RiCE: Research in Chemical Engineering

Volume 5. No 1, 2026

Journal homepage: https://ejournal.ump.ac.id/index.php/rice

Effect of Chitosan Content and Freezing Temperature on the Properties of Freeze-Thawed Hydrogels

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Article Info

Submitted:
29 January 2026

Revised:
18 March 2026

Accepted:
24 March 2026

ABSTRACT

Climate change characterized by increasing temperatures and drought frequency has led to decreased agricultural land productivity, especially in areas with limited water availability. One alternative to overcome this problem is the use of hydrogel as a soil moisture retention material which is able to absorb and store water. This study aims to determine the effect of chitosan concentration (1%, 2%, and 3%) and freezing temperatures (–20°C, –30°C, and –40°C) on the physical characteristics of Polyvinyl alcohol (PVA)-chitosan-based hydrogels synthesized using the freeze–thaw method. The parameters analyzed include gel fraction, swelling ratio, and compressive strength. The results showed that most treatments did not have a significant effect on the physical properties of the hydrogel, especially on the gel fraction and compressive strength. The gel fraction values ranged from 8.42% to 23.55% and the compressive strength ranged from 0.0031 MPa to 0.009 MPa. However, variations in temperature and chitosan concentration affect the swelling ratio. The swelling ratio ranged from 12.98% to 27.90%. PVA-chitosan hydrogels composition and a freezing temperature of –20°C showed the highest potential for water retention despite limited mechanical strength. Therefore, this hydrogel still has potential as an alternative planting medium, especially for maintaining soil moisture in dry areas, with the limitation that the formulation and synthesis process need to be further refined.

Keywords: Hydrogel, Planting medium, Freeze-thaw, PVA, Chitosan.

1. INTRODUCTION

In Indonesia, water management, especially in the agricultural sector, is becoming more difficult due to global warming. This is caused by excessive greenhouse gas emissions, such as methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O), which cause climate change that directly impacts water distribution. As a result, farmers often face severe weather and drought, especially in agricultural areas. Without the use of very low technology, irrigation efficiency ranges from 30-50%. Excessive irrigation application can result in a large portion of irrigation water being wasted as excessive runoff, evaporation and transpiraton (20-30%), and percolation (30-40%). Prolonged drought during the dry season can reduce water availability for plants. In this situation, high evapotranspiration can lead to a decline in groundwater levels. Water shortages negatively impact not only plants but also the soil. Without water, essential plant nutrients cannot dissolve properly, reducing nutrient availability and potentially causing wilting or reduced crop yields [1].

The application of polymer hydrogels is an additional option to increase irrigation efficiency in agricultural land [2]. The word "hydro" is synonymous with water, meaning a gel capable of absorbing and storing water hundreds of times its weight. Hydrogels can be added to growing media as an alternative to reducing the frequency of plant watering [3].

Hydrogels can be synthesized through either physical or chemical cross-linking methods; however, chemical cross-linking may generate harmful residual compounds. Physical cross-links can be formed using the freeze–thaw method, in which repeated cycles of extreme temperature changes induce intermolecular interactions within the polymer network. This approach is widely recognized as an effective technique for hydrogel cross-linking [4].

The synthetic polymer used in this study is poly(vinyl alcohol) (PVA), which is known for its high tensile strength and flexibility. Owing to its hydrophilic nature, PVA can enhance the gel properties of chitosan by reducing gelation time and improving the mechanical strength of the resulting hydrogel. The incorporation of PVA is intended to improve the swelling capacity of the hydrogel and to meet the performance requirements for growing media applications. It is therefore expected that the enhanced hydrogel characteristics, particularly mechanical strength, will be suitable for such applications [5]. However, PVA is a homopolymer that tends to exhibit brittle behavior. Consequently, to improve its mechanical performance during practical use, PVA is commonly blended with other polymers [6]. Chitosan is one of the polymers widely used in hydrogel formulations for this purpose.

Chitosan is a naturally abundant, biodegradable polymer derived from the deacetylation of chitin. Owing to its biodegradability, biocompatibility, and non-toxic nature, chitosan has been widely applied in biomaterials, pharmaceutical, and agricultural fields. These favorable properties make chitosan an attractive reinforcing component in hydrogel systems with diverse functional applications. Consequently, the combination of chitosan with PVA is considered an effective strategy to improve the mechanical properties of hydrogel formulations.

A mini-review by Michalik and Wandzik [7] reported that PVA-chitosan hydrogels prepared using the freeze-thaw method increased soil water retention from 4% to 10% after 30 days. In addition, these hydrogels supported the slow release of nutrients and enhanced the early growth of plants such as okra. These findings indicate that PVA-chitosan hydrogels fabricated via the freeze–thaw method have significant potential as environmentally friendly growing media.

Based on the description above, this study used polyvinyl alcohol (PVA) and chitosan as natural polymers, using chemical-free crosslinking through an efficient and environmentally friendly freeze-thawing process. The hydrogels were then tested for equilibrium swelling ratio, gel fraction, and compressive strength, thus demonstrating their potential benefits in agriculture.

2. MATERIALS AND METHODS

2.1 Materials

The materials used in this study are poly(vinyl alcohol) (PVA), a synthetic polymer, and chitosan, a natural and biodegradable polymer that plays a role in strengthening the hydrogel structure.

2.2 Experimental procedure

2.2.1 Preparation of PVA–Chitosan Hydrogel

In this study, polymerization was performed using PVA and chitosan. First, 10 grams of PVA was weighed into 100 ml of distilled water. Chitosan was weighed according to the percentage variations (1%; 2%; 3%) and then dissolved in 100 mL of distilled water. The PVA solution was heated to 90 °C for 60 minutes, then the Chitosan solution was heated to 50 °C for 60 minutes; both solutions were then stirred using a magnetic stirrer to ensure homogeneity. A 1% acetic acid solution was also added to the Chitosan to facilitate its dissolution in water. Once the solutions are homogeneous, they are mixed and heated on a hotplate at 70 °C for 15 minutes. Before undergoing the freezing process, the solution is stored at room temperature to prevent the formation of uneven ice crystal structures and to stabilize the hydrogel structure. Once the solution has reached room temperature, the freezing process can be carried out at -20 °C, -30 °C, and -40 °C for 18 hours, followed by 6 hours thawing process to aid in the formation of hydrogel pores and enhance the strength of the hydrogel bonds. This freeze-thaw cycle is repeated three times. After the freeze-thaw process, the sample is dried in an oven at 45°C for 24 hours, after which data analysis is performed.

2.2.2 Preparation of Acetic Acid Solution

To prepare 1% acetic acid solution, take 1 ml of acetic acid and dissolve it in 20 ml of distilled water in a beaker until completely dissolved. Then transfer the solution to a 100 ml volumetric flask and add distilled water up to the mark until the solution is homogeneous.

2.3 Methods of analysis

2.3.1 Analysis of Gel Fraction

The gel fraction is defined as the ratio of the dry hydrogel mass after the soaking and drying process to its initial dry mass and is used to characterize the degree of cross-linking within the hydrogel network. The measurement procedure begins by weighing the dried hydrogel sample, followed by immersion in water for 24 h to remove unbound or soluble components. The hydrogel is then redried in an oven at 45 °C for 24 h to eliminate residual moisture. After drying, the sample is reweighed to determine the remaining insoluble mass. A higher gel fraction indicates a larger proportion of insoluble material, reflecting a higher degree of cross-linking within the polymer network [8]. The percent degree of cross-linking can be determined by:

$$\text{Gel Fraction (\%)} = W1/W0 \times 100\% \quad (1)$$

Where W1 is the weight of the dry hydrogel after soaking and W0 is the weight of the dry hydrogel before soaking [5].

2.3.2 Analysis of Swelling Ratio

The swelling ratio is a key indicator of a polymer’s water absorption capacity and is a critical parameter for assessing the application potential of superabsorbent polymer (SAP) materials. This parameter is determined by comparing the mass of the hydrogel after water absorption with its initial dry mass. The swelling ratio is widely used as a primary measure of hydrogel performance, particularly for materials intended for absorbent applications (Astrini et al., 2016). In general, an increased degree of cross-linking within the hydrogel network restricts polymer chain mobility, resulting in a reduced swelling capacity. The swelling behavior reflects the amount of water absorbed by the hydrogel and can be quantified from the mass difference before and after immersion in a specific solution. The swelling percentage is then calculated using the following formula:

$$\%\text{Swelling} = (Ws\text{-}Wd)/Wd \times 100\% \quad (2)$$

In this case, WS indicates the weight of the hydrogel after absorbing water (wet condition), while Wd is the weight of the hydrogel in a dry state before the swelling process occurs [2].

2.3.3 Analysis of Compressive Strenght

The compressive strength of a material reflects its mechanical performance, as increases in compressive strength are generally associated with improvements in other physical properties. Compression testing provides key mechanical parameters, including compressive strength, elastic modulus, and deformation behavior of the specimen. Compressive strength is defined as the maximum compressive stress that a material can withstand before failure or significant deformation occurs [9].

3. RESULTS AND DISCUSSION

The freeze–thaw method is a widely used physical approach for hydrogel synthesis that does not require chemical cross-linking agents. This process relies on the formation of physical cross-links through repeated freeze–thaw cycles, resulting in a stable and porous hydrogel network. In this study, the hydrogel was synthesized from a blend of poly(vinyl alcohol) (PVA) and chitosan, both of which contain active hydroxyl (–OH) and amine ($-NH_2$) functional groups capable of forming intermolecular hydrogen bonds.

During the freezing stage, the PVA–chitosan solution is subjected to low temperatures, typically ranging from −20 °C to

−80 °C. Under these conditions, water molecules crystallize to form ice crystals, which induce phase separation by concentrating the polymer components (PVA and chitosan) within the unfrozen regions. This concentration effect compresses the polymer chains into confined domains, promoting chain entanglement and the formation of physical cross-links [10].

During the subsequent thawing stage, the ice crystals melt; however, the polymer network structure formed during freezing remains intact. This freeze–thaw process is repeated for multiple cycles, typically between 3 and 10 cycles, with each cycle enhancing cross-link density and network homogeneity. An increased number of freeze-thaw cycles strengthens intermolecular interactions between polymer chains, resulting in improved mechanical strength and a higher gel fraction of the resulting hydrogel [11].

This method enables the fabrication of environmentally friendly hydrogels that are suitable for biological applications, as it does not involve the use of hazardous chemical cross-linking agents. Furthermore, the porous structure generated through freeze–thaw cycling enhances water retention capacity, which is a critical property for growing media applications.

3.1. Effect of Chitosan Percentage and Freezing Temperature on Gel Fraction

The results of this study indicate that variations in chitosan concentration and freezing temperature in the freezing–thawing process did not significantly affect the gel fraction of hydrogel. It can be seen from statistical analysis using a factorial design at a significance level of 0.05 which revealed that the percentage of chitosan do not have a significant effect on gel fraction (p = 0.517). Similarly, the freezing temperature also showed no statistically significant influence on gel fraction (p = 0.613), as both p-values exceeded the chosen significance threshold (0,05). The interaction between concentration of chitosan and temperature also doesn’t have the significance effect on the gel fraction base on p value that is less than 0,05.

Figure 1 show factorial plot which show the main effect of the concentration of chitosan and freezing temperature on gel fraction. Both factors do not affect significantly on gel fractions. The interaction between concentration and temperature also does not affect the gel fraction as seen in Figure 2.

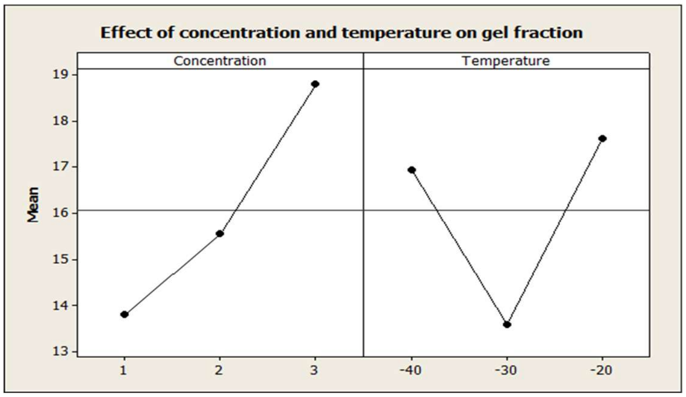

Figure 1. Main effect of concentration of chitosan and temperature on gel fraction

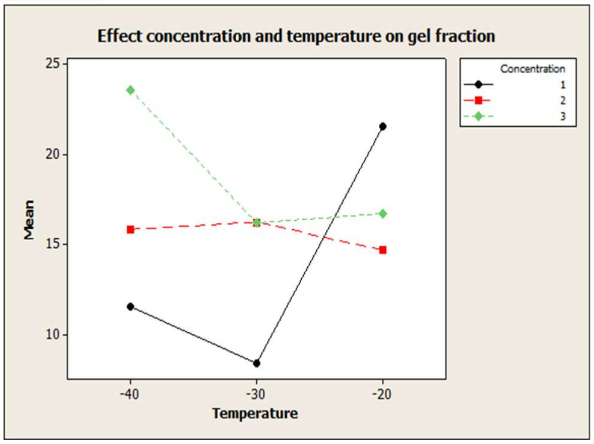

Figure 2. Effect of the interaction both factors on gel fraction.

This finding suggests that the formation of the hydrogel network is primarily governed by the main physical crosslinking mechanism of the base polymer, while the contribution of chitosan concentration and freezing temperature remains insufficient to significantly alter the degree of gel formation.

In the freezing–thawing method, gel fraction formation is mainly attributed to the development of crystalline regions during the freezing stage, which subsequently act as physical crosslinking points upon thawing. This mechanism is largely controlled by the freezing–thawing cycles and the intrinsic properties of the primary polymer (e.g., polyvinyl alcohol). Therefore, the range of freezing temperatures applied in this study may not have been wide enough to induce meaningful differences in the number or stability of the crystalline crosslinks formed, resulting in relatively uniform gel fraction values across different freezing conditions.

Furthermore, the incorporation of chitosan at various concentrations did not significantly contribute to the gel fraction because chitosan primarily functions as a secondary polymer or filler within the hydrogel matrix. The interactions between chitosan and the primary polymer are predominantly physical in nature, such as hydrogen bonding, rather than permanent covalent crosslinks that directly increase network density. Consequently, while chitosan may influence other properties of the hydrogel such as swelling behavior, biocompatibility, or mechanical characteristics its effect on gel fraction remains limited.

The absence of a significant effect from both variables may also be attributed to the dominance of an already established gelation mechanism under near-optimal conditions. Once a stable hydrogel network is formed, moderate variations in processing parameters may no longer result in measurable changes in gel fraction. This observation is consistent with previous studies reporting that the gel fraction of hydrogels prepared via the freezing–thawing method is more sensitive to the number of freezing–thawing cycles than to freezing temperature variations or the addition of secondary polymers.

Overall, these results demonstrate that chitosan concentration and freezing temperature are not critical parameters in determining the gel fraction of the hydrogel system under the conditions investigated. Therefore, optimization of gel fraction in this system is more effectively achieved by controlling other factors, such as the number of freezing–thawing cycles, the concentration of the primary polymer, or the application of additional crosslinking strategies.

3.2. Effect of Chitosan Percentage and Freezing Temperature on Swelling Ratio

The results demonstrated that the swelling ratio increased with increasing chitosan content, particularly within the concentration range of 1–3% (w/v). Statistical analysis using a factorial design at a significance level of 0.05 yielded a p-value of 0.020 for chitosan concentration, indicating a statistically significant effect on the swelling ratio. In contrast, the freezing temperature showed no significant influence on swelling behavior, as indicated by a p-value of 0.067, which exceeded the significance threshold. The interaction between concentration of chitosan and temperature also has the significance affect as indicated by a p-value 0f 0,013. These results can be seen in the table below.

Table 1. Analysis of variance for swelling ratio (%).

```
Factor         Type   Levels  Values
Concentration  fixed       3  1; 2; 3
Temperature    fixed       3  -40; -30; -20


Analysis of Variance for Swelling ratio (%), using Adjusted SS for Tests

Source                      DF   Seq SS   Adj SS  Adj MS     F      P
Concentration                2  1055187  1055187  527594  6,27  0,020
Temperature                  2   623180   623180  311590  3,70  0,067
Concentration*Temperature    4  2005413  2005413  501353  5,96  0,013
Error                        9   757302   757302   84145
Total                       17  4441083
```

These findings are consistent with previous work by Ceylan et al. [12], who reported that increasing the chitosan concentration in PVA–chitosan hydrogels prepared via the freeze–thaw method enhanced water absorption capacity. This behavior is attributed to the increased formation of hydrophilic networks and greater volumetric expansion upon water uptake. In addition, hydrogels containing higher chitosan content exhibited a more open and porous microstructure, facilitating faster and more extensive water penetration.

Although freezing temperature can theoretically influence hydrogel microstructure through ice crystal formation, which may alter pore size and network arrangement, the present results indicate that its effect on swelling ratio is not statistically significant within the temperature range investigated. This suggests that the swelling behavior of PVA-chitosan hydrogels is governed primarily by polymer composition and intermolecular interactions rather than by nominal freezing temperature, provided that an adequate number of freeze-thaw cycles (typically more than three cycles) is applied.

3.3. Effect of Chitosan Percentage and Freezing Temperature on Compressive Strength

Compressive strength is a critical parameter for evaluating the mechanical performance of hydrogels, particularly for growing media applications, where structural stability under soil pressure is essential. Based on the results of this study as shown in table 2, variations in chitosan content within the PVA–chitosan formulation did not significantly affect the compressive strength of hydrogels prepared using the freeze–thaw method (p=0,588). Statistical analysis using a factorial design at a significance level of 0.05 showed that freezing temperature also had no significant effect on compressive strength, as indicated by a p-value of 0.588. The interaction of the concentration of chitosan and temperature also does not affect the compressive strength.

Table 2. Analysis variance for compressive strength

```
Factor         Type   Levels  Values
Concentration  fixed       3  1; 2; 3
Temperature    fixed       3  -40; -30; -20


Analysis of Variance for Compressive strength (MPa), using Adjusted SS for
     Tests

Source                       DF     Seq SS     Adj SS     Adj MS     F      P
Concentration                 2  0,0000016  0,0000016  0,0000008  0,56  0,588
Temperature                   2  0,0000016  0,0000016  0,0000008  0,56  0,588
Concentration*Temperature     4  0,0000048  0,0000048  0,0000012  0,85  0,529
Error                         9  0,0000128  0,0000128  0,0000014
Total                        17  0,0000208
```

In freeze-thaw cross-linked systems, the primary hydrogel network is predominantly formed through the crystallization of PVA rather than chitosan. During freezing, PVA chains are forced into close proximity and form physical cross-links in the form of microcrystalline domains, which act as the main load-bearing components of the network. In contrast, chitosan does not readily crystallize and is largely dispersed within the PVA matrix, contributing minimally to network reinforcement. Consequently, increasing the chitosan concentration does not result in a substantial change in the mechanical strength of the hydrogel.

4. CONCLUSION

Based on the results of this study, chitosan concentration did not have a statistically significant effect on gel fraction or compressive strength. Factorial design analysis conducted at a significance level of 0.05 yielded p-values of 0.517 for gel fraction and 0.588 for compressive strength. In contrast, chitosan concentration significantly influenced the swelling ratio, as indicated by a p-value of 0.020. Freezing temperature did not significantly affect gel fraction, swelling ratio, or compressive strength, with p-values of 0.613, 0.067, and 0.588, respectively, all exceeding the significance threshold.

ACKNOWLEDGMENT

I would like to thank to Directorate General of Higher Education, Central Executive Board of Muhammadiyah for giving fund to this research.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Haryanto: Conceptualization, sepervision, methodology, data curation, writing original draft.
Gita Ayu Lestari: Investigation, resources.

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