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Synthesis of Hydrogel from Chitosan and Glycerol Using Cross-linking Method: Citric Acid as a Crosslinker

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Abstract

This study focuses on creating a biodegradable hydrogel made from chitosan and glycerol, with citric acid used to link everything together. The goal is to improve the hydrogel's strength and ability to absorb water, making it ideal for medical uses. Chitosan is a biopolymer known for its biodegradability and biocompatibility, making it widely used in pharmaceuticals, biomedicine and agriculture. Glycerol, as a plasticizer has been chosen to enhance the flexibility and mechanical strength. In the presence of citric acid, which served as a cross-linking agent, this research was done by mixing the material in solvent form to synthesis the hydrogel. By varying the concentration of citric acid (1%, 2% and 3%), the effect it had on the hydrogel was evaluated using Thermogravimetric Analysis (TGA). TGA results revealed that increasing the citric acid concentration elevated the onset degradation temperature from 48.92 °C (1% citric acid) to 59.25 °C (2% citric acid) and 64.15 °C (3% citric acid). Hydrogel A (3% citric acid) exhibited the highest thermal stability due to a more densely cross-linked network structure, while Hydrogel C (1% citric acid) demonstrated a broader degradation temperature range (48.92 °C to 171.66 °C), indicating a less cross-linked and more flexible structure.

1. Introduction

Hydrogels are remarkable materials capable of holding large amounts of water while maintaining their structure, making them highly valuable in medical applications like wound dressings. Their soft, flexible nature closely mimics human tissue, creating a moist environment that encourages faster and more effective healing. Among the natural polymers used to make hydrogels, chitosan is especially promising due to its excellent biocompatibility and biodegradability, which means it is safe for the body and breaks down naturally without harmful effects [1].

Among natural polymers used in hydrogel fabrication, chitosan derived from the deacetylation of chitin has gained prominence due to its biodegradability, non-toxicity, bioadhesiveness, and intrinsic antimicrobial activity. These characteristics make it an ideal material for wound dressings, as it not only protects wounds from infection but also helps accelerate healing by encouraging new cell growth and collagen formation [2]. However, chitosan hydrogels often suffer from brittleness and limited flexibility, especially when dried or subjected to mechanical stress. To address these limitations, plasticizers such as glycerol are incorporated to improve mechanical flexibility and moisture retention. Glycerol intercalates between polymer chains, reducing

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intermolecular forces and enhancing elasticity, while its hygroscopic nature helps maintain moisture levels, which is beneficial for skin-contact and cellular environments [3].

Further enhancement of mechanical strength, stability, and water resistance is achieved through chemical crosslinking. Citric acid is an effective and environmentally friendly crosslinker due to its multifunctional structure, enabling formation of stable covalent bonds with polymer chains via esterification and amidation reactions. Heating induces citric acid dehydration to cyclic anhydrides, which react with hydroxyl groups on chitosan to form a robust three-dimensional network. Secondary ionic interactions with metal ions can further stabilize the hydrogel. Citric acid crosslinking improves mechanical properties, swelling behaviour, and biocompatibility, making it suitable for advanced biomedical applications such as drug delivery and tissue engineering [4].

A critical aspect of hydrogel performance is its water content and thermal stability, which directly influence its mechanical properties, swelling behaviour, and biocompatibility. Thermogravimetric Analysis (TGA) is widely used to quantify the water content in hydrogels by measuring weight loss as the sample is heated. In chitosan-based hydrogels, TGA typically shows an initial weight loss between 40°C and 150°C due to evaporation of free and bound water, providing insight into the hydration state of the polymer network [3]. Complementary to TGA, Differential Scanning Calorimetry (DSC) characterizes thermal transitions such as glass transition temperature (Tg) and melting points, revealing how water interacts with the polymer matrix. DSC can detect melting of freezable water near 0°C and shifts in Tg caused by water plasticization, which are important for understanding hydrogel stability under physiological conditions [5]. Together, these techniques offer comprehensive information on hydrogel hydration and thermal properties, essential for optimizing their biomedical functionality.

2. Methodology

2.1 Material

Chitosan Hydrochloride (KHL Global Sdn Bhd), an industrial-grade material with 98% purity and a degree of deacetylation of at least 95%, was used in this study. Glycerin (Dchemie Malaysia), with a purity of 99.5% and meeting USP/BP standards, was used as a plasticizer. Food-grade anhydrous citric acid (Dchemie Malaysia) was used as the hydrogel matrix and crosslinking agent, respectively. All the chemicals were purchased from reliable suppliers and used as received without any further purification. Distilled water was used for preparing all solutions to ensure consistency and purity.

2.2 Preparation of Hydrogel

Hydrogel is prepared using a simple technique of mixing and drying, with chitosan added to each formulation or solution, where the chitosan will be the base material in producing the hydrogel. The chitosan in use is a soluble compound which is easier to dissolve in water. The chitosan solution is prepared either by directly mixing chitosan powder with water or in an acidic solution for a faster reaction time. Furthermore, citric acid was used as a crosslinker with varied values of 1%, 2%, and 3% (w/v). Then, glycerol was used as plasticizer with a varied concentration of 1% to 2% (w/v). The outline of the method used is aligned based on Fig. 1.

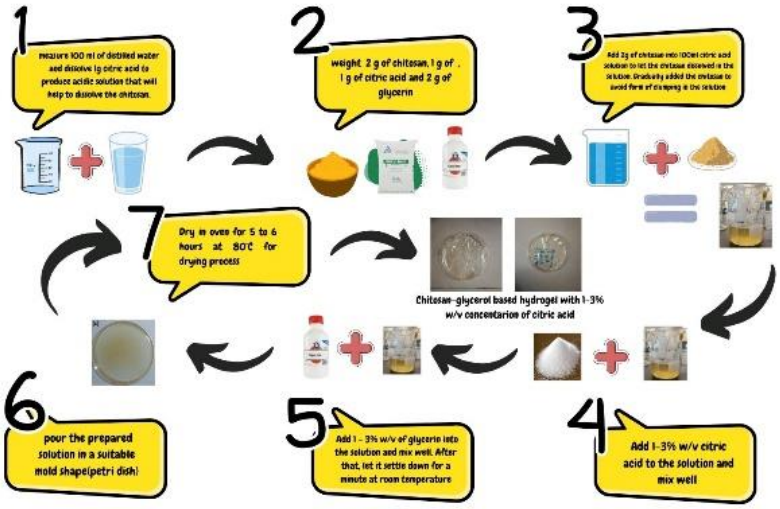


Fig. 1 *Step by step in synthesizing hydrogel chitosan based*

2.2.1 Preparation of Acidic Solution

To prepare an acidic solution for dissolving chitosan using citric acid, a concentration of about 1% (w/v) citric acid in distilled water is typically effective. This means dissolving 1 gram of citric acid in 100 mL of distilled water and mixing it well. This mild acidic environment helps protonate the amino groups in chitosan, making it soluble, similar to how acetic acid works. According to [4], citric acid was used in this range to successfully dissolve or crosslink chitosan, highlighting its effectiveness and additional benefits due to its three carboxyl groups.

2.2.2 Preparation of Chitosan Solution

Chitosan Hydrochloride was accurately weighed using an analytical balance to obtain 2 g of chitosan powder. Then, 2 g of chitosan were gradually added into 100 mL of citric acid solution. The mixture was stirred gently and continuously to prevent the formation of clumps, ensuring a homogeneous solution

2.2.3 Incorporating Citric Acid as Crosslinking Agent

Citric acid was weighed accurately in the range of 1 to 3 grams (corresponding to 1–3% w/v) and gradually added to the prepared chitosan solution while stirring continuously for 30 minutes to ensure complete dissolution and uniform mixing.

2.2.4 Incorporating Glycerol

Glycerol then was introduced into the solution of chitosan-citric acid with a concentration of 1% w/v which is equivalent to 1 gram of glycerol. This material will react with the chitosan polymer chains and form hydrogen bonds with them, which will further function as a plasticizer for the hydrogel [6].

2.2.5 Gelation Process

After mixing, the chitosan-citric acid solution was poured into a petri dish. The solution was then heated at 70–80 °C for five to six hours to promote crosslinking through esterification reactions between the citric acid and the hydroxyl and amino groups of chitosan, resulting in the formation of a stable three-dimensional hydrogel network. After that, the hydrogel was cooled to room temperature and stored in sealed containers until further use or characterization.

2.3 Characterizations

2.3.1 Thermogravimetric Analysis (TGA)

Thermogravimetric Analysis (TGA) was performed to determine the weight loss of the hydrogel at a certain set of temperature. The arrangement of the equipment was set as the temperature was ranged to be 30 °C to 180 °C whereas flowrate was set to 20 m^2/s and the gas used was nitrogen gas.

3. Result and Discussion

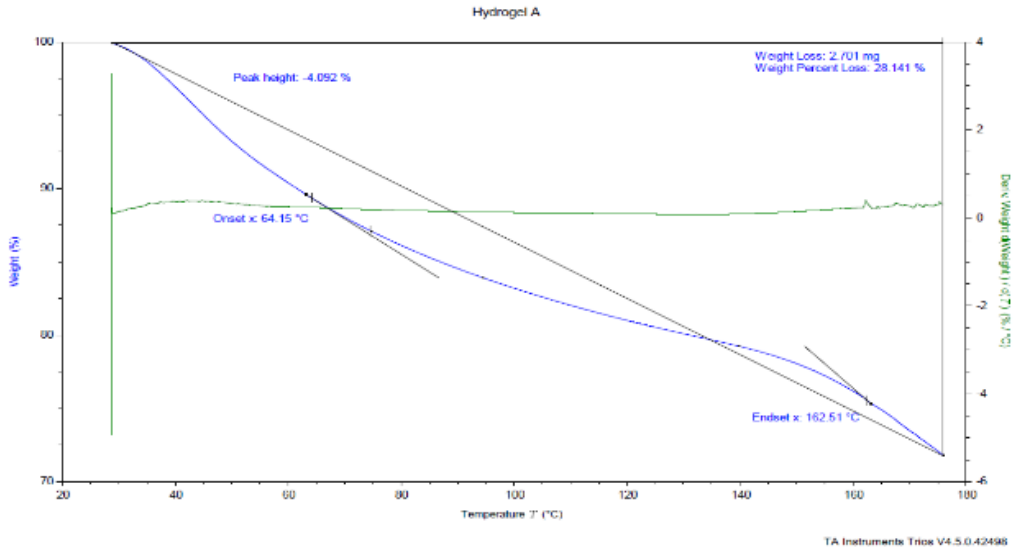
3.1 TGA analysis

TGA is widely used to quantify the water content in hydrogels by measuring weight loss as the sample is heated. This analysis method provides data for thermal stability and decomposition analysis. Fig. 2 shows a differentiation between three types of hydrogels, which are labelled as Hydrogel A with a composition of 2 g chitosan, 3 g citric acid and 1g glycerol for the first hydrogel. The second hydrogel is Hydrogel B with 2 g chitosan, 2 g citric acid and 1g glycerol. Lastly, the third hydrogel is Hydrogel C with 2 g chitosan, 1 g citric acid and 1 g glycerol.

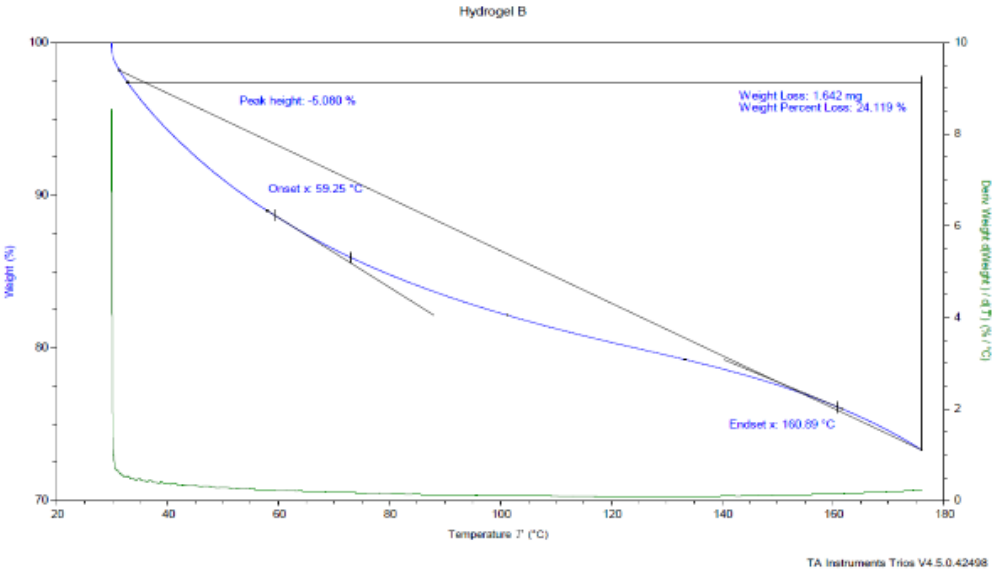
From Fig. 2 (a), thermal degradation of Hydrogel A initiated at 64.15 °C and terminated at 162.51 °C. Within this temperature interval, the sample experienced a weight loss of 28.141% relative to its initial mass. This higher onset degradation temperature indicated that Hydrogel A possessed a stronger and more thermally stable network structure compared to the other formulations. Meanwhile, in Fig. 2 (b), degradation for Hydrogel B began at 59.25 °C and continued until reaching 160.89 °C. During this process, the material exhibited a total weight loss of 24.12% of its original weight. Overall, the variance in weight loss percentage and onset degradation temperature between Hydrogel A and Hydrogel B showed a margin of ± 4.

In Fig. 2 (c), Hydrogel C began degrading at a lower onset temperature of 48.92°C, yet the degradation process spanned across a broader range, concluding at 171.66°C. The sample lost 24.12% of its original weight

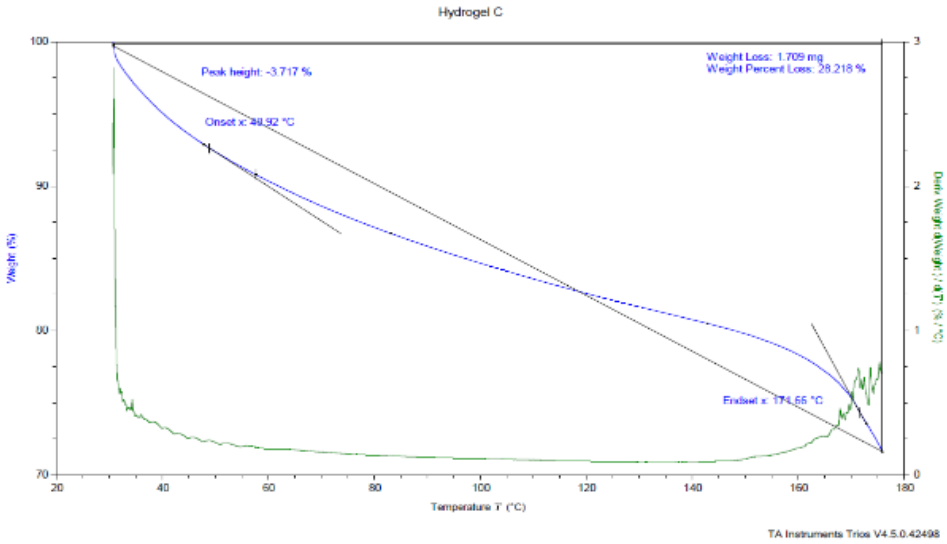
(with the inset curve recording 25.210%). This broader range of degradation temperature suggested that Hydrogel C possessed a less cross-linked and more flexible polymer structure. Overall, this occurrence is due to the difference in the concentration of citric acid, which will lead to a variation in the strength of bonding between molecules.



(a)



(b)



(c)

Fig. 2 *TGA results of (a) Hydrogel A; (b) Hydrogel B; (c) Hydrogel C*

4. Conclusion

In summary of the conducted experiment, the synthesize of hydrogel using chitosan as the base material with glycerol as support material as well as citric acid as the crosslinker with a varied amount was conducted and type of hydrogel was produced. The produced hydrogel, which is Hydrogel A, Hydrogel B, and Hydrogel C is concluded to have different strength in bonding between molecules with Hydrogel A having the highest strength follow by Hydrogel B and Hydrogel C. This will further lead to stronger chitosan polymer chains for keeping its properties intact for future usage of research.

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Conflict of Interest

Authors declare that there is no conflict of interest regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: ***study conception and design:*** *Muhammad Nor Azzumar Abdullah, Nadzri Najhan, Syafrizal Shuhaili, Mohd Khairul Nizam Mohd Zuhan;* ***data collection:*** *Muhammad Nor Azzumar Abdullah, Nadzri Najhan, Syafrizal Shuhaili, Mohd Khairul Nizam Mohd Zuhan;* ***analysis and interpretation of results:*** *Muhammad Nor Azzumar Abdullah, Nadzri Najhan, Syafrizal Shuhaili, Mohd Khairul Nizam Mohd Zuhan;* ***draft manuscript preparation:*** *Muhammad Nor Azzumar Abdullah, Nadzri Najhan, Syafrizal Shuhaili, Mohd Khairul Nizam Mohd Zuhan. All authors reviewed the results and approved the final version of the manuscript.*

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