	—	—	2.0	—	—	—	—

properties and porosity of the membrane and will probably be used in membrane homogeneity measurement. Liquid wicking rate is the major factor that reflects the performance of NC membranes. The distribution of the liquid wicking in the membrane strip can occur in two ways, either through travel perpendicularly through the membrane or through slip flow on one side of the membrane strip (lateral flow liquid distribution).[15] This study was focused on lateral flow liquid distribution, as the membranes produced were designed to function as lateral flow membranes in immunodiagnostic test analysis.

The ability of NC membranes to bind protein is commonly accepted as a universal property of these membranes. NC membranes have been used widely in a vast variety of fields of study, such as immunochromatography testing, protein immobilization analysis, and western blotting.[16] In immunodiagnostic tests, proteins are the most common samples applied to a solid membrane surface; therefore, the protein-binding capacity is a critical property of the membrane. The interaction of proteins with microporous membranes will significantly reflect the membrane morphology.

The objective of this study was to adjust several parameters accordingly to obtain the optimum formulation of the membrane in a lateral flow application with controlled membrane morphology. This study elucidated the influence of initial additive concentration and water content introduced into the casting solution on the membrane microstructure. Furthermore, the effects of the resultant membrane structures on the membrane performance, such as membrane protein-binding ability and solute lateral wicking time on the membrane strip, were also studied to confirm the effectiveness of the resultant membrane.

EXPERIMENTAL

Materials

The NC used in this study contained 11.8–12.3% nitrate and 30% alcohol and was supplied by Sigma (St. Louis, MO). The solvent, methyl acetate (MA), was reagent grade and was purchased from Merck (Darmstadt, Germany), whereas distilled water and isopropanol (IP; Merck) were used as nonsolvents in the casting dope. The wetting agent and additives used in this study, including glycerol, PEG 200, PEG 600, and PEG 3000, were purchased from Merck, whereas the ionic surfactant, sodium hexadecane sulfonate (SHS), was purchased from Sigma Aldrich. Bovine serum albumin (BSA) was purchased from Sigma, and the bicinchoninic acid working reagent was purchased from Merck. The 0.05*M* buffer tris (hydroxymethyl)aminomethane/HCl and 0.05*M* phosphate buffer at pH 7.0 were prepared for characterization. All chemicals were reagent grade and were used without further purification.

Porous membrane preparation

NC was dried in a vacuum oven at 50°C overnight before being used to remove the excess alcohol in the polymer. Nine different compositions of casting dope used in this experiment are shown in Table I. The NC membranes were prepared with the dry-phase-inversion method.[17] The casting process was carried out at ambient temperature and performed with a membrane autocasting machine, where the casting solutions were cast onto a glass plate with a casting blade. The depth of the casting was 600 μm for all of the membranes; a casting speed of 100 rpm was used. Before use, the membranes were dried in a vacuum oven overnight at 40°C. To check the reproducibility, at least two specimen membranes were manufactured from each solution.[18]

Field emission scanning electron microscopy (FESEM) analysis

The surface and cross section of the membrane morphologies were observed by FESEM (VPFESEM SUPRA 35VP, Germany). Membrane samples were first coated with a conducting layer to prevent the sur-

face from being charged up. From the images, average pore sizes were measured. At least six spots per sample and three samples per membrane were measured to confirm the reproducibility of the experimental data.[13] Thickness measurement of the membrane layer was applied by FESEM and further confirmed with a microthickness gauge (Mitutoyo 7301, Japan).

Determination of the membrane porosity

The porosity of the membrane (ε) was calculated according to the equation given by Yamane et al.[19] and Meier:[13]

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

The synthesized membranes shown in Table I were cut into 2 cm × 2 cm samples, and their thicknesses were measured, where V_A, the apparent volume of the membrane, was calculated from the film thickness and the film surface area (2 cm × 2 cm). The samples were then dried in an oven to exclude any contamination of water vapor in the membrane. V_E, the existent volume of the membrane, was then determined through the polymer density and the membrane sample weight. At least six samples from each membrane were used to determine the porosity to confirm the reproducibility of the experiment data.[13,17]

Measurement of the membrane wicking flow

The liquid flow time (wicking time) measurement was conducted on 2 cm wide membrane strips cut from the synthesized NC membranes. The medium used for wicking was deionized water or phenol red buffer solution, which consisted of phenol red (Sigma) dissolved in 0.05*M* tris(hydroxymethyl)aminomethane/HCl buffer solution. These test mediums were chosen as they did not bind to the NC membrane. Phenol red buffer solution was used for better visibility of the migration front. The experiment was conducted at room temperature (27°C) and ambient pressure. Times were measured for the wicking medium to migrate to heights of 2, 3, and 4 cm standardwise after initial contact between the membrane and test medium.

Determination of the protein bound to the NC membrane

The NC membranes were cut into 12 mm diameter samples, and the total membrane volume was calculated. Membrane samples were incubated in 3 mL of BSA with phosphate buffer (pH 7.0, 3 mg/mL) and shaken for 3 h at 25°C. Samples were then washed (repeated two times) with phosphate buffer to remove unbound protein on the membrane surface. Each sample replicate was transferred into a test tube. Subsequently, 2.0 mL of the bicinchoninic acid working reagent (Merck) was added, and the test tubes were incubated at 37°C for 30 min. The liquid content was then sent for photometric measurement at a wavelength of 562 nm. With the preliminarily plotted standard curve, the recorded corrected absorbance readings for the samples were interpolated.

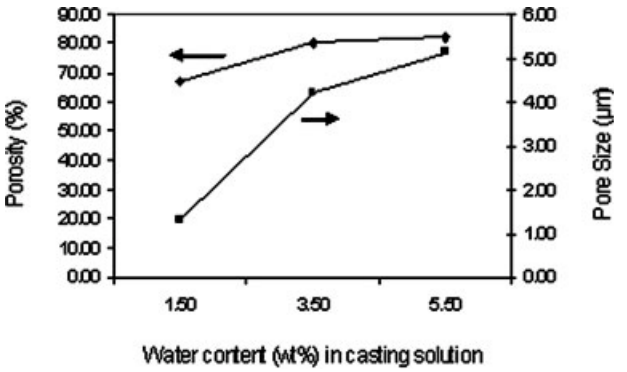

Figure 1 Effect of the water content on the membrane porosity and pore size.

RESULTS AND DISCUSSION

Effect of the water content on the membrane morphology

The effects of water on the NC membrane's porosity and pore size were determined by the preparation of various membranes with different water contents in the casting solutions. The differences in water content refers to samples M7, M8, and M9 in Table I. Figure 1 shows an apparent increase in the porosity with increasing water concentration in the casting solution. On the basis of the experimental results, the membrane porosities were 66.9, 80.0, and 82.3% for 1.5, 3.5, and 5.5 wt % water content in the casting solutions, respectively. The increasing membrane porosity was due to the low solubility of water compared to the solvent in the casting solution. When the water content in the casting solution was increased, it greatly decreased the homogeneity of the casting solution. Finally, this thermodynamically unstable casting solution enhanced the precipitation rate and formed a more porous membrane film.

Figure 1 shows that the average membrane pore size increased from 1.3 μm (1.5 wt % water content) to 5.1 μm (5.5 wt % water content). This was because the presence of water in the casting solution enhanced crystallization, and the gelation process took place during the drying of the cast films. The initial composition of the casting dope moved closer to the region of the liquid–liquid phase separation,

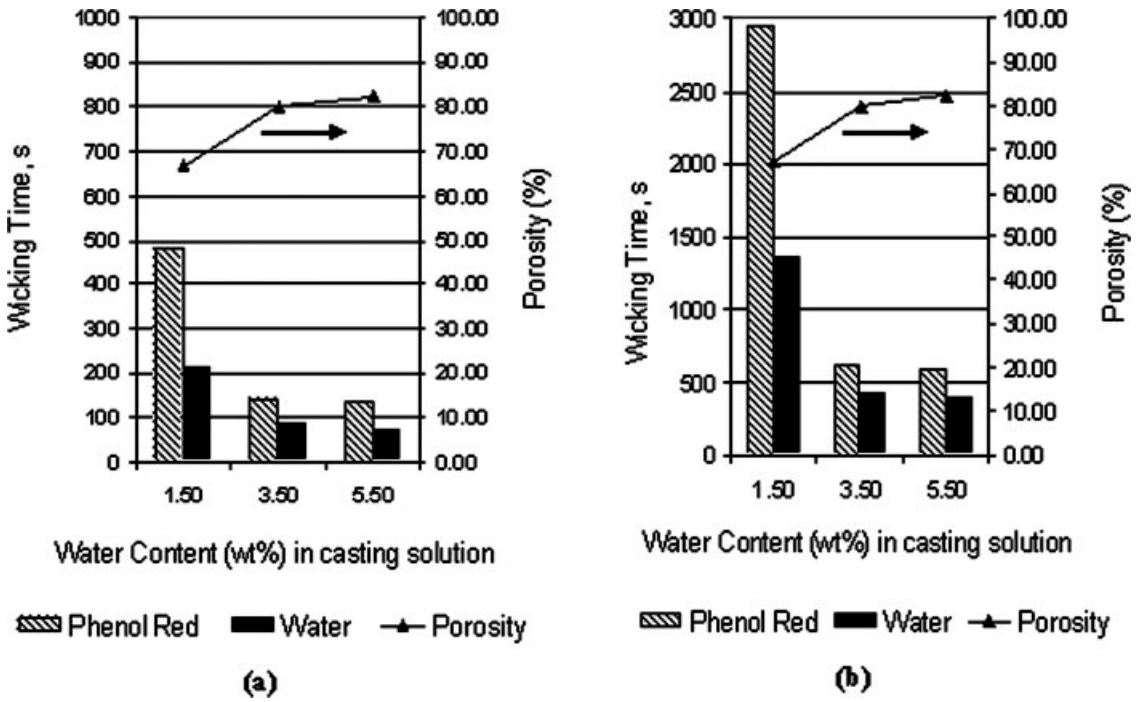

Figure 2 (a) Wicking time needed for the testing medium (distilled water and phenol red) to migrate (a) 2 and (b) 4 in heights on the membrane strip.

which resulted in bigger pores appearing on the membrane top layer. All of these data proved that the addition of water as pore former was a simple and effective way to manipulate the membrane surface and cross-section morphology. A similar observation was also reported by Young and Chen[5] during their wet-casting membrane-formation process.

Effect of the water content on the membrane lateral flow rate

The lateral flow wicking test was used because NC membranes are widely used to manufacture lateral flow devices. The data in Figure 2 show that an increase in porosity was directly correlated to a decrease in wicking time. Figure 2(a) indicates the wicking time needed for the test medium (deionized water or phenol red) to migrate 2 cm in height on the membrane strip, whereas Figure 2(b) shows the migration up to 4 cm in height. Both types of testing medium (water and phenol red) showed the same wicking trend, whereby the higher the membrane water content was, the shorter the required wicking time was.

As shown in Figure 2(b), the membrane with 1.5 wt % water content in the casting solution apparently took longer to migrate to 4 cm in height on the membrane strip compared to the membranes formed by the addition of 3.5 and 5.5 wt % of water in the casting solution. This difference indicated that the amount of water (1.5 wt %) used to prepare the membranes was not sufficient to generate porous structure in the membrane. The faster wicking ability of the NC membranes with 3.5 and 5.5 wt % water content in the casting solution was mostly due to the presence of interstitial moisture (higher water content in the casting solution) and porous spaces (higher membrane porosity) in the membrane layer. This finding from this study could be later applied to the manufacture of immunodiagnostic assays because assays use the lateral flow of fluids through a membrane for target analyte analysis, which is based on the membrane surface morphology and pore size.[3] The finding was supported by Greene and Tannenbaum,[20] who found that dependency of the membrane lateral migration rate relied solely on the porosity and pore size of the membrane.

On the other hand, due to the effects of gravity, wicking time from one point to another point on the membrane strip with the same distance was not strictly proportional. Longer wicking time was needed with an increase in the migration distance from gravity. As an example, shown in Figure 2(a), for the membrane with 3.5 wt % water content, the phenol red solution took 144 s to migrate 2 cm in height on the membrane strip. For the same membrane, the phenol red solution needed 615 s to migrate 4 cm in height on the membrane strip [Fig. 2(b)]. This implied that the wicking rates declined with an increase in wicking distance from gravity.

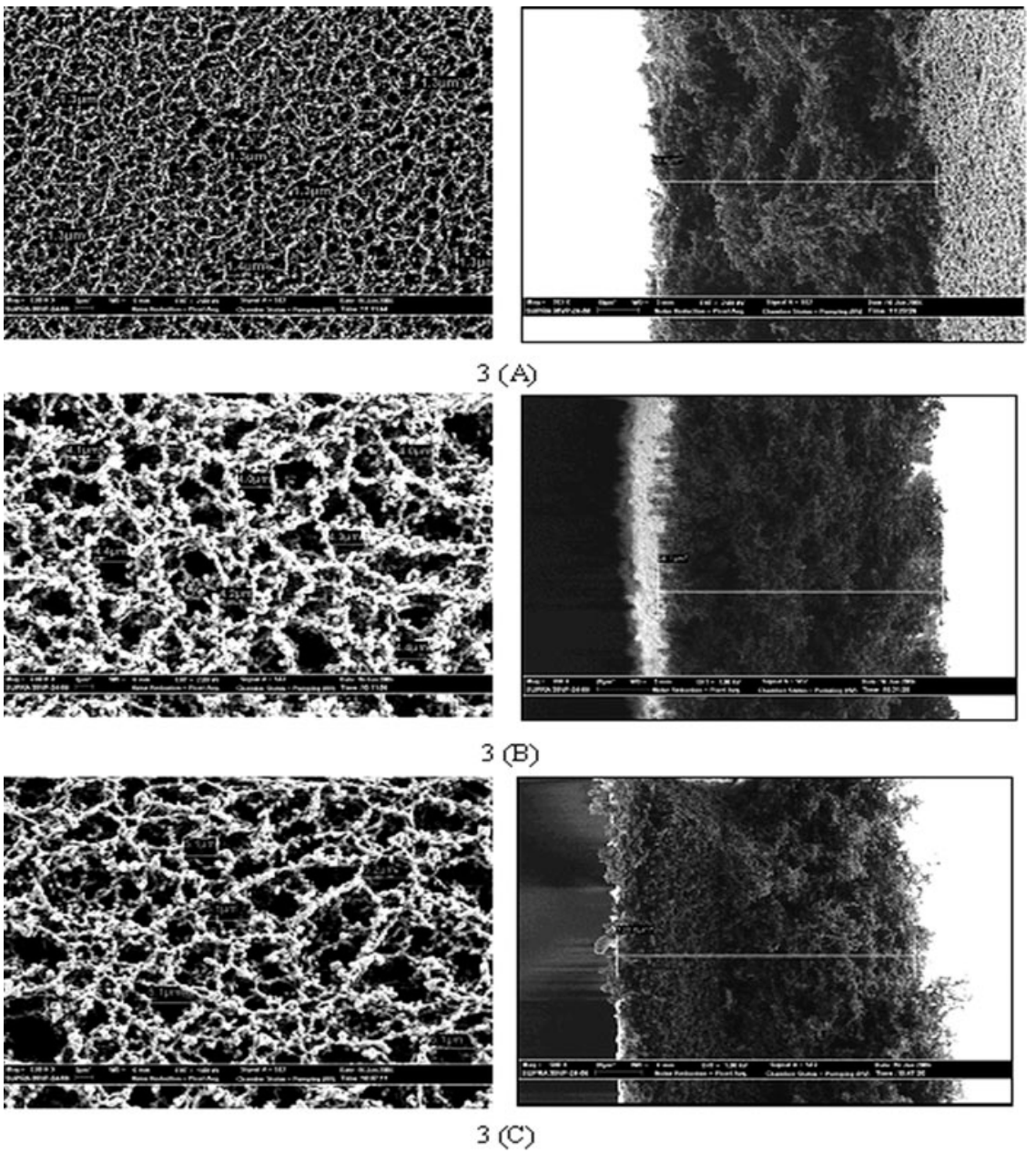

Figure 3 FESEM micrograph of the (left) membrane surface structure and (right) thickness for water contents of (A) 1.5, (B) 3.5, and (C) 5.5 wt % in casting solutions.

FESEM observation of the membrane morphology for different water contents during casting

FESEM was used to obtain information on the surface pore size and the morphology of the NC membranes. Pore distribution and the thickness of the membranes by different amounts of water in the casting solutions were observed. Membrane prepared with addition of 1.5 wt % water [Fig. 3(A)] in the casting solution showed a smooth surface, with small pores finely distributed homogeneously all over the membrane surface. Meanwhile, the addition of 3.3 wt % [Fig. 3(B)] and 5.5 wt % [Fig. 3(C)] water in the casting solution showed bigger and interconnecting pores in the membrane surface. This implied that increasing water concentration in the casting solution formed more porous NC membranes. Meier et al.[13] observed a similar trend by using water as pore former in the production of microporous cellulose acetate membranes for controlled drug permeation.

As shown in the FESEM micrographs, the higher the concentration of water in the casting solution was, the more significant the increase in membrane pore size was [Fig. 3(B,C)] but at the expense of the membrane surface becoming rougher.

It was expected that the membrane thickness was smaller than the initial cast thickness because of sol-

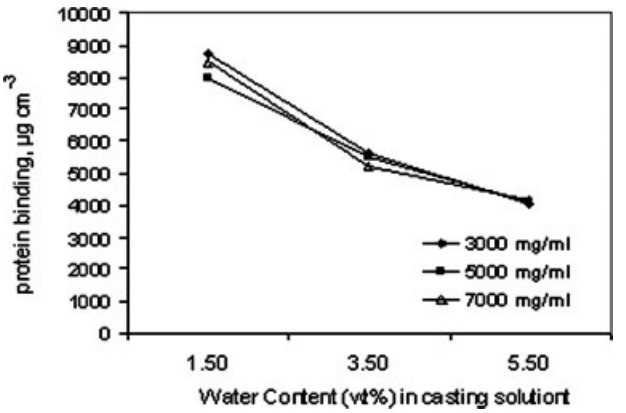

Figure 4 Effect of the water content on the membrane protein-binding ability.

vent loss during membrane formation. However, a more porous membrane would increase the thickness of the membrane. With the same initial casting thickness, the final membrane thicknesses were increased from 69.4 to 94.1 and 130.6 μm for 1.5, 3.5, and 5.5 wt % water, respectively.

Effect of the water content on the membrane binding ability

In immunodiagnostic tests, proteins are the most common sample applied to a solid membrane surface. The interaction of proteins with membrane pore structures can substantially influence the effectiveness of such application.[16]

Protein binding is primarily influenced by pore size. Figure 4 shows that whenever the water content in the casting solution was increased (where the membrane pore size was increased), the protein-binding capacity of the membrane decreased. This was due to the relative decrease in the availability of the membrane surface area. For the membrane incubated in 3000 ppm BSA solution, the membrane binding capacity dropped from 8744 (1.3 wt % water) to 5613 (3.3 wt % water) and 4045 μg/cm^3 (5.3 wt % water). This was because membranes formed by higher water content in the casting solution had bigger pores and, subsequently, decreased the total membrane surface area necessary for binding to take place. As long as the incubation time was enough for BSA solution adsorption, there was not much difference in the binding capacity for different protein solution concentrations ranging from 3000 to 7000 ppm.

Effect of different additives on the membrane morphology

Additives, such as glycerol, PEG, methylcellulose, water, and SHS, in the casting solution have been reported as being used to improve the mechanical properties of the polymer matrix[13] and to radically change the membrane morphology[21] to enhance membrane performance.

Figure 5 shows that the membrane containing glycerol exhibited the highest porosity (80%) followed by membrane containing different molecular weights of PEG and SHS. This was due to the fact that glycerol with the smallest molecular size could easily diffuse during the membrane-formation process. The average porosity of the membrane was effectively enhanced by the introduction of a small amount of glycerol in the casting dope. This meant that the addition of glycerol in the NC membrane casting solution served not only as plasticizer but also as a pore former in the membrane-formation process.

Generally, PEG has been used to increase membrane porosity. PEG 3000 (M6) was capable of creating a greater porosity than PEG 600 (M4), where the porosities were 78 and 77.3%, respectively. However, in our observations, PEG 200 (M1) reduced the membrane porosity compared to the membrane without any additive (M3) introduced into the casting dope. The synthesized membrane without any additive was able to form 76.9% porosity, whereas the membrane with PEG 200 (M1) was only able to form 73.7% porosity during membrane formation. With the addition of PEG 200 in the casting dope (M1), a thin layer was formed between the cast solution and the air interface. Hence, the solvent in the cast solution could diffuse out at a higher rate compared to the water vapor from air inflow into the membrane during dry phase inversion. Smaller and closer pores were formed on the membrane surfaces. In other words, PEG 200 was not capable of working as a pore-forming agent but only as a pore-reduction agent. This finding was supported by Kim and Lee,[22] who used PEG 200 as an additive in the formation of poly(ether imide) asymmetric membranes.

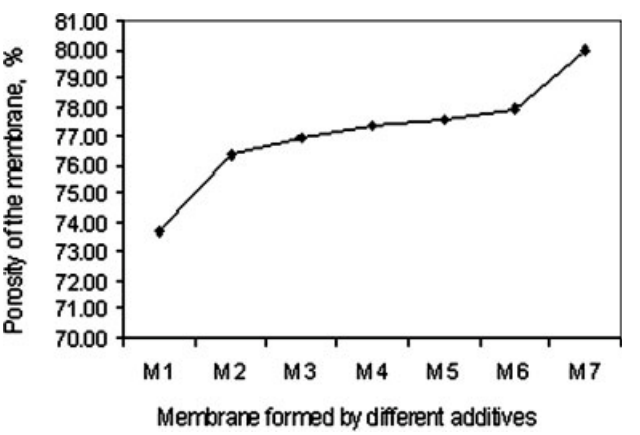

Figure 5 Effect of different additives on the membrane porosity.

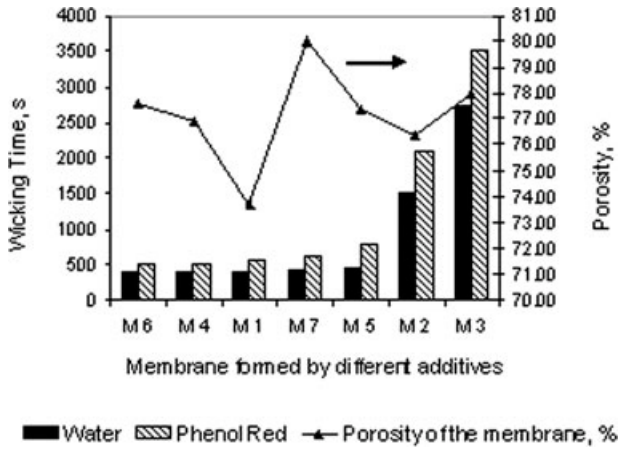

Figure 6 Effect of different additives on the membrane wicking time per 4-cm height of the membrane strip.

Compared to the membrane without any additives (M3), M2 showed a lower porosity (76.4%), but the porosity of M5 (77.6%) was higher than that of M3. According to Alsari et al.,[23] SHS concentrations below critical micelle concentration lower the porosity of the membrane. In contrast, SHS concentrations above the critical micelle concentration enhance the membrane surface porosity. In this study, the concentration of SHS used in M5 was higher than that of M2. The increase in membrane porosity in M5 compared to that in M3 (the membrane with no additive included) may have been due to the fact that the concentration of SHS was above the critical micelle concentration. In other words, M2 with a lower SHS concentration was below the level of critical micelle concentration.

Effect of different additives on the membrane lateral flow rate

Figure 6 shows a series of capillary rise times by deionized water and phenol red solution for membranes with different choices of additives. As shown in Figure 6, an increase in porosity had no direct correlation with wicking time for different types of additives introduced into the membrane casting solution. The membrane with PEG 3000 (M6) showed the fastest capillary rising time, followed by the membranes with PEG 600 (M4), PEG 200 (M1), glycerol (M7), SHS 2.0 (M5), and SHS 0.5 (M2) and without additive (M3). This implied that the membrane wicking time was not only governed by the pore size and porosity of the membrane but also largely by the different properties of the individual additives.

As shown in Figure 6, for the same kind of additives added into the casting solution, the wicking time of the membrane was proportionate to the membrane surface porosity. The wicking times of the NC membranes with PEG [PEG 200 (M1), PEG 600 (M4), and PEG 3000 (M6)] added as additives into the casting solution decreased when the porosity of the membrane increased from 73.70% [PEG 200 (M1)] to 77.60% [PEG 3000 (M6)]. This finding provided evidence that minor variations in the pore size and porosity of the membranes could have had significant impacts on the migration rate.

On the other hand, the capillary rising time for the membrane with no additive was slow (2744 s) for deionized water and 3504 s for phenol red solution to migrate 4 cm in height on the membrane strip. This corresponded to the literature result in which the absence of additive reduced the wicking rate, which thus caused more time to be required to achieve a specific migration front for both water and phenol red as the test media.[5] The overall observations discussed in this section mean that additives are important elements in the membrane-formation process, where they could modify the membrane morphology effectively and enhance the performance of membranes to be used as a lateral flow membranes used in immunological assays.

Effect of different additives on the membrane binding ability

As shown in Figure 7, the protein-binding capacity was highest for the casting solution containing glycerol (M7) followed by the membranes with PEG 200 (M1), SHS 0.5 (M2), PEG 600 (M4), SHS 2.0 (M5), no additives (M3), and PEG 3000 (M6). The membrane containing glycerol (M7) used as wetting agent and pore former exhibited a higher protein-binding capacity (5613 $\mu g/cm^3$) for 3 h of membrane incubation in BSA solution. Glycerol is generally used to improve the mechanical properties of the polymer

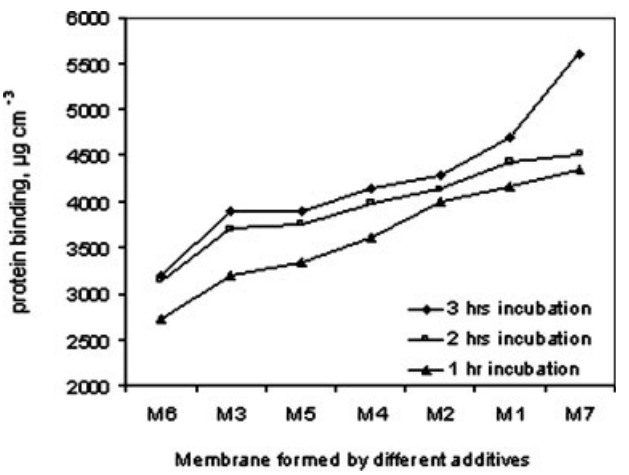

Figure 7 Effect of different additives on the membrane protein-binding ability.

matrix by weakening the intermolecular forces between the polymer chains.[13] This could ultimately increase the free volume of the membrane for protein binding. The membranes containing SHS (M2 and M5) gave lower protein bindings compared to the membrane containing glycerol (M7). The membranes with SHS as surfactant were only able to bind 3902 $\mu g/cm^3$ (M5) and 4291 $\mu g/cm^3$ (M2) of protein for 3 h of membrane incubation in BSA solution. This was primarily due to the role of the surfactant as a blocking agent, which reduced the nonspecific interactions and potentially reduced the protein-binding capacity. In addition, surfactants also deterred the capture reagents from binding firmly onto the membrane.

NC membranes with cast solutions containing PEG showed smaller protein-binding capacities compared to membranes with glycerol or SHS as additives. This was mainly due to the fact that manmade pore formers such as PEG are known to interfere with protein binding. Their mode of action may be a combination of effects that inhibits one or more of the forces essential for protein–membrane binding.[21]

The experimental data in Figure 7 show that membrane binding capacities were 3203, 4139, and 4694 $\mu g/cm^3$ (3 h of membrane incubation in BSA solution) for NC membranes containing PEG 3000, PEG 600, and PEG 200, respectively. These results suggest that when the same type of additive (PEG) is introduced but with different molecular weights, the membrane binding capacity seemed to depend on the membrane pore size and porosity rather than the wettability of the membrane. The overall membrane porosity and pore size increased with increasing molecular weight of PEG additive. As pore size increased, the membrane protein-binding capacity decreased because of the related decrease in the availability of the membrane surface area for binding. Also, the longer the protein–membrane samples were incubated in the shaker, the higher the protein-binding capacity was. This implied that the membrane had to have a sufficient membrane incubation time for the binding mechanism to take place.

CONCLUSIONS

Thin-film lateral flow NC membranes were prepared via dry-phase-inversion method with different amounts of water and different kinds of additives. These additives acted as wetting agents and plasticizers, which effectively controlled the morphology and performance of the membranes. In this study, increasing water content in the casting solution (samples M7, M8, and M9) resulted in increasing membrane pore sizes and porosities but rougher membrane surfaces. Higher water contents in the casting solution contributed to faster membrane wicking speeds or shorter wicking times but at the expense of membrane protein-binding abilities. Also, different properties of the additives (glycerol, PEG, and SHS) in samples M1–M7 gave different membrane performances and morphologies. Membrane performances in wicking time and binding ability were not only governed by the membrane surface and cross-section morphology but were also largely affected by the different properties of the individual additives. An increase in membrane porosity had no direct correlation with the membrane lateral wicking time and binding ability for the different types of additives introduced into the membrane casting solution. Among all of the additives used in this study, glycerol was chosen as the most suitable additive in NC membrane formulation because it was able to improve the membrane performances for both lateral wicking time and protein-binding ability. The effects of additives and pore formers were proven to be a practical method for manipulating the final membrane structures and performances of the membranes. In this study, the optimum lateral flow membrane performance was obtained by preparation of a membrane with glycerol as an additive and 3.5% water content in the casting solution.

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