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H-PVA Cross-Linking Mechanism Prepared By Freeze-Thaw And Annealing: Optimizing Gel Fraction And Swelling

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Abstract. PVA has attracted attention as a material in the formation of hydrogels for drug release media. Crosslinking is one of the keys to hydrogel formation. Gel fraction, swelling ratio, and degree of crystallinity are parameters used to determine the enhancement of crosslinking and the formation of mechanical properties of hydrogels. H-PVA was synthesized through freeze-thaw and annealing processes and optimized by Box-Behnken design. Analysis of the degree of crystallinity revealed that the freeze-thaw and annealing processes formed and increased crosslinking. This is supported by data from the gel fraction analysis, which indicates that the number of crosslinks increases and the swelling ratio decreases, indicating that the hydrogel structure is strong enough to withstand water absorption. The results of the design of the experiment (DOE) showed that simultaneously the model was able to explain the presence of crosslinks formed in H-PVA, as seen from the p-value <0.0001 and the statistical test results $R^2 = 0.99$ (close to 1). Optimal conditions were found at a PVA concentration of 18.972 (%w/v), a freeze-thaw cycle of three times, and an annealing temperature of 92 ^{o}C.

1 Introduction

The mechanical characteristics of the hydrogel become one of the elements that demand attention and influence on the drug release mechanism, necessitating research and development. Gel fraction, gel swelling and degree of crystallization are examples of mechanical qualities [1]. The gel fraction influences the number of cross-links produced in the hydrogel during the freeze-thaw process. Swelling is a rise in the volume of a material caused by contact with a liquid, gas, or vapor, and is indicative of the substance diffusing through certain materials [2]. When the biopolymer comes into contact with a liquid, such as water, swelling occurs owing to a thermodynamic fit between the polymer chain and water, as well as the existence of a tensile force induced by the polymer chain's cross-linking action [3].

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Hydrogels are three-dimensional networks of polymers joined by cross-links, and their forming elements are hydrogen bonding between polymer chains. The resulting three-dimensional network structure produces a hydrogel that is both stable and insoluble in water [4]. Aside from that, it exhibits properties such as the capacity to swell in water or biological fluids (swelling), high water diffusion, and is flexible and elastic. Because the hydrophilic functional groups are hydrated by water and hydrogen bonding, hydrophilicity is one of the elements of the hydrogel that plays a role in determining the mechanical characteristics. Hydrogels' hydrophilicity can be controlled by elements such as -OH, -COOH, -COONH2, and SO3H groups [5].

Polyvinyl Alcohol (PVA) is a synthetic polymer that is hydrophilic or water soluble and is generated by a hydrolysis process. It is non-toxic, biocompatible, biodegradable, and has high strength and hardness. Because of its qualities, it has sparked a lot of interest in the use of PVA as a hydrogel preparation material [6].

Cross-links are network structures that produce new bonds, either ionic or covalent. The freeze thaw technique is a physical approach for creating hydrogels that works by crosslinking the polymer mixture through cold cycles of up to -20oC and raising the temperature to room temperature [7]. This approach turns liquid polymers into solids or gels by preventing the polymer from changing shape. The technique of connecting (typically chemically) numerous polymer chains together in order to improve their characteristics [8]. Cross-linking is typically accomplished by a chemical reaction using a reagent having multi-functional groups that are reactive with the functional groups of the polymer being changed. Hydrogel polymers are frequently changed using this process [9].

Annealing is characterized as heating to an appropriate temperature and then cooling to achieve the required mechanical qualities such as crystallinity, gel fraction, swelling, toughness, and material stability [10]. This technique, in addition to enhancing or preserving the mechanical characteristics of the hydrogel, can remove the usage of harmful crosslinking chemicals. The annealing process in drug delivery systems is responsible for maintaining the rhythm or changing the rate of medication release [11].

In this study, a hydrogel based on polyvinyl alcohol (PVA) was created to identify the optimal and best hydrogel mechanical characteristics as an ideal drug delivery system, such as gel fraction, swelling, and degree of crystallinity. To study the process, Behnken's Design Box approach is employed as a Response Surface Methodology (RSM). RSM is a statistical tool for assessing the impact of process factors and their interactions on chosen responses. Each factor's level is independent in each model. The Box-Behnken design approach, which contains three factors that may predict the highest optimum value, can be utilized as a design model in the RSM process. To maximize the mechanical characteristics of the PVA hydrogel, Behnken's RSM box design was used. PVA content, number of freeze-thaw cycles, and annealing temperature were the factors studied.

2 Materials and Methods

2.1 Materials

The materials used are Polivinil Alcohol Mw 85.000 – 124.000 (99% hydrolyzed) was purchased from Sigma Aldrich. Distilled water was obtained from Chemical Engineering Departement, University of Indonesia.

2.2 Formulation of PVA based Hydrogels

PVA powder with different concentration (10%, 15%, 20%) were dissolved in 20 ml distilled water. The PVA solution was heated using oven at temperature 90 °C for 3 hours. After all the PVA has dissolved, the temperature is then lowered to room temperature. The solution was transferred to the mold and then frozen at -20°C for 12 hours and thawing at room temperature for 12 hours, according to variations of freeze-thaw cycles 1, 2, 3 times. After the freeze-thaw process, the hydrogel is then annealead on variations of annealing temperature (60, 80, 100 °C) for 90 minutes.

2.3 Determination of Degree Crystallinity

The XRD test serves to analyze the degree of crystallinity of PVA hydrogel. The test was performed using a Shimadzu XRD 7000 Maxima-X tool with a Cu tube. Diffraction analysis of dry samples was carried out at room temperature (25 °C) using a K∞Cu radiation analyzer with λ = 1.54 and scanning at a speed of 2 °/min in the 2θ range of 9–90 °.

To determine the degree of crystallinity (X_C), it is important to deconvolute the XRD spectrum of the sample to find the areas of the amorphous and crystalline peaks [12, 13]. XC was calculated using Equation (1) [14]:

$$X_C(\%)=\frac{A_C}{A_T}\times 100 \qquad (1)$$

Where A_C and A_T are the crystalline peak areas and the total amorphous and crystalline peak areas, respectively.

2.4 Gel Fractions

Gel fraction testing was carried out by weighing 3 grams of hydrogel as (Wd), then wrapped in gauze and soaked in 6 mL of distilled water for 24 hours. The soaked hydrogel was dried again in the oven at 50°C for approximately 90 minutes and weighed as (W1). To determine the gel fraction (G) in the PVA hydrogel formed based on the freeze-thaw and annealing methods, it was determined using equation 2 [15].

$$G(\%)=\frac{W_1}{W_d}\times 100 \qquad (2)$$

Where G = Gel Fraction, W_d = weight of hydrogel, W_1 = weight of dried hydrogel.

2.5 Swelling Ratio

The hydrogel was immersed in 20 mL phosphate buffer saline (PBS) pH 7.4 at room temperature. The hydrogel was periodically taken out of the PBS, dried with tissue and weighed until a constant weight is observed . The swelling ratio of the hydrogels were calculated with the following equation 2 [16].

$$\text{Swelling ratio, } Q_t=\frac{W_s-W_d}{W_d}\times 100 \qquad (3)$$

Where Qt = swelling ratio at time t, W_s = weight of the swollen hydrogel at time t, and W_d = weight of the dried gel.

2.6 Optimization (Box Behnken Design) of the PVA Hidrogel

The effect of PVA concentration, the number of freeze-thaw cycles and annealing temperature were analyzed using Box Behnken design to evaluate the effect of factors and determine the optimum PVA hydrogel characteristics gel fraction and swelling. Box Behnken design with three factors and three levels (-1, 0, +1) was used for the experiment with a total 15 run (experiments).

Table 1. Independent Variables and Coded Levels.

Independent Variable	**Symbol Code**	**Level**		
		-1	**0**	**1**
PVA Concentration (%)	X_1	10	15	20
Number of freeze-thaw cycle	X_2	1	2	3
Annealing Temperature (°C)	X_3	60	80	100

Table 2. Results of H-PVA Box-behnken experimental design- Actual and Prediction

Run Order	**Variabel**			**Respon 1: YG (%)**		**Respon 2: YQ_t (%)**	
	X_1	**X_2**	**X_3**	**Actual**	**Prediction**	**Actual**	**Prediction**
1	1	0	-1	70.84	69.92	210.47	208.93
2	0	1	-1	69.93	70.70	208.91	210.95
3	0	0	0	79.42	79.42	197.56	197.70
4	0	-1	-1	55.79	56.13	236.61	236.98
5	0	-1	1	74.41	74.64	229.55	228.52
6	1	-1	0	63.68	64.27	219.70	220.87
7	1	0	1	87.05	87.24	186.21	187.07
8	1	1	0	80.67	80.81	178.99	178.49
9	1	0	-1	54.50	54.31	243.35	242.48
10	0	0	0	78.79	79.42	196.68	196.70
11	-1	-1	0	50.96	50.81	249.97	250.48
12	0	1	1	81.61	81.27	190.78	190.41
13	0	0	0	80.04	80.42	198.87	197.70
14	-1	0	1	64.15	65.06	232.81	234.35
15	-1	1	0	57.06	57.47	230.89	229.71

The data obtained were analyzed using analysis of variance (ANOVA) and the response surface to determine the functional relationship between the independent process variables and the desired response. The second-order polynomial regression equation describes the significance of the relationship between the independent variables in the response. The polynomial equation is described as follows:

$$Y=\beta_0+\sum_{i=1}^{k}\beta_i X_i+\sum_{i=1}^{k}\beta_{ii}X_i^2+\sum_{i=1}^{k}\sum_{j=i+1}^{k}\beta_{ij}X_iX_j \qquad (4)$$

Where Y is the desired response, βO represents a constant coefficient, Xi and Xj represent independent variables, k represents the number of independent variables, and βi, βii, and βij represent linear coefficients, quadratic coefficients, and interaction coefficients [17].

3 Results and Discussion

3.1 Crosslinking PVA Hydrogel

The PVA hydrogel formation system is based on the formation of cross-links, which is to see or determine how good the characteristics of the PVA hydrogel are obtained through two methods, namely freeze-thaw and annealing. Crosslinking is enhanced through freezing, thawing, and annealing processes based on hydrogen bonding interactions. PVA is known to contain hydroxyl (-OH) groups; these groups play an important role in the physical crosslinking process [18]. Firstly, PVA is dissolved in water, and the OH groups begin to bind to each other. In the freezing process, ice crystals are formed, which then cause phase separation between the water solution and the PVA OH groups. During the thawing process, PVA is free to form hydrogen bonds and crystals, so cross-linking occurs [19]. These bonds are strengthened through the annealing process, where the hydrogen bonds formed increase, leading to the formation of crystal structures (crystallization), scheme of hydrogel crosslinking were showed in Figure 1 [11, 20].

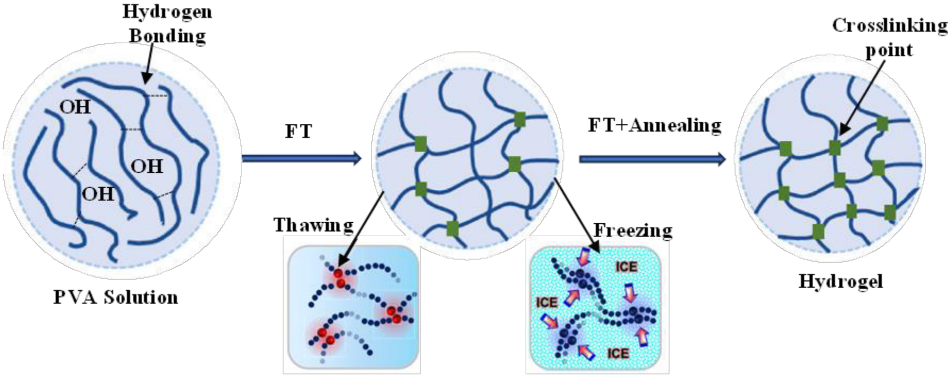

Fig. 1. Scheme of H-PVA crosslinking mechanism

The increase in cross-linking can also be seen from the degree of crystallinity. Table 3 compares the degree of crystallinity of PVA hydrogels without and using crosslinkers. The results show that for research [8], using crosslinker as a hydrogel adhesive media, PVA concentration of 10%, freeze-thaw cycle 2 times, as well as annealing temperatures RT and 90 ºC produce degrees of crystallinity ranging from 20-33%. While in this study without the use of crosslinker, PVA concentration of 10-20%, freeze-thaw cycle 1-3 times, and annealing temperature of 60-100 °C produces a degree of crystallinity ranging from 25-63%. This proves that the freeze-thaw and annealing process has an important role in the formation of hydrogel crystal structure (crystallization). In addition, the increase in the degree of crystallinity is also influenced by the formulation of making hydrogels, for example in this study with a concentration of PVA 10%, freeze-thaw 2 cycles and annealing temperature of 60 ºC obtained degree of crystallinity 25.15%. The small degree of crystallinity if associated with the theory of cross-linking mechanism may be due to low the content of OH groups (PVA concentration), freeze-thaw cycle and annealing temperature causes OH groups do not have enough time to be free to form hydrogen bonds and crystals. Therefore, the right formulation of PVA concentration, freeze-thaw and annealing is needed to make PVA hydrogels with good and stable crystallinity.

Table 3. Comparison of Crystallinity of PVA hydrogels at different reaction temperatures prepared in different processing methods

PVA Concentration (%)	**Crosslinker**	**FT**	**Annealing Temperature (C)**	**Degree of Crystallinity (%)**	**Reference**
10	hydrochloric acid and glutaraldehyde	2	-	20,9	[8]
10		2	90	32,8	
10	-	1	60	25,15	This Study
15	-	2	80	43,44	
20	-	3	100	63,14	

Based on this, PVA concentration, freeze-thaw cycle and annealing temperature are potential independent factors for box-behnken design. These factors are the basis for knowing their effect on the mechanical properties of the PVA hydrogel formed, such as the characteristics of the gel fraction and gel swelling. In this study, the gel fraction and gel swelling resulting from the experimental design software are shown in Table 2. The fraction percentage and swelling of PVA hydrogel obtained from this study showed varying values. The lowest gel fraction percentage was 50.96% and the highest was 81.61%, while for swelling gel the lowest value was 178.99% and the highest was 249.97%. From table 2, it is observed that the number of cross-links formed based on the gel fraction value is the lowest and highest for experiments 11 and 12, while for the mechanical properties of the hydrogel formed through the water absorption scheme based on the swelling ratio value, the low absorption and high absorption are seen in experiments 8 and 11. The larger gel fraction value means that the hydrogel has a high number of cross-links, while a lower gel swelling value indicates that the large number of cross-linked networks formed can resist water absorption into the hydrogel [3, 15, 21]. The results of experiment 11 show that the low gel fraction value forms little cross-linking, which causes the mechanical properties of the hydrogel to be brittle, resulting in a lot of water being absorbed in the hydrogel. In research [7] mentioned that the degree of crosslinking has an influence on the nature of the network, if the network formed is not tight and strong, it results in a gap for water to enter, causing the mechanical properties of the hydrogel to be more vulnerable to water. If observed at a glance, this is influenced by the hydrogel formulation, which has a 10% PVA concentration, a freeze-thaw cycle once, and an annealing temperature of 80 °C.

3.2 Optimization of The Procedure

The fraction and swelling of PVA hydrogels were optimized using response surface methodology. A Box-Behnken matrix design with a total of 15 experiments consisted of three independent variables X_i (i.e., PVA concentration, number of freeze-thaw cycles, and annealing temperature) and two response outcomes studied (i.e., gel fraction and swelling). A quadratic model was used to analyse the data on two characteristics of the PVA hydrogel (gel fraction and swelling ratio). Equations (5) and (6) were obtained as polynomial equations generated after modelling the data, and these show the interaction and curvature effects between the independent variables (X) and the two response variables (Y) analysed (gel fraction (YG) and swelling ratio (YQ_t)).

$$YG = 79.42 + 9.45X_1 + 5.55X_2 + 7.02X_3 + 2.72X_1X_2 + 1.64X_1X_3 - 1.73X_2X_3 - 8.81X_1^2 - 7.51X_2^2 - 1.47X_3^2 \quad (5)$$

$$YQ_t = 197.7 - 20.21X_1 - 15.78X_2 - 7.5X_3 - 5.4X_1X_2 - 3.43X_1X_3 - 2.77X_2X_3 + 11.97X_1^2 + 10.22X_2^2 + 8.54X_3^2 \quad (6)$$

where X_1, X_2, and X_3 are PVA concentration, number of freeze-thaw cycles and annealing temperature, respectively. Based on the individual coefficients in the polynomial equation (5), the independent variables (X_1, X_2, X_3) individually affect the response (YG), as indicated by the positive constant value in the model. While the interaction between the independent variables does not significantly affect the gel fraction, This is reflected in the negative value of the model. However, the opposite is true for polynomial equation (6), where the independent variable X has no significant effect on the response YQ_t (swelling ratio), as seen from the negative value of the model. The significance of the polynomial equation can be validated by the resulting regression coefficient (R^2), F-value, and P-value. The selected reference statistic values of this study for the YG response are regression coefficient R^2 = 99.30%, F-value = 221.66, p-value <0.0001; YQ_t is regression coefficient R^2 = 99.18%, F-value = 188.43, p-value <0.0001. This proves that the predicted and observed values are close, so the model can be represented in predicting the gel fraction (increase in the number of crosslinks) with an error value of less than 5% [22]. Data on the significance of the independent variables of PVA hydrogel preparation consisting of PVA concentration, freeze-thaw cycle, and annealing temperature on the response, namely the percentage of gel fraction and swelling ratio, are presented in Table 4.

Table 4. ANOVA Analysis of Quadratic Model of Gel Fraction and Swelling Ratio

Source	**Gel Fraction**					**Swelling Ratio**				
	Sum of Squares	**df**	**Mean Square**	**F-value**	**p-value**	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**
Model	1868.05	9	207.56	221.66	< 0.0001	6934.49	9	770.50	188.43	< 0.0001
A- PVA Concentration	714.26	1	714.26	762.77	< 0.0001[b]	3266.62	1	3266.62	798.85	< 0.0001[b]
B- Freeze-thaw Cycle	246.54	1	246.54	263.28	< 0.0001[b]	1993.08	1	1993.08	487.41	< 0.0001[b]
C- Annealing temperature	394.14	1	394.14	420.90	< 0.0001[b]	449.82	1	449.82	110.00	0.0001[a]
AB	29.63	1	29.63	31.64	0.0025[a]	116.77	1	116.77	28.56	0.0031[a]
AC	10.78	1	10.78	11.51	0.0194[a]	47.07	1	47.07	11.51	0.0194[a]
BC	12.04	1	12.04	12.85	0.0158[a]	30.67	1	30.67	7.50	0.0409[a]

A^2	286.79	1	286.79	306.26	$< 0.0001^b$	528.68	1	528.68	129.29	$< 0.0001^b$
B^2	208.43	1	208.43	222.58	$< 0.0001^b$	385.57	1	385.57	94.29	0.0002^a
C^2	7.97	1	7.97	8.51	0.0331^a	269.33	1	269.33	65.87	0.0005^a
Residual	0.0168	5	0.0196			0.0045	5	0.0149		
Lack of Fit	0.0139	3	0.0023	3.35	0.0524	0.0018	3	0.0031	4.99	0.0512
Pure Error	0.0009	2	0.0006			0.0005	2	0.0003		
Cor Total	1868.0668	14				6934.4945	14			
Std Dev	0.0968					1.022				
R^2	0.9975					0.9971				
Adjusted R^2	0.9930					0.9918				
Predicted R^2	0.9657					0.9577				
Adeq Precision	46.1106					43.5968				

$^a0.0001 \leq p < 0.05$: signifcant
$^bp < 0.0001$: highly significant

The difference between the experimental data and the predicted value of the model is explained by the residual mean square (MS) value in the analysis of variance (ANOVA) [23]. This study produced a residual mean square (MS) value of YG and YQ_t of 0.0196 and 0.0149, respectively, so it can be concluded that the model used is good enough and accurate in explaining the closeness between the experimental results and the predicted value obtained from the experimental design. The closeness between the experimental data and the predicted values is clarified by the coefficient of determination (R^2) obtained, which is 0.9975 (99.75%) for YG and 0.9971 (99.71%) for YQ_t, so it can be stated that the model is able to explain the experimental data. The relationship between the experimental results obtained and the predicted value of the model can be validated with the actual vs. predicted diagram, while the reliability and significance of the model can be validated by looking at the average probability percentage, for each response illustrated in Figures 2a and 2b.

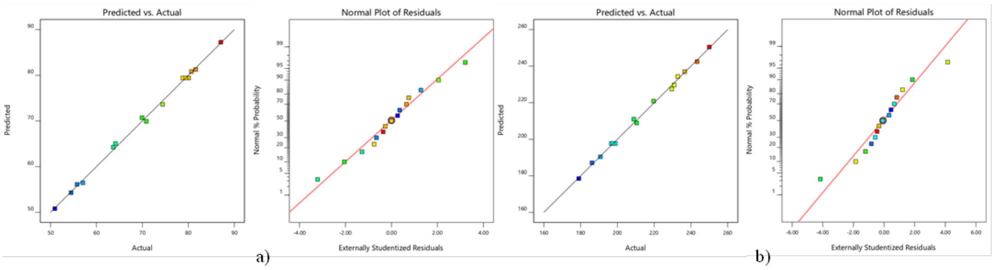

Fig. 2. Probability plots (Normal VS Internally Studentized Residual): a) Gel Fraction, b) Swelling ratio

On a normal probability plot, the diagonal line shows the data predicted by the experimental design. This applies vice versa, for the points around the diagonal line show the experimental results obtained. The closer the experimental data points are to the diagonal line, the more normally distributed the residual values are [24]. This normal distribution analysis aims to observe the magnitude of deviation from the model. In this study, the normal probability plot shows points that spread and approach the diagonal line, so it can be said that the model given by the experimental design can fulfill the assumption of normality.

3.3 Effect and Interaction between Variables on Gel Fraction and Swelling Ratio

Figure 3 presents a response surface plot of the crosslinks formed as seen through the values of gel fraction percentage (Figure 3a) and swelling ratio (Figure 3b). Response surface plots make it possible to understand the effects of interactions between two or more parameters and to determine the optimal value of each variable to minimize or maximize a given one. Based on the response surface plot images, it shows that the percentage gel fraction (Figure 3a) increases with increasing PVA concentration, number of freeze-thaw cycles, and annealing temperature up to a certain critical point. However, this is inversely proportional to the swelling ratio (Figure 3b), which decreases as the three variables increase. The highest percentage of gel fraction and swelling ratio is shown by the red contour plot area of the response surface, while the smallest percentage of gel fraction is depicted in the blue contour plot. In this study, the contour plot shows that more than 80% of the amount of crosslinking (gel fraction) and less than 180% of water absorption (swelling ratio) was obtained when the freeze-thaw process was repeated 2-3 cycles with an annealing temperature of 90–100 °C and a PVA content of 18–20%.

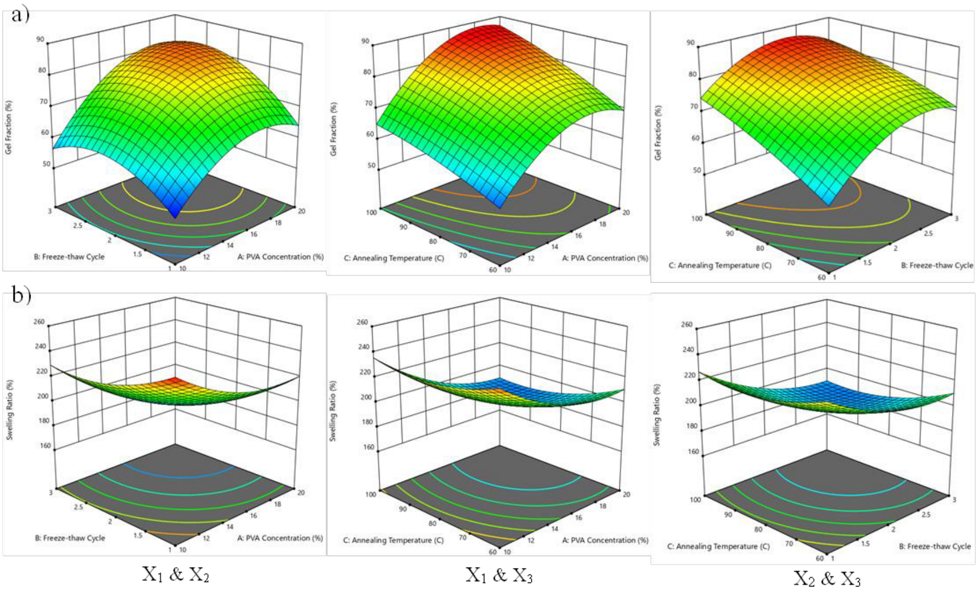

Fig. 3. Response Surface Methodology of PVA Hydrogel: a) Gel Fraction, b) Swelling ratio

According to [25] the highest gel fraction percentage and the lowest swelling ratio occurred at 10% PVA concentration with a 3-cycle freeze-thaw process at 110 °C annealing temperature. Increasing PVA concentration, the number of freeze-thaw cycles, and the annealing temperature can increase hydrogen bonding in PVA hydrogels, thereby increasing the number of cross-links (gel fraction) and strengthening the mechanical structure of hydrogels, which can slow down water absorption. One of the reasons for this phenomenon is that the capillarity of the pores of the sample subjected to the freeze-thaw process will lead to the formation of two phases, the frozen phase consisting of the solvent and the unfrozen liquid microphase consisting of soluble molecules including monomers [26]. These monomers interact with each other and form long chain polymers with hydroxyl groups randomly located on both sides of the chain. Hydrogen bonding between hydroxyl groups leads to the formation of crystallites. During the thawing process, the hydrogel produces an interconnected porous network resulting in the formation of cross-links [27]. In subsequent freeze-thaw cycles, the number of hydrogen bonds between chains increases which in turn increases the crosslink density. The annealing process produces more crystallites and also increases the crosslink density of PVA. The higher the crosslink density, the lower the swelling ratio [28]. As for the swelling ratio, it decreases as the PVA content increases because the higher the PVA content, the smaller the pore size of the gel. And the capillarity of the pores depends on the pore size [29]. Therefore, an increase in PVA content leads to a decrease in swelling ratio. PVA hydrogels prepared by freeze-thaw and annealing processes are physically crosslinked with polymer crystallites. Hydrogels with more PVA content had higher crosslink density under freeze-thaw and annealing, and therefore had higher gel fraction.

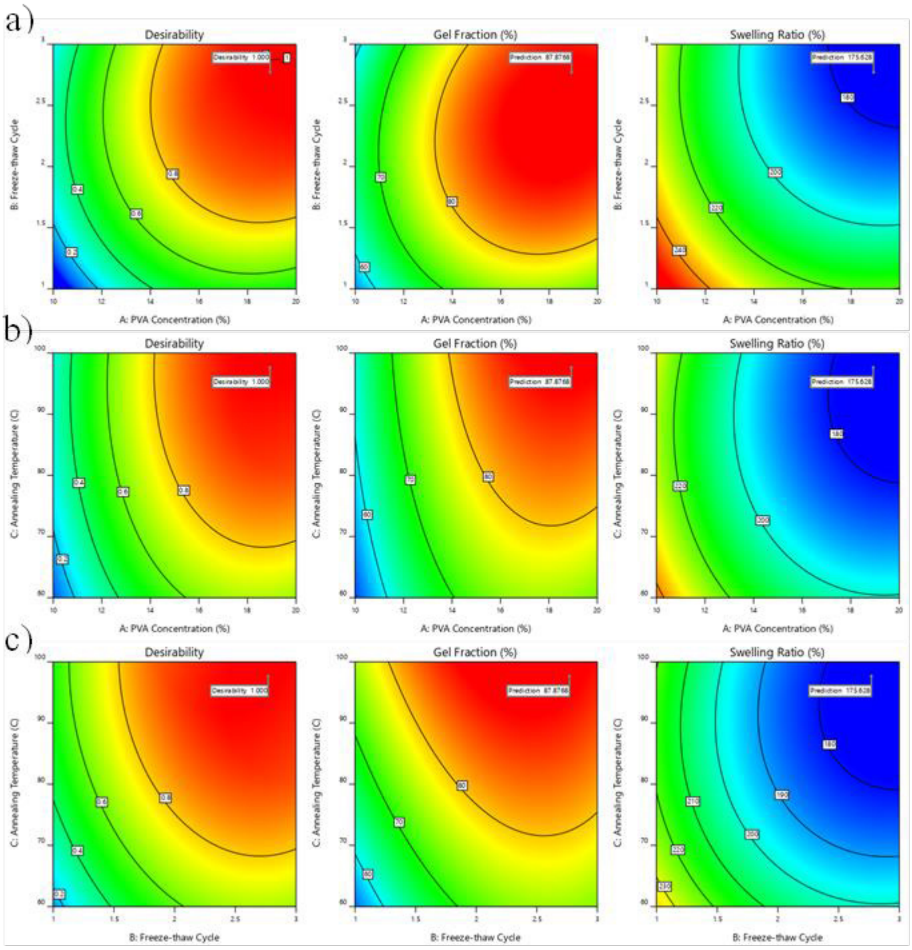

Fig. 4. Countour plots the effect of a. X1 and X2. b. X1 and X3. c. X2 and X3 on Gel Fraction and Swelling Ratio

The results of the desirability function analysis for each independent variable and the prediction of the response at optimal conditions can be observed from the response desirability countor plots. The desirability countour plots show that the percentage of gel fraction increases as the value of independent process variables such as PVA concentration, freeze-thaw cycle, and annealing temperature increases until it reaches a certain critical value. While in the desirability countour profile, the percentage of swelling ratio decreased as the value of the independent process variables increased until it reached a certain critical value. The optimum condition for increasing cross-linking and slowing down water absorption based on gel fraction and swelling ratio can be seen from the line plot between the independent variables and the response value, which is close to the core of the countour (desirability 1). In this study, the optimum condition was obtained at a PVA concentration of 18.972 (%w/v), a number of freeze-thaw cycles of 3, and an annealing temperature of 92 °C, with an optimum response in the form of a gel fraction percentage of 87.8768% and a swelling ratio of 175.628%.

4 Conclusion

The process of making PVA hydrogels with freeze-thaw and annealing to increase cross-linking and strengthen the PVA chain structure showed potential for application, with the acquisition of a high percentage of gel fraction, up to 87.8768%, and the best swelling ratio of 175.628%. RSM was used based on BBD to obtain the highest gel fraction percentage and the lowest swelling ratio. The BBD design showed that increasing PVA concentration, freeze-thaw cycle, and annealing temperature significantly affected the gel fraction and swelling ratio. The optimum condition was obtained at a PVA concentration of 18.972 (%w/v), a number of freeze-thaw cycles of 3, and an annealing temperature of 92 °C. Therefore, the results of this study can be used as a reference to strengthen the mechanical structure of hydrogels by increasing the number of cross-links seen through the percentage of gel fraction and swelling ratio in PVA hydrogels.

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