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Abstract: A mixture-process design methodology, i.e., the crossed design, is proposed for experimental analysis and optimization. Five mixture materials for membrane formulation and two process factors for casting condition were fixed in the design methodology. The study was to generate a regression model for each of the responses, based on the experimental data and analysis variance of the study. Based on the response models, the optimal blend and casting condition were predicted. The highest desirability function, D, which prevailed from the optimization was 0.66. This optimal blend composition of the cast solution and the process condition in crossed design is proven to increase the membrane performance through the high membrane porosity, the high protein binding ability, and the fast lateral wicking rate.

Keywords: Crossed design, membrane, methodical analysis, mixture, optimization

INTRODUCTION

Lateral flow polymeric membrane is the key element in producing medical and healthcare analysis devices, such as rapid diagnostic test strip (1,2). In diagnostic test strips, different polymer materials, surface

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properties, structure, and dimensions are able to influence various test methods such as the lateral flow immuno-chromatography test (1), bacteria filter binding assay (2) and protein immobilization (3). Hence, by controlling the membrane structure (4) and morphology, various immunological analysis could be performed effectively and accurately.

Among the membranes used in the immunological analysis, nitrocellulose (NC) has gained importance in biomedical application, due to their ability in high binding capacity and large void volume between the membrane pores. This feature offers good accessibility and large surface area for potential adsorption of protein molecules. As compared to the analogous membrane, such as cellulose acetate or cellulose triacetate membrane, NC membrane is able to reach the required membrane's sensitivity level that applied in the immunoassay, where the NC membrane is capable of binding 50–80 $\mu g/cm^2$ of single-stranded DNA while the cellulose acetate membrane only binds 1 $\mu g/cm^2$ (5,6).

In immunological application, pure NC membrane has the optimal protein binding capacity in the range of 50 to 80 $\mu g/cm^2$ (5,6). The membrane is targeted to have as high a porosity as possible in order to increase the interconnection between the membrane pores and consequently hasten the lateral wicking speed of the membrane. In order to get those membrane's characteristics, there is a need to understand the membrane formation mechanism, which is based on the casting formulation and the casting conditions that are used in the fabrication process (4). The formation mechanism was said to be rather complex as there are various fabrication factors to be considered during the casting stage (7), for example, mixture factors that should be taken into account including the choices of casting materials, composition of casting materials and the gelation-crystallization behaviour of the polymer (5,8–10). At the same time, some of the casting process factors such as the surrounding humidity, casting speed, casting thickness, evaporation time, and the drying temperature have to be considered simultaneously (11,12). By manipulating phase transition during the initial stage of casting blends and the casting process factors, the membrane morphology can be controlled and the porous membranes can be prepared at the desired pore size, porosity, thickness, and surface roughness (13–15).

In order to overcome the complexity of membrane formation mechanism, an accurate statistical analysis method is needed to analyze the factors that can affect membrane formation. Some researchers have tried to optimize their membrane formulation (4,16,17) and performances (18,19) by using mathematical processing methods such as mixture and factorial design. In mixture experimental design, the total number of the components is held constant. The responses of synthesized membranes vary when the proportions of the blends change. However, mixture design considers

the mixture formulation without concerning the process factors. It does not take into account any process factor that purportedly affects the membrane formation. To date, there are no reports on membrane optimization which consider both the mixture and the casting process factors.

It is nearly impossible to mull over all the varieties of mixture components and casting processes with conventional experimental study on membrane formation. Designing an experiment that is able to optimize the membrane performance with a minimum experimental run is greatly desired. Lee and Gilmore (20) proposed a more accomplished design method, i.e., crossed design to analyze the polyhydroxyalkanoates production. Crossed design is a design method that combined both mixture and process factors. It minimizes cost and the time frame of study while providing information-rich data and analysis (20–22). The comprehensive crossed mixture-process design is able to calculate the complex interaction between compositional variables with process factors (20). Other successful applications of this crossed design method in solving formulation problems were documented (19,23,24). In other words, the crossed design method consumes less time and resources compared to conventional experimental work, and ultimately provide an ample amount of information with a minimal experiment run (23).

The current study elucidates the crossed design that combines mixture components (polymer, solvent, nonsolvent, glycerol, and water) and process factors (evaporation time and drying temperature). This experimental design is aimed to illustrate the interaction between membrane performances against the mixture solution and membrane casting condition, in order to improve their performances as lateral flow membrane in medical analysis. The paper emphasized as to how the membrane performance can be affected by changing the composition of casting solution as well as changing the evaporation time and drying temperature. Two major membrane performances of concern are the membrane protein binding ability for diagnostic analysis and the membrane wicking ability which allow the wicking medium to be transported laterally across the membrane to reach a diagnostic analysis control line. The final regression models obtained from this study are expected to be able to pinpoint the optimal membrane cast solution with advantageous casting process parameters.

EXPERIMENTAL

Membrane Preparation

The raw materials involved in membrane formulation are nitrocellulose (NC) polymer with 11.8–12.3% nitrate and 30% of alcohol, methyl

acetate (MA) (the solvent), iso-propanol (IP) (non-solvent), glycerol, and water. The membranes were prepared using dry phase inversion method according to the design blends and process factors as stated in Table 1. Dry phase inversion method refers to the process which a polymer solution (liquid phase) inverts into a swollen three-dimensional macromolecular network (solid state) (5,25). The casting process was carried out in ambient temperature and performed by using a Membrane Auto Casting Machine where the casting solutions were cast onto a glass plate by means of a casting blade. The solvent in the initial cast membrane was evaporated according to the evaporation time as listed in Table 1. After solvent evaporation, the membrane was thoroughly dried in accordance with the drying temperature as tabulated in Table 1. Explicit details on materials and membrane preparation work can be referred from the previous work (9,11).

Determination of Membrane Porosity and Yield Thickness

The porosity of the synthesized membranes was calculated based on an equation provided by Yamane et al. and Meier (14,26).

$$\varepsilon = \frac{V_A - V_E}{V_A} \times 100\% \tag{1}$$

The apparent volume of the membrane, V_A, was calculated from the film thickness and the film surface area (2 cm × 2 cm), where the membrane thickness was determined by using a micro thickness gauge (Mitutoyo 7301, Japan). The samples were then dried in an oven to exclude any contamination of water vapor in the membrane. V_E, the existing volume of the membrane, was determined through the polymer density (1.23 g cm^{-3}) and the membrane sample weight (9,11). At least six samples from each membrane were used to determine the porosity to confirm the reproducibility of the experiment data.

Measurement of Membrane Wicking Flow

Synthesised membranes were cut into 2 cm wide and 10 cm long strips for in-plane liquid distribution or lateral liquid wicking time measurement. Deionized water was used as the wicking medium. The experiment was conducted at room temperature (27°C) and at ambient pressure. Time measurement started when the wicking medium had migrated 4 cm along the membrane strip after initial contact between the membrane and the deionized water (i.e., the wicking medium) (9,11).

Table 1. Design layout and responses for mixture-process crossed-design

Run	Mix: A: x_1 Polymer NC actual (g) %	Mix: B: x_2 Solvent MA actual (g) %	Mix: C: x_3 Non-solvent IP actual (g) %	Mix: D: x_4 Water actual (g) %	Mix: E: x_5 Glycerol actual (g) %	Process: F: z_1 Drying temperature actual °C	Process: G: z_2 Evaporation time actual min	Resp. 1 Y_1 Protein binding μg/cm^3	Resp. 2 Y_2 Wicking time sec/4 cm	Resp. 3 Y_3 Porosity %	Resp. 4 Y_4 Yield thickness μm
1	4.00	75.00	15.00	4.00	2.00	27.00	5.00	4783	472	72	96
2	4.00	78.50	12.50	4.00	1.00	40.00	5.00	4688	643	74	91
3	4.00	75.00	15.00	4.00	2.00	40.00	5.00	5283	550	71	101
4	5.00	81.00	10.00	2.00	2.00	27.00	5.00	5864	533	70	120
5	5.00	76.50	12.50	4.00	2.00	30.25	1.25	3945	381	74	122
6	4.00	79.50	12.50	2.00	2.00	27.00	0.00	4835	410	75	127
7	5.00	81.00	10.00	2.00	2.00	36.75	1.25	4400	668	74	117
8	4.00	79.50	12.50	2.00	2.00	40.00	5.00	6535	941	68	91
9	4.00	79.50	12.50	2.00	2.00	40.00	5.00	6938	964	68	91
10	4.50	78.50	12.50	3.00	1.50	40.00	0.00	4451	665	75	116
11	5.00	76.00	15.00	2.00	2.00	40.00	0.00	5024	819	74	116
12	5.00	76.00	15.00	3.00	1.00	27.00	5.00	5015	523	76	109
13	4.00	83.00	10.00	2.00	1.00	27.00	0.00	4020	393	78	128
14	5.00	75.00	15.00	4.00	1.00	27.00	0.00	3455	481	79	120
15	5.00	80.00	10.00	4.00	1.00	40.00	0.00	3064	323	77	114
16	4.50	76.50	15.00	2.00	2.00	27.00	0.00	5096	511	75	117
17	5.00	74.00	15.00	4.00	2.00	27.00	0.00	4053	302	79	132
18	4.50	75.50	15.00	4.00	1.00	40.00	0.00	4171	708	76	109
19	5.00	74.50	15.00	4.00	1.50	40.00	5.00	4251	808	75	104

20	4.50	75.50	15.00	4.00	1.00	27.00	5.00	4143	500	77	107
21	4.00	78.50	12.50	4.00	1.00	27.00	0.00	4010	454	78	115
22	4.25	77.25	13.75	3.50	1.25	33.50	2.50	4354	690	75	102
23	4.50	78.50	12.50	3.00	1.50	27.00	5.00	4676	428	75	108
24	4.00	80.50	12.50	2.00	1.00	30.25	3.75	4776	637	76	97
25	4.00	80.00	10.00	4.00	2.00	27.00	5.00	4752	440	70	89
26	4.00	82.00	10.00	2.00	2.00	27.00	5.00	5798	502	69	92
27	4.50	75.50	15.00	4.00	1.00	40.00	0.00	4245	731	77	113
28	5.00	80.00	10.00	4.00	1.00	27.00	5.00	4441	500	75	106
29	5.00	76.00	15.00	2.00	2.00	40.00	0.00	5687	1027	73	100
30	5.00	79.50	12.50	2.00	1.00	40.00	0.00	4690	1006	72	97
31	4.00	78.50	12.50	4.00	1.00	40.00	0.00	3662	513	77	109
32	5.00	74.50	15.00	4.00	1.50	40.00	0.00	4066	715	77	117
33	5.00	76.50	12.50	4.00	2.00	40.00	5.00	4153	641	74	116
34	5.00	80.00	10.00	4.00	1.00	40.00	5.00	3678	511	77	111
35	4.00	78.00	15.00	2.00	1.00	27.00	0.00	5068	690	76	100
36	5.00	76.50	12.50	4.00	2.00	40.00	0.00	3461	486	77	128
37	5.00	81.00	10.00	2.00	2.00	40.00	5.00	6029	985	72	115
38	5.00	76.00	15.00	3.00	1.00	40.00	5.00	5258	935	74	100
39	4.00	78.00	15.00	2.00	1.00	27.00	5.00	5127	755	71	84
40	4.00	75.00	15.00	4.00	2.00	40.00	0.00	5320	550	74	121
41	5.00	79.50	12.50	2.00	1.00	40.00	5.00	5656	1109	72	93
42	5.00	76.00	15.00	3.00	1.00	40.00	0.00	4581	867	74	106
43	4.00	83.00	10.00	2.00	1.00	40.00	0.00	4722	609	75	92
44	4.00	80.00	10.00	4.00	2.00	27.00	0.00	3970	295	75	121
45	5.00	76.50	15.00	2.00	1.50	27.00	0.00	4519	558	77	120

(*Continued*)

Table 1. Continued

Run	Mix: A: x_1 Polymer NC actual (g) %	Mix: B: x_2 Solvent MA actual (g) %	Mix: C: x_3 Non-solvent IP actual (g) %	Mix: D: x_4 Water actual (g) %	Mix: E: x_5 Glycerol actual (g) %	Process: F: z_1 Drying temperature actual °C	Process: G: z_2 Evaporation time actual min	Resp. 1 Y_1 Protein binding $\mu g/cm^3$	Resp. 2 Y_2 Wicking time sec/4 cm	Resp. 3 Y_3 Porosity %	Resp. 4 Y_4 Yield thickness μm
46	4.00	83.00	10.00	2.00	1.00	33.50	2.50	4710	695	76	100
47	4.00	80.00	10.00	4.00	2.00	40.00	0.00	4688	463	73	114
48	5.00	74.50	15.00	4.00	1.50	40.00	0.00	3577	687	78	127
49	4.00	78.00	15.00	2.00	1.00	40.00	5.00	6284	1200	70	84
50	5.00	79.50	12.50	2.00	1.00	27.00	0.00	3600	550	78	127
51	4.00	80.00	10.00	4.00	2.00	40.00	0.00	4697	423	73	117
52	4.50	78.50	12.50	3.00	1.50	27.00	0.00	4688	446	75	118
53	5.00	81.00	10.00	2.00	2.00	27.00	0.00	4611	390	72	125
54	5.00	76.00	15.00	2.00	2.00	40.00	5.00	6426	1254	69	94
55	4.00	83.00	10.00	2.00	1.00	27.00	5.00	5071	620	75	95

Determination of Protein Binding Ability

The synthesised membranes were cut into 12 mm diameter samples and the total membrane volume was calculated. Membrane samples were incubated in 3 ml of Bovine Serum Albumin (BSA) with phosphate buffer (pH 7.0, 3 mg mL^{-1}) and shaken for 3 hours at 25°C. Unbound BSA on the membrane surface was then washed out using a phosphate buffer (repeated two times). Each sample replicate was transferred into a test tube. Subsequently, 2.0 ml of Bicinchoninic Acid (BCA) working reagent was added, and the test tubes were incubated at 37°C for 30 minutes. The BSA concentration was detected using a spectrophotometer (Spectronic Genesys, USA) at 562 nm wavelength. With the preliminarily plotted standard curve, the corrected absorbance readings for the samples were interpolated.

Statistical Approach – Crossed Design

Before the experiment was designed, the materials suitable for membrane formation (9) and the casting parameters (11) were selected from preliminary study. In this study, five membrane casting components combined with two process variables (membrane evaporation time and drying temperature) were evaluated. Both the mixture and the process factors were interconnected, which rendered a total of 55 experimental runs, as shown in Table 1. The experimental run selection points are based on a D-optimal mathematical algorithm that minimizes the volume of the confidence ellipsoid for the coefficients. The crossed design uses the CONVERT algorithm to find the vertices (27). Four responses which were taken into consideration consist of membrane porosity, membrane wicking time, membrane protein binding ability, and final membrane thickness. The crossed design programme (Design Expert^{TM}, version 6.0.6) was used to analyze the experiment data and to optimize the membrane formulation with process factors. The selected model has the highest polynomial order with significant terms and the model is not aliased (23).

RESULTS AND DISCUSSION

ANOVA and Regression Analysis

Table 1 shows the design layout in terms of the actual component values, the actual process factor values, and the responses for each experiment. All experimental data were mathematically processed and some experimental response models were produced.

First analysis to be considered is based on the ANOVA output. It produces statistics such as Sum of Squares (SS), Mean Square (MS), Estimate Coefficients, Standard Error, F values and Prob > F to fit the models in crossed design, as shown in Tables 2 to 5. From the analysis, $|Linear \times Linear|$ model was chosen to fit the five mixture components and two process factors of this study. Every individual and its interaction effects have a single degree of freedom and their SS values were computed as tabulated in Tables 2 and 4. These SS values were then used to calculate F-test which considers the model's reliability and feasibility (16).

$$F = \frac{MS_{Effects}}{MS_{Error}} \tag{2}$$

where, F is the F-test values, $MS_{Effects}$ is the variance between the model's coefficients, and MS_{Error} is the standard deviation between model and experimental value. F-value of 30.52 for membrane protein binding ability (Table 2) and 33.78 for membrane wicking time (Table 4) implies that these models are significant. For such F-values, there is 99.99% chance that the developed model is noise-free.

Besides, values of "Prob > F" less than 0.05 is desirable for the model to be significant. The 45 out of 55 experimental runs were used to estimate the "Lack of Fit" of the experiment. The insignificant values shown in Table 2 (0.93 for protein binding ability) and Table 4 (1.58

Table 2. ANOVA, variance analysis and regression model from crossed design for membrane protein binding ability

	Sum of squares (SS)	Mean square (MS)	F value	Prob > F	
Model	3.382E+07	3.382E+06	44.44	<0.0001	Significant
Linear mixture	2.477E+07	6.193E+06	81.37	<0.0001	
x_1 z_1	7.675E+05	7.675E+05	10.09	0.0027	
x_1 z_2	1.553E+06	1.553E+06	20.41	<0.0001	
x_2 z_1	1.968E+06	1.968E+06	25.86	<0.0001	
x_2 z_2	2.091E+06	2.091E+06	27.47	<0.0001	
x_3 z_1	2.131E+06	2.131E+06	28.01	<0.0001	
x_4 z_1	5.383E+05	5.383E+05	7.07	0.0109	
Residual	3.349E+06	7.610E+04			
Lack of Fit	2.925E+06	7.500E+04	0.89	0.6391	Not Significant

ANOVA for Crossed Linear (Mixture: x_1, x_2, x_3, x_4, x_5) x Linear (Process: z_1, z_2) Model.

Table 3. ANOVA and variance analysis for membrane protein binding ability

Component	Coefficient estimate (real component, actual factor)	Standard error	
x_1-Polymer	98550.02	728.09	
X_2-Solvent	−902.98	91.80	
X_3-Nonsolvent	−16250.47	148.80	
X_4-Water	21874.94	348.01	
X_5-Glycerol	62842.49	697.92	
X_1 z_1	−4558.20	751.86	
X_1 z_2	6001.99	619.51	
X_2 z_1	230.40	91.64	
X_2 z_2	−155.50	90.62	
X_3 z_1	949.47	151.08	
X_4 z_1	−2057.74	354.51	
R-Squared	0.9099	Adj R-Squared	0.8894
Adeq Precision	32.229	Pred R-Squared	0.8593

for membrane wicking time) confirmed the reliability of the best-fit model equations for membrane performances.

It can be observed from Table 2 that the interaction between the cast solution blends and the drying temperature has more significant terms compared to the interaction between the cast solution blends and the

Table 4. ANOVA, variance analysis and regression model from crossed design for membrane wicking time analysis

	Sum of squares (SS)	Mean square (MS)	F value	Prob > F	
Model	939.17	93.92	46.63	<0.0001	Significant
Linear mixture	710.29	177.57	88.16	<0.0001	
x_1 z_1	11.77	11.77	5.84	0.0198	
x_2 z_1	45.05	45.05	22.37	<0.0001	
x_2 z_2	67.16	67.16	33.34	<0.0001	
x_3 z_1	66.93	66.93	33.23	<0.0001	
x_4 z_1	26.00	26.00	12.91	0.0008	
x_5 z_1	11.97	11.97	5.94	0.0189	
Residual	88.62	2.01			
Lack of Fit	81.99	2.10	1.59	0.3227	Not significant

ANOVA for Crossed Linear (Mixture: x_1, x_2, x_3, x_4, x_5) x Linear (Process: z_1, z_2) Model.

Transform: Square root.

evaporation time. Figure 1 further strengthen this observation, where it clearly shows that there are more interaction points between the drying temperature lines (Fig. 1a) compared to the evaporation time lines (Fig. 1b). This implies that the changes in the drying temperature can contribute to different membrane performances, in either increasing or decreasing of the protein binding ability. In contrast, the changes in the evaporation time will results in one trend of the membrane performance,

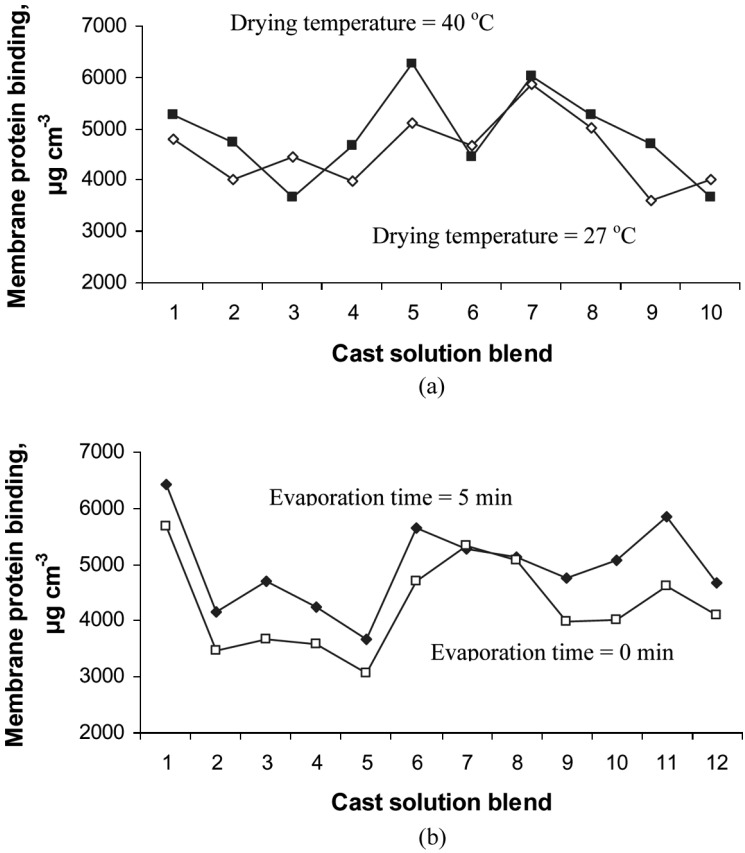

Figure 1. Interaction between different cast solution blends and (a) drying temperature (b) evaporation time for membrane protein binding. In (a) cast solution blend 1: run 1 & 3, 2: run 13 & 43, 3: run 28 & 34, 4: run 44 & 47, 5: run 39 & 49, 6: run 52 & 10, 7: run 4 & 37, 8: run 12 & 38, 9: run 50 & 30, 10: run 21 & 31. In (b) cast solution blend 1: run 4 & 53, 2: run 33 & 36, 3: run 2 & 31, 4: run 55 & 13, 5: run 19 & 48, 6: run 39 & 35, 7: run 25 & 44, 8: run 34 & 15, 9: run 54 & 29, 10: run 41 & 30, 11: run 3 & 40, 12: run 23 & 52.

or in other words, for the same membrane casting solution, the increase of the evaporation time will increase the membrane protein binding ability.

To predict respective membrane performances, the responses' regression models were generated by crossed design. The generated models are fitted to the experiment data to predict optimal condition. In the crossed design, the interaction model was considered.

$$Y = f(x_1, x_2, x_3, x_4, x_5) \quad g(z_1, z_2) + e \tag{3}$$

Y is the responses of the study, $f(x_1, x_2, x_3, x_4, x_5)$ represents the blending properties of the cast solution, $g(z_1, z_2)$ refers to the membrane casting process factors and e is the estimated error in the models. The final response models for the protein binding and membrane wicking time obtained from the current crossed design in terms of actual components and actual factors were shown below:

Membrane protein binding model:

$$\begin{aligned} Y_1 = {} & 98550.02 \times x_1 - 902.98 \times x_2 - 16250.47 \times x_3 + 21874.94 \\ & \times x_4 + 62842.49 \times x_5 - 4558.20 \times x_1 \times z_1 + 6001.99 \\ & \times x_1 \times z_2 + 230.40 \times x_2 \times z_1 - 155.50 \times x_2 \times z_2 + 949.47 \\ & \times x_3 \times z_1 - 2057.74 \times x_4 \times z_1 \end{aligned} \tag{4}$$

Membrane wicking time model:

$$\begin{aligned} SquareRoot\ (Y_2) = {} & -340.73 \times x_1 + 28.17 \times x_2 - 3.41 \times x_3 + 332.71 \\ & \times x_4 - 485.50 \times x_5 + 11.54 \times x_1 \times z_1 - 0.26 \times x_2 \times z_1 + 2.60 \times x_2 \times z_2 \\ & + 3.35 \times x_3 \times z_1 - 15.78 \times x_4 \times z_1 + 11.10 \times x_5 \times z_1 \end{aligned} \tag{5}$$

Models obtained from Eqs. (4) and (5) provide information regarding mixture components and process factors which influence the synthesised membrane performances and responses. To confirm the reliability of the regression models, standard errors displayed in Tables (3) and (5) explained the regression deviation related to the estimated coefficients for the membrane protein binding ability (Eq. (4)) and wicking time (Eq. (5)). 95% confidence interval level was set, where the model's coefficients are believed to fit well within 95% of the experiment data.

The R^2, Predicted R-Squared (Pred-R^2), and Adjusted R-Squared (Adj-R^2) must be in reasonable agreement before any regression models can be accepted. In the ANOVA model, the R^2 is equal to 0.9099 for protein binding ability and 0.9138 for membrane wicking time. Besides that,

Table 5. ANOVA and variance analysis for membrane wicking time analysis

Component	Coefficient estimate	Standard error	
X_1-Polymer	−340.73	3.73	
X_2-Solvent	28.17	0.47	
X_3-Nonsolvent	−3.41	0.76	
X_4-Water	332.71	1.79	
X_5-Glycerol	−485.50	3.60	
X_1 z_1	11.54	3.87	
X_2 z_1	−0.26	0.52	
X_2 z_2	2.60	0.39	
X_3 z_1	3.35	0.79	
X_4 z_1	−15.77	1.85	
X_5 z_1	11.10	3.73	
R-Squared	0.9138	Adj R-Squared	0.8942
Adeq Precision	26.596	Pred R-Squared	0.8616

high Adj-R^2 and Pred-R^2 support good explanation on the reliability of regression models for the protein binding ability (Adj-$R^2 = 0.8894$ and Pred-$R^2 = 0.8593$) and the membrane wicking time (Adj-$R^2 = 0.8942$ and Pred-$R^2 = 0.8616$). The good agreement between the R^2, Adj-R^2 and Pred-R^2 values have demonstrated the viability of crossed regression models for the protein binding and wicking time prediction.

Diagnostic Statistic

The major diagnostic plots in Figs. 2 and 3 are to determine the residual analysis of crossed design, ensuring that the statistical assumptions fit the analysis data. Figures 2a and 3a display the "normal % probability" plot versus the "studentized residuals," which examine whether the number of standard deviations between actual and predicted response values followed the normal distribution (20). The normal distribution analysis (Figs. 2a and 3a) is used to determine whether the simulated models obtained from the study are sufficient or otherwise. From plotted "studentized residuals" versus "predicted values" (Figs. 2b and 3b), the constant variance assumption can be confirmed. All points of experimental runs should be scattered randomly within the constant range of residuals across the graph, i.e., within the horizontal lines at point of ±3.0.

Based on the "box-cox plot" for power transformation graphs (Figs. 2c and 3c), the transformation for particular response with the best lambda value was recommended. In the power transformation, mathematical functions such as square root, natural log, inverse, power, or logit

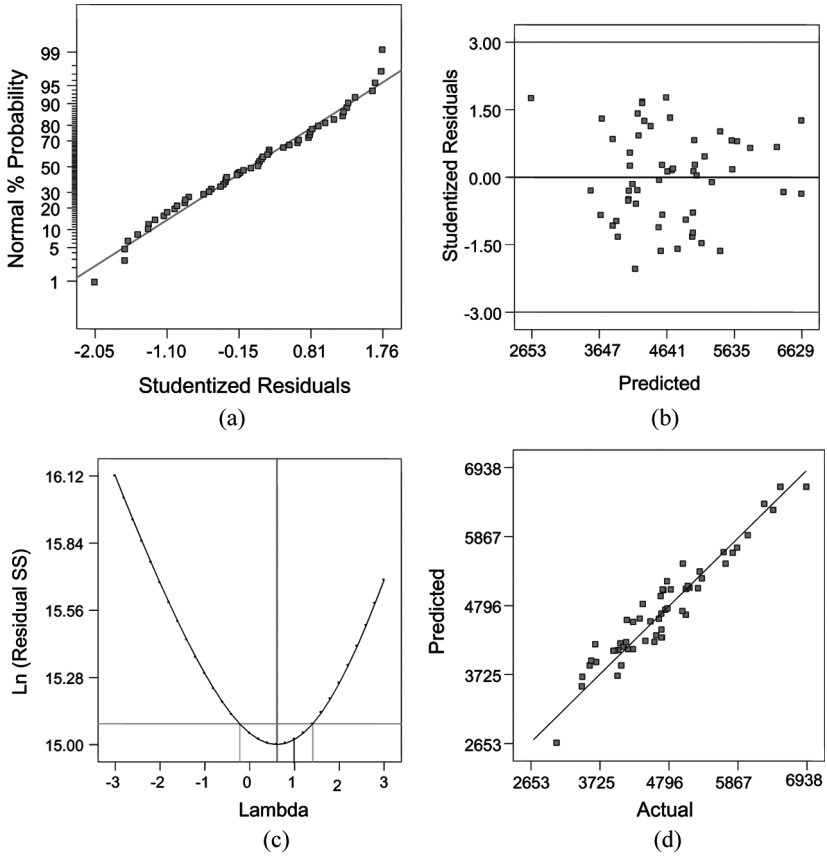

Figure 2. Diagnostic plots for membrane protein binding regression model (a) normal distribution analysis (b) studentized residuals versus predicted (c) box-cox (d) actual versus predicted.

was applied to all the response data. For estimated value of membrane protein binding ability (Y_1), no transformation is needed (Fig. 2c). As observed from Fig. 3c, the estimated value of membrane wicking time (Y_2) was transformed to $\sqrt{Y_2}$.

The last diagnostic analysis displayed in Figs. 2d and 3d were the "actual values" versus the "predicted values" plot. Actual values obtained from the experimental studies were compared to the estimated values from regression models. All points should place along and near the diagonal line to confirm the reliability of the models. Points above the diagonal line were points which were over estimated and vice versa. The entire diagnostic statistics in Figs. 2 and 3 were well fitted

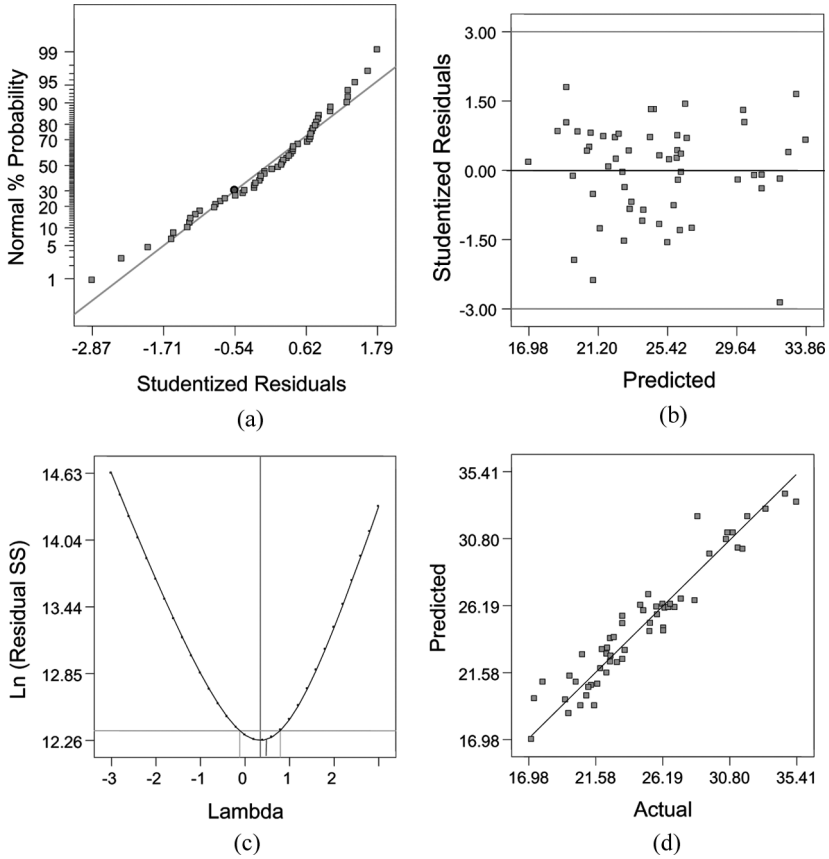

Figure 3. Diagnostic plots for membrane wicking time regression model (a) normal distribution analysis (b) studentized residuals versus predicted (c) box-cox (d) actual versus predicted.

in acceptable variance range. Thus, the regression models obtained from the crossed design can be further used as the predictor for membrane formulation and performance optimization.

Model Graph and Contour Plot

Mixture components displayed as contour plot in this crossed design study is considered complex as there are 5 mixture components that are able to affect the formulation process. From a previous preliminary study (9), the effects of solvent and nonsolvent composition on the

responses of membrane performance were fairly subtle compared to the polymer, water, and additive composition in the casting solution. Hence, polymer, water, and additive were chosen as component X1, X2, and X3, respectively in the mixture contour plots.

3D surface contour plots displayed in Figs. 4 and 5 were used to estimate the membrane protein binding ability and wicking time responses, which generated at different level of process factors. It is apparent from the response contour plots (Figs. 4 and 5) that both the evaporation time and drying temperature applied in the membrane formation process causes great changes on the responses of the membrane. These contour plots are in good agreement with the regression models obtained from the ANOVA analysis, and both of contour plots have linear effects on all cast solution blends.

On the other hand, Fig. 6 discloses the effects of cast solution blends on the membrane performance. A variety of polymer compositions were used at the same process factor condition. In Fig. 6, the varied behavior of the membrane responses on protein binding ability indicates significant interactions between process factors and mixture.

As revealed in Fig. 4, membrane with excellent protein binding ability can only be produced with high evaporation time and high drying temperature during the membrane casting process. However, low evaporation time and low drying temperature were required in membrane formation process to hasten the membrane wicking time (Fig. 5). Hence, it is necessary to implement optimization in order to achieve the most suitable formulation and process factor, capable of producing a highly effective membrane.

Optimization

The complexity of membrane casting formulation and casting process factors contribute to unpredicted membrane performances. By using crossed design, well-analysed statistical regression models have been produced as stated in Eqs. (4) and (5). A couple of factors in crossed design can be further developed and optimized through a desirability function (D) for multiple responses based on Eq. 6

$$D = \left[\prod_{i=1}^{N} d_i^{r_i}\right]^{\frac{1}{\sum r_i}} \tag{6}$$

where, D is the total desirability function combined from all the required responses, N is the number of responses in the measure, r_i referred to the

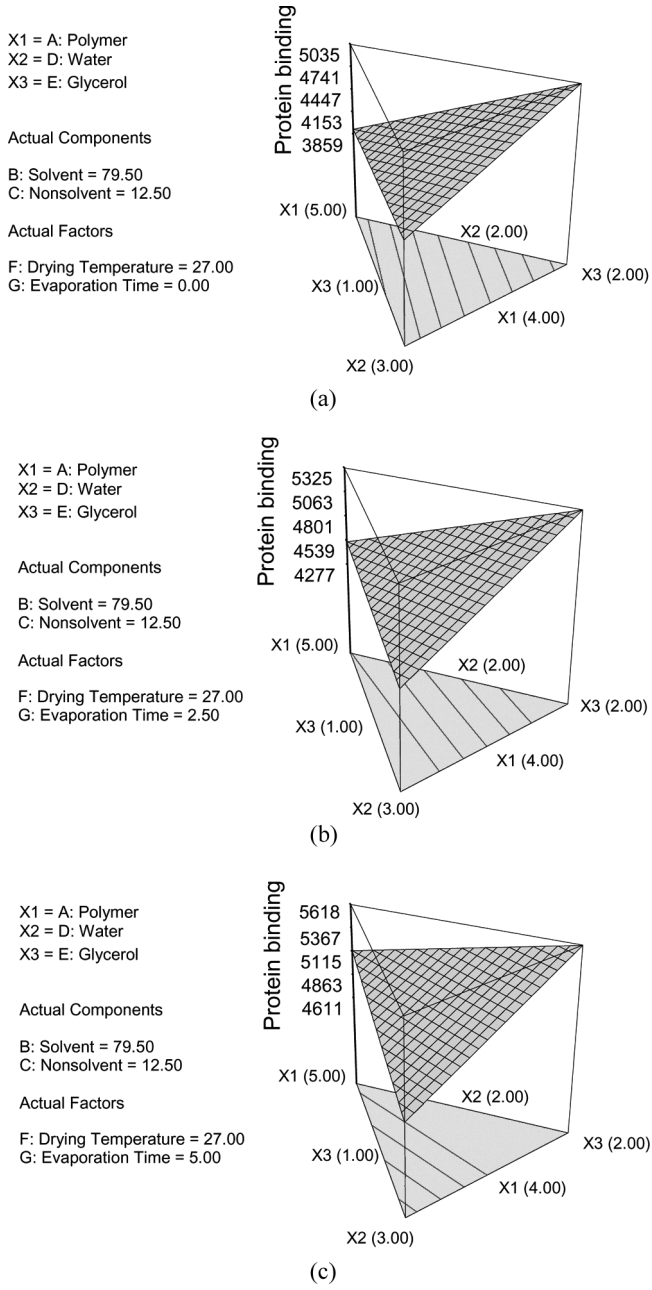

Figure 4. 3D surface contour plots for membrane protein binding at different cast solution and evaporation time (a) 0 min (b) 2.5 min (c) 5 min.

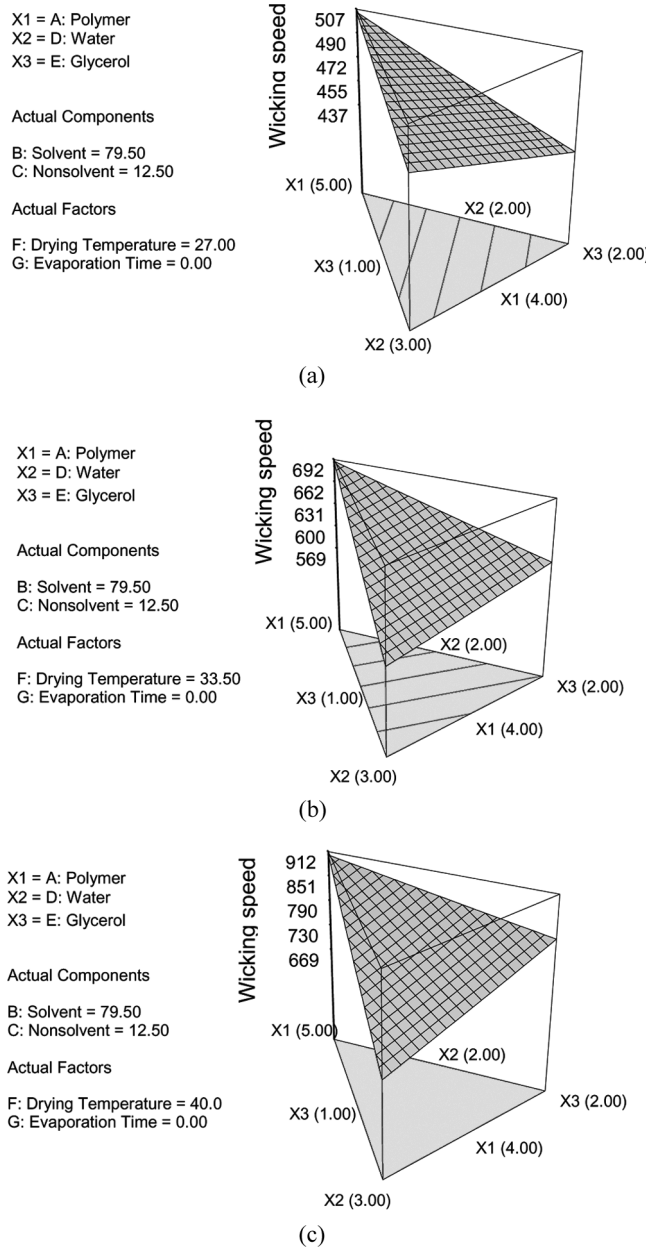

Figure 5. 3D surface contour plots for membrane wicking speed at different cast solution and drying temperature (a) 27°C (b) 33.5°C (c) 40°C.

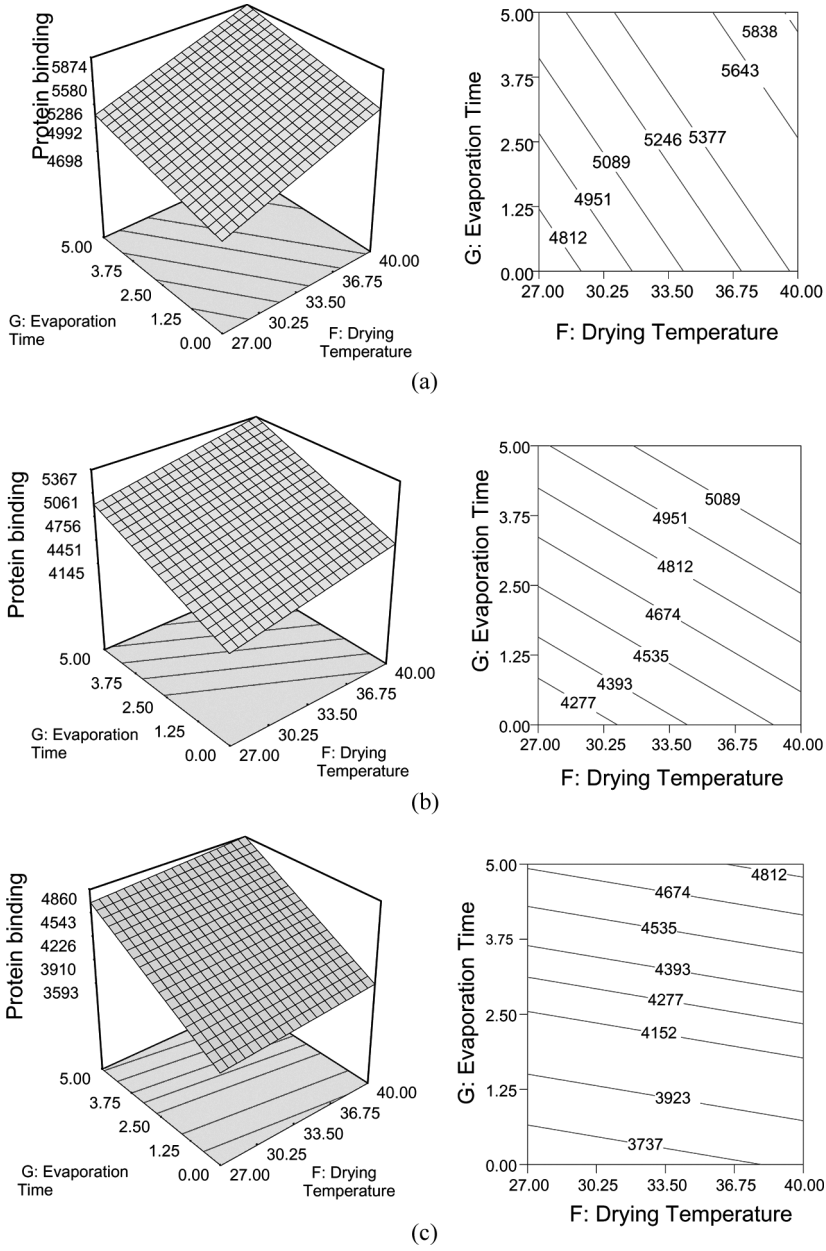

Figure 6. 3D surface contour plots for membrane protein binding at different process factors and polymer content (a) 4% (b) 4.5% (c) 5%.

Table 6. Numerical optimization and verification for mixture-process crossed design

NC, x_1 (g) %	MA, x_2 (g) %	IP, x_3 (g) %	Water, x_4 (g) %	Glycerol, x_5 (g) %	Drying temperature, z_1 °C	Evaporation time, z_2 min	Protein binding μg/cm^3	Wicking time sec/4 cm	Porosity %	Desirability	
4.00	82.00	10.00	2.00	2.00	27.00	3.05	5282	423	73.17	0.66	Selected
4.23	80.37	10.92	2.49	2.00	28.11	5.00	5485	534	69.22	0.65	
4.00	80.35	11.05	2.61	1.99	27.71	5.00	5378	518	69.02	0.64	
4.47	80.82	10.71	2.00	2.00	27.00	2.23	5084	449	71.75	0.64	
4.34	80.02	11.64	2.00	2.00	27.00	1.76	5078	456	72.35	0.63	
4.00	81.45	10.00	2.55	2.00	32.53	3.15	5340	535	70.77	0.63	
Verification of optimised membrane performances											
4.00	82.00	10.00	2.00	2.00	27.00	3.05	5202	395	73.82		
Standard Deviation between predicted values from model and actual values from experiment											
							1.57%	6.62%	0.89%		

importance of particular responses varies from the least important (1), to the most important (5) and d_i is the partial desirability function for specific responses (22).

Desirability functions optimize the casting formulation and process factor by primarily adjusting the responses' required target, such as minimum, maximum, and within range (22). A combination of all goals from various responses and factors will renders an optimization value and desirability function (D), which is also assigned with a value ranging from 0 (away from the target) to 1 (target fitted).

Achieved functions D (0.66, 0.65, 0.64, and 0.63), from Table 6 are considered significant since 5 mixture components and 2 process factors were involved and 4 simultaneous responses are considered. Desirability value of 0.66 is selected as the optimized point for mixture-process crossed models. The predicted and optimized design point (x_1: 4%, x_2: 82%, x_3: 10%, x_4: 2%, x_5: 2%, z_1: 27°C and z_2: 3.05 min) is expected to be able to produce a highly porous membrane structure with high protein binding ability and fast lateral wicking time.

Subsequently, in order to prove the reliability of the generated models by crossed design, the optimised membrane formulation selected from Table 6 is cast based to the predicted design point of membrane formulation and casting process. The actual experimental responses of the membrane were stated in Table 6. The actual values obtained from experimental works (protein binding ability $= 5202\,\mu g\,cm^{-3}$, solution wicking time $= 395\,sec\,4\,cm^{-1}$, membrane porosity $= 73.82\%$) were found to be in good agreements with the predicted values (protein binding ability $= 5282\,\mu g\,cm^{-3}$, solution wicking time $= 423\,sec\,4\,cm^{-1}$, membrane porosity $= 73.17\%$) from the crossed design models. All the standard deviation values between predicted and actual data were fall within 7%, as shown in Table 6. This shows that the models generated in this crossed design study are both reliable and feasible.

CONCLUSIONS

The aim of this study is to identify the interaction between the complexity of membrane casting solution and process factors in order to achieve the required membrane performances. Five casting solution (polymer, solvent, nonsolvent, glycerol and water) and two casting process factors (drying temperature, evaporation time) were considered. The complexity of membrane formulations and casting process has been well analysed by crossed design method. Crossed design technique provides information on variables and their associate levels of responsibility in affecting the membrane performance. Regression models obtained from the analysis

can be used to predict the membrane responses. Optimisation of the membrane casting formulation in consideration of casting process factors have been carried out with results shows that the optimal membrane formulation and process factors are—4% polymer, 82% solvent, 10% nonsolvent, 2% water, 2% glycerol with 27°C drying temperature and 3.05 min evaporation time. The outcome is proven to be able to perform with high protein binding ability and fast lateral wicking time for the diagnostic test strip application. The optimal membrane casting formulation and process factors obtained from this study can then be further applied in future membrane application studies.

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NOMENCLATURE

English Notations

$V_A =$ Apparent volume (cm^3)
$V_E =$ Existing volume (cm^3)
$x_1 =$ Nitrocellulose (NC) content (wt. %)
$x_2 =$ Methyl Acetate (MA) content (wt. %)
$x_3 =$ Iso-propanol (IP) content (wt. %)
$x_4 =$ Water content (wt. %)
$x_5 =$ Glycerol content (wt. %)
$z_1 =$ Drying Temperature (°C)
$z_2 =$ Evaporation Time (min)
$Y_1 =$ Protein binding ($\mu g/cm^3$)
$Y_2 =$ Lateral wicking time (min)
$Y_3 =$ Porosity (%)
$Y_4 =$ Yield Thickness (μm)
$F =$ F-test (Dimensionless unit)
$D =$ Desirability function (Dimensionless unit)
$N =$ Number of responses in the measure (Dimensionless unit)
$r_i =$ Importance of particular response (Dimensionless unit)
$d_i =$ Partial desirability function for specific responses (Dimensionless unit)

Greek Notation

$\epsilon =$ Membrane porosity (%)

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