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Swelling and re-swelling performance of glutaraldehyde crosslinked wet chitosan hydrogels

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ABSTRACT

Chitosan (Chi) is frequently used in hydrogels because of its natural characteristics. It is environmentally friendly, biodegradable, and does not include any harmful substances. Chitosan hydrogels were chemically synthesized by cross-linking with glutaraldehyde (GA) at various ratios ranging from 1% to 10% (w/w), employing two types of molecular weight chitosan. The degree of swelling of wet hydrogels (non-drying) was examined at pH 2.0, 5.6, and 7.4. After the swollen hydrogels dried, their re-swelling capabilities were examined in distilled water. Chemically cross-linked chitosan hydrogels were found to be stable, so the hydrogels were able to swell repeatedly 5 times without losing any of their swelling properties. The storage modulus properties of chitosan hydrogels were measured using a Discovery Hybrid Rheometer-1 at different temperatures. The storage moduli of chitosan hydrogels remained stable as temperature increased, with maximum values of 0.01 MPa and 0.02 MPa for low and medium molecular weight hydrogels. The viscosity of chitosan solutions was determined using two instruments: a Discovery Hybrid Rheometer-1 at 25 °C at different shear rates, and a Brookfield Viscometer. The viscosity of low and medium molecular weight chitosan solutions were measured using a Brookfield Viscometer, resulting in values of 101.5 and 333.5 cps, respectively.

I. INTRODUCTION

Hydrogels are 3-dimensional hydrophilic polymeric networks. These hydrogel polymers are hydrophilic because they have polar groups like hydroxyl (-OH), amidic (-CONH-), carboxylic (-COOH), and sulphonic ($-SO_3H$) [1]. Superabsorbent polymers can absorb water, saline water, or physiological fluid in a ratio ranging from 10 to 1,000 times their own weight [2]. Intermolecular forces like hydrogen bonding, electrostatic forces, and hydrophobic interactions are used to create physically crosslinked hydrogels which have relatively low mechanical strength and uncontrollable pore size. Some crosslinker molecules are used to make chemically crosslinked hydrogels. Chemically cross-linked hydrogels, on the other hand, exhibit relatively higher stability and mechanical strength because they are formed by chemical bonds between the polymer chains [3]. Chemical cross-linking increases the degree of crystallinity and hydrophobicity and decreases swelling in chitosan hydrogels [4]. Many studies have been conducted on the impact of covalent crosslinking on chitosan modification. Cross-linker type, and its density directly affect the final properties of hydrogels such as mechanical strength, mucoadhesiveness, swelling and drug release [5]. Deacetylating chitin produces chitosan, which has an amine group [6]. Chitosan is a biodegradable and biocompatible polymer because it is metabolized by certain human enzymes, such as lysozymes. It has been used in pharmaceutical and medical materials, such as the hydrogel system [7–9]. Chitosan is one of the most used semi-synthetic polymers due to its numerous advantages, as mentioned above [10, 11]. Ruiz et al. demonstrated that chitosan hydrogel cross-linked with

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glutaraldehyde is effective at adsorbing palladium ions. It has been discovered that the use of glutaraldehyde crosslinked chitosan hydrogel systems as an adsorbent, particularly for wastewater treatment, holds great promise [12]. According to Ozbas and Gürdag's (2016) study, 5-fluorouracil-loaded, glutaraldehyde-cross-linked chitosan hydrogels demonstrated efficient wound healing activity as well as anti-tumor and anti-metabolite properties in comparison to non-drug loaded hydrogels [13]. Pan et al. used hyaluronic acid hydrogel to deliver recombinant human bone morphogenetic protein to dental implants. This improved bone growth around the implants. This method does not require making a wet hydrogel on-site, which leads to better alkaline phosphatase activity and osterix expression [14]. The ability of hydrogels to re-swell can be utilized in various applications. Soft, wet, and biocompatible hydrogels have become appealing options for flexible electronics, as they have similar characteristics to biological tissues and can be easily customized [15]. Superabsorbent polymers are very compatible with concrete and have been found to improve self-healing and self-sealing in fractured concrete. The ability of superabsorbent polymers to re-swell is a key factor in determining how well they seal themselves in concrete [16]. Shur et al. conducted a study on the kinetics of hydrogel re-swelling in living organisms using microcomputed tomography. Their findings demonstrated that the shape of the implant and the presence of water had a major impact on the re-swelling process [17]. Superabsorbent polymers have the potential to mitigate water evaporation in arid regions. However, due to their limited salt resistance, water retention capacity, and swelling rate, they are less suitable for use in areas with scarce water supplies. Tian et al. reported that hydrogels are highly successful in preventing soil water evaporation. After 16 hours at 25 °C, the hydrogels retained 97.7% of the water, and after 5 cycles at 40 °C, they absorbed 832 g of water per gram of hydrogel. This finding presents new opportunities for their application [18]. Lipatova et al. synthesized chitosan hydrogels. When compared to films made from chitosan that have not been treated, films made by drying hydrogel have less crystallinity and can absorb more water vapor and Cu^{2+} ions. Additionally, these films have the ability to hold up to 5000% of water after re-swelling without undergoing any damage [19]. The most important difference of this study from the studies in the literature is that the synthesized hydrogels were not subjected to any drying process. The swelling properties of non-dried hydrogels were examined at different pHs. The swollen hydrogels were dried, and their swelling properties were examined again in distilled water.

II. EXPERIMENTAL METHOD

2.1 Materials and Preparation Techniques

The low and medium molecular weight chitosan, with a deacetylation degree of ≥ 75%, were acquired from Sigma-Aldrich. The following chemicals were also acquired from Sigma Aldrich: acetic acid, sodium acetate, disodium citrate, hydrochloric acid, sodium hydroxide, and glutaraldehyde (25% solution in water). All the other compounds were of high purity.

2.1.1 Solubility

Chitosan (0.1 g, 1% wt.) solution was prepared with acetic acid (1% wt.) in a centrifuge tube. After the dissolving process was completed, the mixture was further agitated for 40 minutes at 250 rpm and 25 °C. The centrifuge tube was kept in boiling water for 10 minutes. After the centrifuge tube was kept in boiling water for

10 minutes, the solution was cooled to room temperature and centrifuged at 1200 rpm. Once the liquid in the centrifuge tube was removed, the sediment at the bottom was rinsed with distilled water and centrifuged again. The insoluble solid part was dried at 60 ºC for 24 hours. The percentage solubility value of chitosan was calculated using the initial and final dry matter amounts as shown in Equation 1.

$$S\ (\%) = \left(\frac{w_{initial} - w_{insoluble\ part}}{w_{initial}}\right) * 100 \quad (1)$$

S (%): solubility percentage of chitosan; $w_{initial}$: mass of chitosan initially weighed; $w_{insoluble\ part}$: The amount of chitosan remaining or insoluble part after the dissolution, heating, centrifugation, rinsing, and drying processes described above, respectively.

2.1.2 Viscosity

In this method, Brookfield viscometer (DV2T) was used to determine the viscosity values of low and medium molecular weight chitosan used in hydrogel preparation. Chitosan solution was prepared by dissolving in 1% wt. Acetic acid. The viscosity of 1% wt. low and medium chitosan solutions were determined using Spindle No: 3 at 100 rpm.

2.1.3 Preparation of hydrogels

The chitosan, with a concentration of 2% (w/v), is dissolved in a 2% (w/v) acetic acid solution by agitating it with a magnetic stirrer. The resulting mixture is then placed in an ultrasonic bath for approximately 30 minutes to eliminate any air bubbles. A 1% (w/w) aqueous solution was prepared with distilled water by diluting a 25% glutaraldehyde solution. Then, the glutaraldehyde crosslinker, prepared as a 1% (w/w) aqueous solution, was added at a rate of 1-10% by weight based on chitosan with an automatic pipette, mixed rapidly to ensure a homogeneous distribution and kept at room temperature for one day until the crosslinking reaction was completed [9]. Figure 1 illustrates the steps involved in determining the gelation time of hydrogels produced with chitosan of medium molecular weight. The gelation times of hydrogels were determined as 60 to 180 seconds (sec.), depending on the molecular weight and cross-linker concentration.

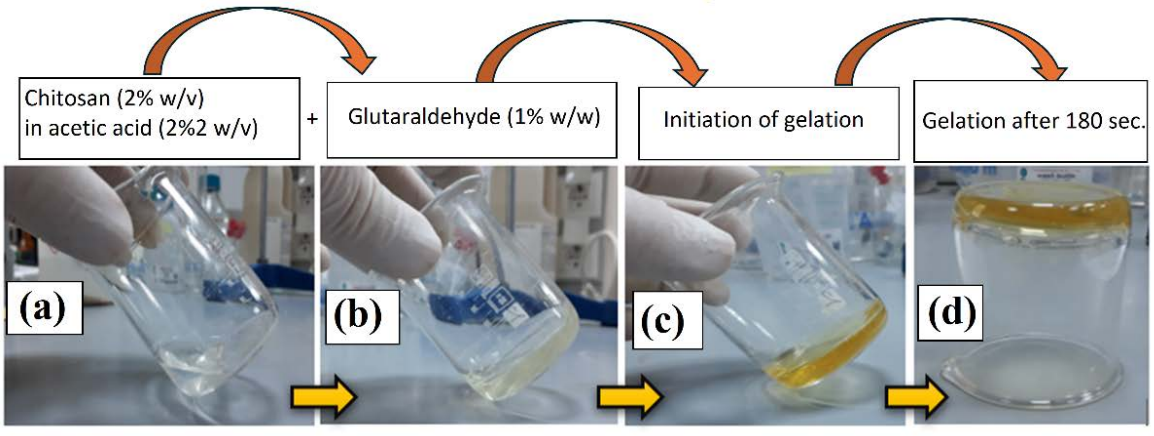

Figure 1. Illustration of the various stages involved in establishing the gelation time of medium chitosan hydrogel crosslinked with 1% glutaraldehyde

Two different molecular weight chitosan, including low and medium molecular weight, were used in this investigation. Chitosan with two different molecular weights were treated with glutaraldehyde at concentrations varying from 1% to 10% (w/w). The given hydrogels' sample codes are included in Table 1.

Table 1. Sample codes of different molecular weights chitosan-based hydrogels

GA (%) (w/w)	Specimens	
	Low Molecular Weight Chitosan Hydrogels	**Medium Molecular Weight Chitosan Hydrogels**
1	L-Chi/GA1	M-Chi/ GA1
3	L-Chi/GA3	M-Chi/ GA3
5	L-Chi/GA5	M-Chi/ GA5
7	L-Chi/GA7	M-Chi/ GA7
10	L-Chi/GA10	M-Chi/ GA10

2.2 FT-IR

Fourier transform infrared (FTIR) spectra of hydrogels were obtained using a Perkin-Elmer Spectrum 100 FT-IR spectrometer with ATR equipment in the range of 4000-650 cm^{-1}, at a resolution of 4 cm^{-1}.

2.3 Swelling Degree

Low and medium molecular weight chitosan hydrogels, at 5 different GA concentrations were cut into the same sizes and weighed. Then, the gels that were left in the swelling environments one by one were removed from these environments at certain periods, and after the excess water on them was removed with filter paper, they were carefully weighed and returned to the swelling environments. This process continued until the gel samples reached swelling equilibrium and the weights were constant. At the end of the process, the water content of the hydrogels that reached their maximum swelling value at equilibrium was calculated using Equation 2 [20].

$$S\ (\%) = \frac{w_s - w_i}{w_i} \times 100 \quad (2)$$

S (%) represents the swelling percentages of the hydrogel, w_s ; weight of the hydrogel in its swollen state, w_i ; the initial weight of the hydrogel. All swelling experiments were carried out on three samples for each GA concentration. The swelling capacities of the hydrogels were assessed in three different environments to simulate the conditions of the stomach, skin, and physiological pH levels. These environments had pH values of 2.0, 5.6 (distilled water), and 7.4, respectively.

In the present study, chitosan hydrogels were not subjected to drying using a freeze dryer or any other drying equipment. The hydrogels that were prepared were subjected to various conditions without undergoing the drying process, and their swelling properties were investigated.

2.4 Re-swelling performance of the hydrogels

Hydrogel specimens were initially dipped in distilled water for one day to allow them to reach their maximum swelling capacity. Hydrogel specimens were then weighed, dried in a vacuum oven at 50 °C, and then placed back into the swelling medium for another day. The procedure was repeated for five cycles. Meanwhile, the

swelling values of the gel samples were measured and the swelling performances after the fifth repetition were evaluated.

2.5 Dynamic mechanical analyzer (DMA)

DMA of chitosan-based hydrogels specimens were performed using a Discovery Hybrid Rheometer-1 (DHR-1). Samples were tested in uniaxial tension mode at a heating rate of 3 °C/min and a frequency of 1 Hz. The flow properties of the chitosan solutions (1% w/v) were determined with the same device at 25 °C and shear rates between 0.5 and 1000 1/s.

III. RESULTS AND DISCUSSIONS

3.1 Solubility

Chitosan solubility is directly affected by factors such as pH, molecular weight, chitin pretreatments, degree of deacetylation and process conditions (temperature, alkali concentration, processing time), ionic strength, and acetyl group distribution, so these parameters can be changed to control solubility. The solubility of chitosan is limited in water and common inorganic acids due to its high molecular weight and crystal structure. The most used solvent for chitosan is 1% acetic acid solution. One of the most critical factors influencing chitosan solubility is the medium's pH. Chitosan's free amino groups allow for protonation in pH < 6 conditions and easy dissolution via electrostatic repulsion of the cationic fields. At pH greater than 6.5, solubility stability weakens, and precipitation occurs as amino groups become less protonated [21–24]. In this study, the solubility percentages of commercially chitosan were determined according to equation 1. The solubility percentages of low and medium molecular weight chitosan were determined as 95.5% and 91.0%, respectively. It was found that increasing molecular weight causes chitosan to be more difficult to dissolve.

3.2 Hydrogel Preparations

Chitosan hydrogels were obtained because of the Schiff base reaction over the aldehyde groups (-CHO) in the structure of glutaraldehyde and the amine groups in the structure of chitosan ($-NH_2$) [25]. It was observed that the yellow color of the chitosan gel changed to orange as the glutaraldehyde concentration in the solution increased. This is the evidence of the reaction between chitosan and glutaraldehyde resulting in the formation of the Schiff base [26]. Hydrogel synthesis was prepared in a 2 ml syringe. The samples used for the swelling test were cut properly with a razor blade and then the swelling test was performed. Figure 2 illustrates the swelling of chitosan hydrogels before and after being treated with various amounts of glutaraldehyde. Also, it has been observed that increasing the cross-linking concentration in the gel content increases color darkening as well as cross-linking speed. In Figure 2a, the first two hydrogels from the left appear to be more rounded, while the next ones appear to have a more regular structure. Here too, the shapes are seen to be smoother as the cross-linker ratio increases. In addition, in Figure 2b, it is seen that the first hydrogel sample from the left cannot fully maintain its structural stability due to its lower cross-linking ratio.

Figure 2. Before (a) and after (b) swelling images of medium molecular weight chitosan hydrogels in distilled water

3.3 FT-IR

FTIR experiments were carried out on both neat chitosan and chitosan hydrogels cross-linked with 7% w/w glutaraldehyde. Figure 3. shows the FTIR spectra for 7% GA/ L-CS and 7%/GA-CS hydrogels.

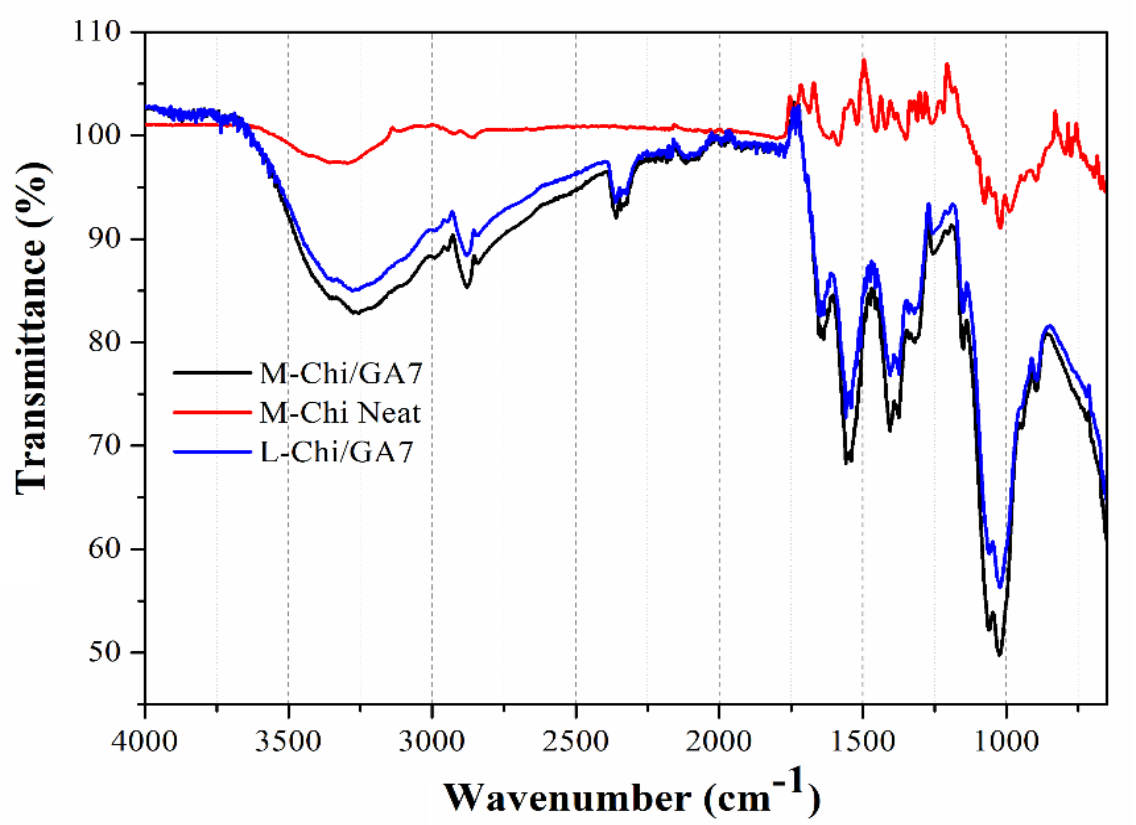

Figure 3. Fourier transform infrared spectra of neat medium molecular weight chitosan (M-Chi), M-Chi/GA7 and L-Chi/GA7 hydrogels

FT-IR spectrum (Figure 3) shows that a broad band ranging from 3500 to 3000 cm^{-1}, which is ascribed to the presence of N-H and O-H hydroxyl groups [1]. An analysis of the data presented in Figure 3 reveals a narrow band at 2880 cm^{-1} corresponding to the C-H asymmetric stretching bond, with sharp peaks in the range of 1677-1566 cm^{-1} attributed to the Amide II groups, indicative of N-H bending vibrations in free amine ($-NH_2$) groups [27]. Upon examination of the FTIR spectra of hydrogels cross-linked with 7% GA, minor peaks are observed at the 1640 cm^{-1} band. The peaks are corresponded to the C=N imine bonds formed between chitosan and GA [28]. The existence of this band validates the Schiff reaction, indicating covalent cross-linking between chitosan and GA. The intensity of the N-H bending vibration band of primary amines, located at 1650-1550 cm^{-1}, diminishes and changes to a lower wavenumber. This is ascribed to the diminished intensity of the N-H band associated with the decreasing $-NH_2$ groups due to the interaction of chitosan with GA. As the GA ratio rises, the intensity of the bands at 1058-1026 cm^{-1} increases, while the bending vibration band of $-NH_2$ is observed at 620 cm^{-1} [29]. FTIR study results support the cross-linking interaction between chitosan and GA. Upon examination of the FTIR spectra of M-Chi and 7% GA cross-linked chitosan hydrogels depicted in Figure 3, it is evident that there is no substantial variation in the spectra of hydrogels samples of differing molecular weights.

3.4 Swelling of Hydrogels

The swelling properties of the hydrogels whose swelling properties were examined without drying were different from the swelling properties of the dried hydrogels published in the literature [9]. The pH of a healthy human epidermal layer is naturally maintained at a slightly acidic level, with the skin's surface ranging from 4.5 to 5.3. The pH of wounds' microenvironment is naturally more alkaline due to the disruption of the acidic environment caused by trauma, which exposes underlying tissues with a physiological pH of 7.4 [30]. pH values of 2.0, 5.6, and 7.4 were selected as ideal options to mimic the acidity levels seen in the stomach, skin, and physiological environments. Figure 4 and Figure 5 show how the chitosan hydrogels swollen when they were put in a medium made by adding NaOH to distilled water to increase the pH to 7.4. Upon analyzing the figures provided, it was seen that higher concentrations of cross-linkers led to a notable decrease in the water absorption percentages of gels. In general, the swelling of hydrogels in distilled water is lower than the swelling values in acid solutions. This situation, resulting from the cationic structure of chitosan, which does not undergo protonation in neutral and basic states, also reduces swelling values with the effect of increasing the GA ratio [31]Based on research by Hoffman et al. (2012) and Peppas et al. (2000), hydrogels can swell up to thousands of times their original size. This can happen depending on the pH and temperature of the environment, as well as the ionic strength of the environment [32, 33]. Berger et al. (2004) reported that the swelling capacity of chitosan-based hydrogels depends on the ambient pH as well as the cross-linker density, and that as the cross-link ratio increases, swelling values decrease and gel stability improves [34]. In contrast to the literature, the gels utilized in this investigation were synthesized, that is, without drying. In the present study, the higher swelling of chitosan gels in distilled water compared to pH 7.4 is related to the ionic strength of the water. Upon immersion in pH 7.4 phosphate buffer, the produced gels experienced water loss instead of swelling (results were not presented). Salts in the buffer solution increased the chemical potential of the solution. The water in the chitosan hydrogels in this solution moved out of the gel to decrease the chemical potential. Therefore, instead of swelling the gel in the phosphate buffer, the gel shrank, and the swelling percentage was negative. Water diffuses towards the side with a higher ionic strength. Thus, the pH of distilled water was adjusted to 7.4 by introducing NaOH to the distilled

water. Lower pH levels were expected to result in a greater swelling value. Nevertheless, when pH 2.0 was once again created using an acetate buffer, the expansion of the wet gel was significantly lower than anticipated. Hydrogels prepared with low-molecular-weight chitosan exhibit greater water retention percentages compared to hydrogels produced with medium-molecular-weight chitosan. As the concentration of cross-linkers in the hydrogels increased, the degree of swelling in the hydrogels reduced, as seen in Fig. 4 and Fig. 5.

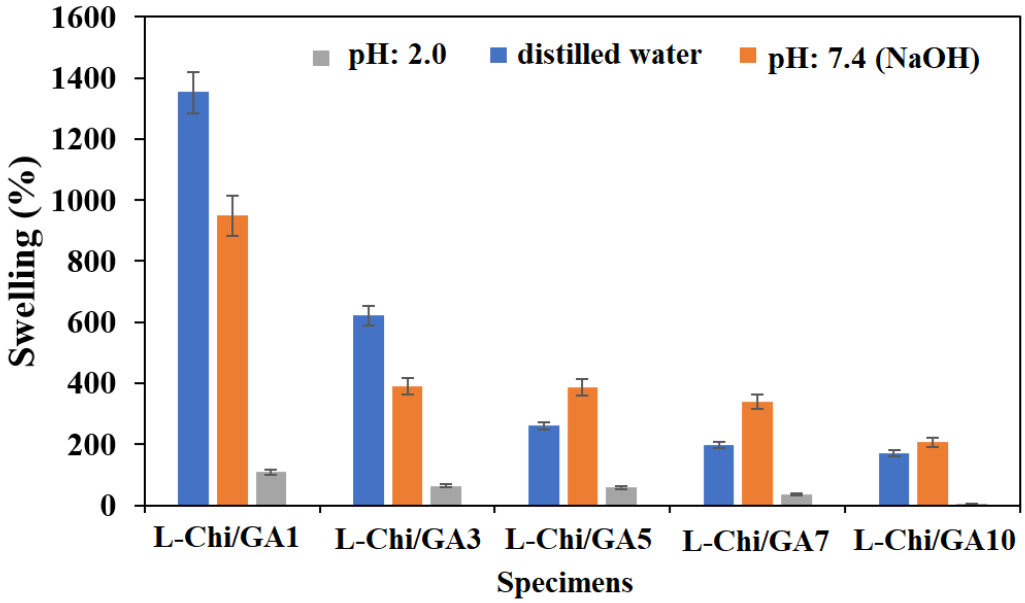

Figure 4. Swelling values of low molecular weight chitosan-based wet* hydrogels in distilled water and at different pHs after 24 hours (* wet hydrogel means that the hydrogels were not dried)

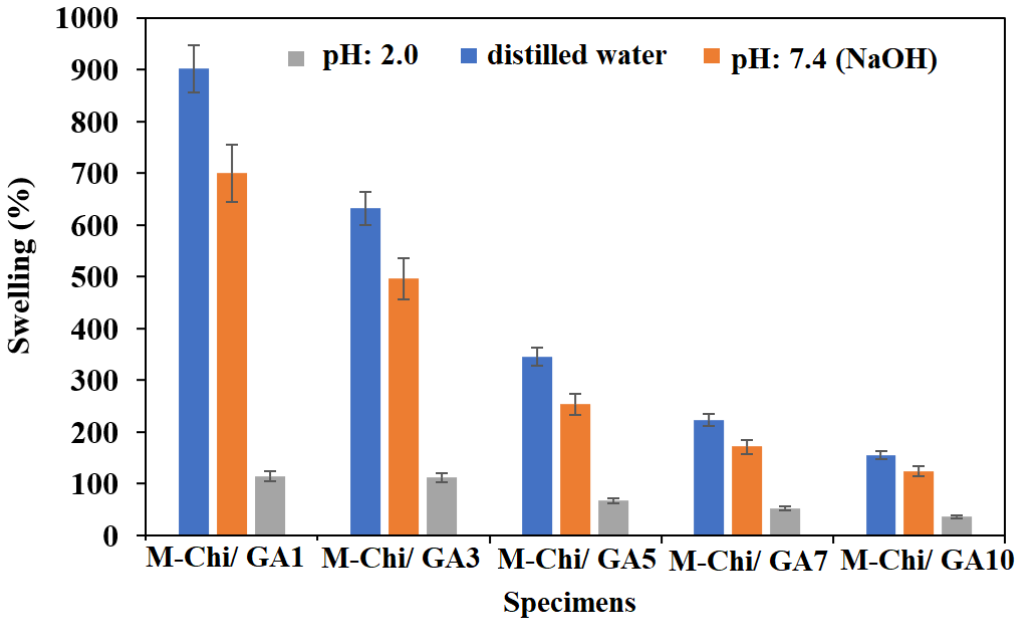

Figure 5. Swelling values of medium molecular weight chitosan-based wet hydrogels* in distilled water and at different pHs after 24 hours (* wet hydrogels means that the hydrogels were not dried)

3.5 Re-Swelling Performance of the Hydrogels

In this study, it was aimed to investigate the effect of the swelling percentage in chitosan hydrogels when immersed in distilled water on the swelling performance after repeated use. The effect of hydrogels prepared with low- and medium-molecular-weight chitosan on the reswelling performance is shown in Figures 6 and 7, respectively. The hydrogels, which reached maximum swelling equilibrium, were dried in a vacuum oven and placed back in the swelling environment, allowing them to swell again. This process was repeated 5 times. Chitosan gels cross-linked with GA revealed a swelling performance ranging from approximately 160% to 1350% in distilled water for both molecular weights. Although there was a slight decrease in the swelling performance of the hydrogels after the first use, there was no significant change in their subsequent re-swelling performance.

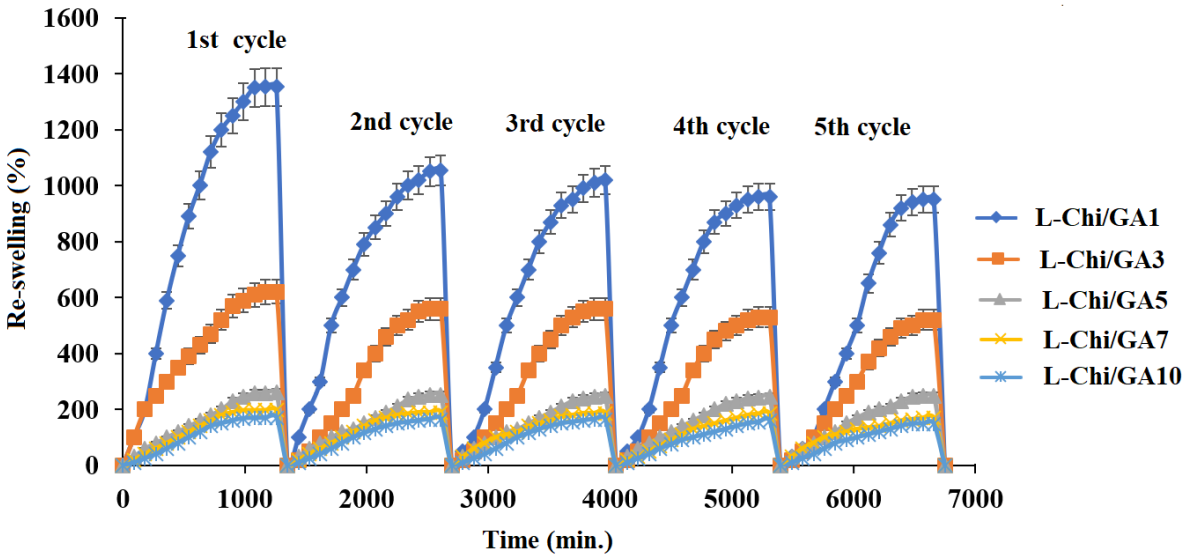

Figure 6. Change of re-swelling performance of Low molecular weight chitosan hydrogels prepared in different GA ratios in distilled water over time after 5 cycles

When the presented graphics are examined (Figure 6 and 7), it is seen that there is a decrease in swelling values with an increasing GA ratio for each cycle. As the crosslinking density increases in the hydrogel network, the hydrogel molecular network becomes denser. Relatively denser crosslinking causes a more rigid hydrogel structure, and this limits the swelling properties of the hydrogels. This increased crosslinking also affects the pore sizes of the hydrogel indirectly and reduces. While there was a noticeable difference in swelling percentages between the first and second uses, there was no substantial difference in the findings from the other uses, and the values were nearly identical. This situation; it has been explained that the hydrophilic groups in the structure of the gels, which are kept in the vacuum oven to be dried after the first use, reduce the swelling rate slightly by releasing the water trapped within them under the influence of heat, but as a result, the gel becomes more stable and firm, and there is no significant difference in the swelling rates in subsequent applications. In addition, the fact that no significant difference was observed in the swelling of the hydrogels after the 2nd cycle may also be due to the removal of unbound free polymers or the breakage of the pieces due to excessive swelling

in the first and second cycles swellings. According to Shu et al. (2001), certain ions and secondary parameters influence the water absorption and release properties of chitosan hydrogels. These elements provide a protective barrier that hinders interactions within the networked structure and reduces swelling [35].

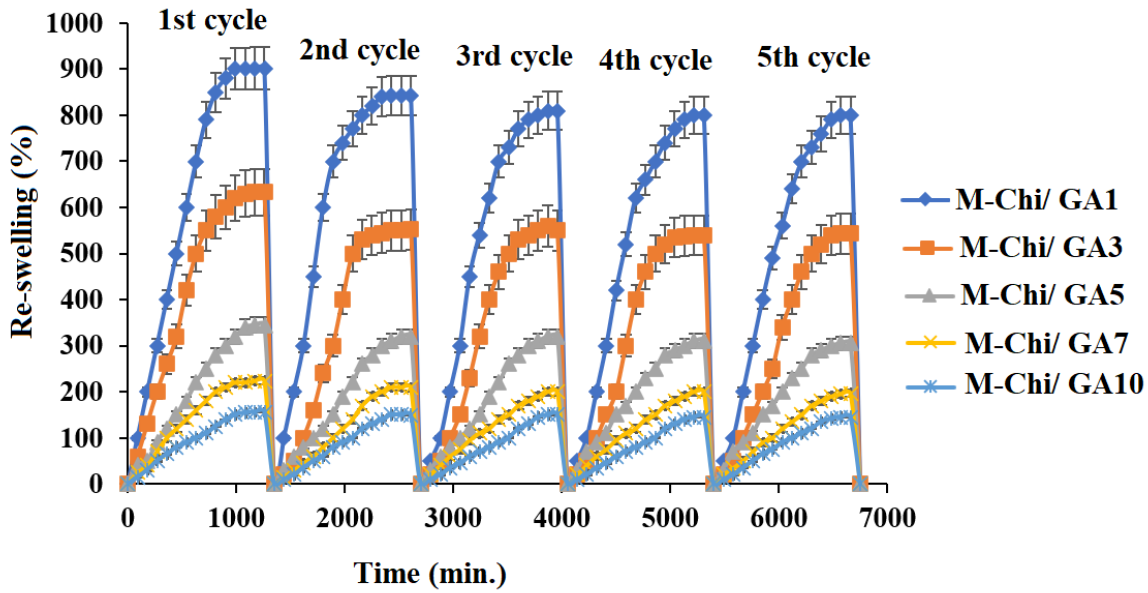

Figure 7. Change of re-swelling performance of Medium Mw chitosan hydrogels prepared in different GA ratios in distilled water over time after 5 cycles

3.6 Dynamic mechanical analysis (DMA)

Dynamic mechanical analysis (DMA) provides measurements of the storage or elastic modulus (G') and the loss or viscous modulus (G"), as well as the mechanical loss tangent (tan delta = G"/G'). The G' values indicate the amount of energy stored in the material under deformation stress, whereas G" characterizes its viscous nature [36, 37].

We used a Discovery Hybrid Rheometer-1 (DHR-1) to find out how the chitosan hydrogels behaved when they were heated from room temperature to 40 °C. In Figures 8 and 9, the storage modulus values of hydrogels prepared with low and medium molecular weight chitosan are shown versus temperature. Since the experiments were conducted at a temperature below the glass transition temperature of chitosan, the storage modulus values did not change depending on the temperature. Because chitosan hydrogels are often utilized at room temperature or somewhat higher, no experiments were undertaken at temperatures near to the glass transition temperature of chitosan. When the number of cross-linkers in the hydrogel is low, the storage modulus is also lower. However, when the percentage of cross-linker ratio increases, the elastic modulus rises as well, resulting in a higher consistency of the hydrogel. But the amount of water in hydrogels has a big effect on their elastic modulus values, and the changes that have been seen cannot be explained by changes in the structure of the molecules [36]. In this study, the storage moduli of both categories of chitosan hydrogels remained relatively stable as temperature increased. The highest storage modulus values found were 0.01 MPa for low molecular weight chitosan hydrogels made with 10% glutaraldehyde and 0.02 MPa for medium molecular weight chitosan hydrogels.

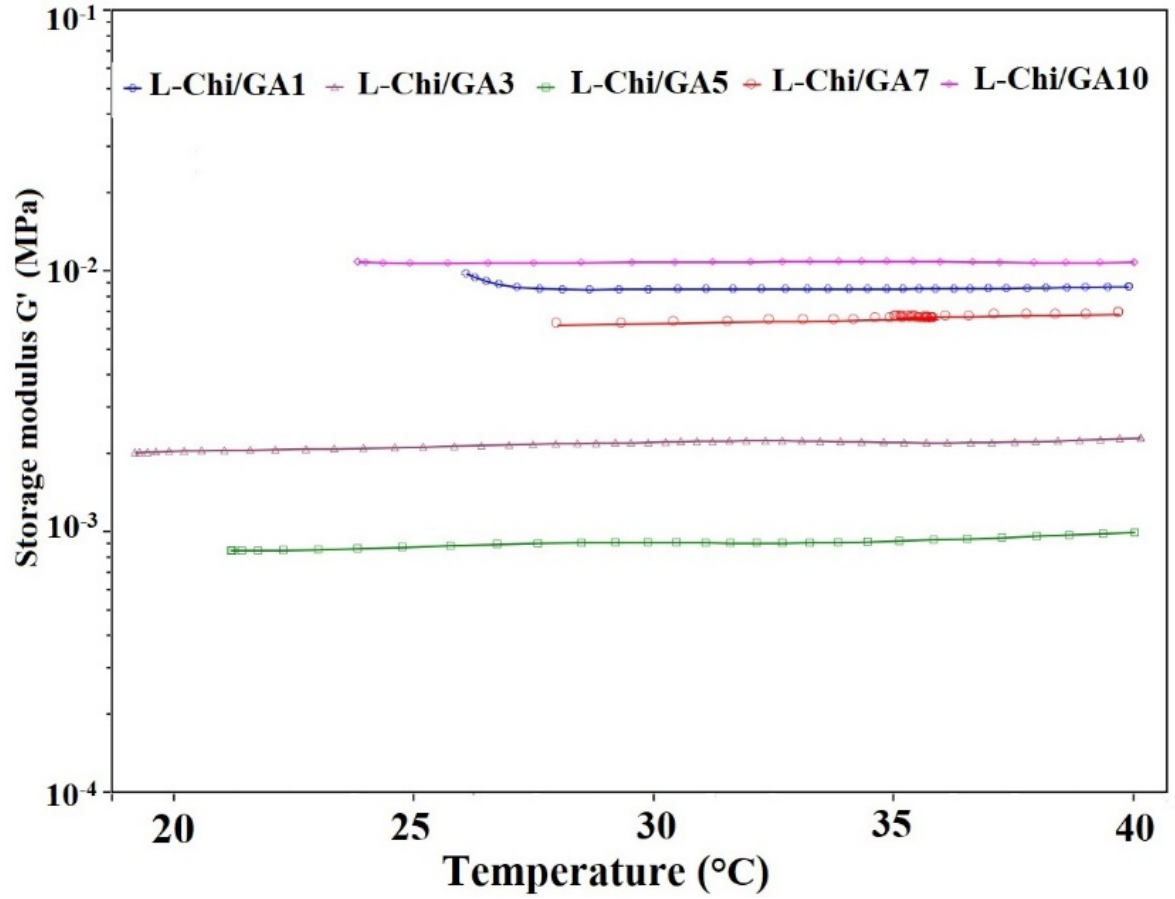

Figure 8. Storage modulus (G′) of low molecular chitosan hydrogels crosslinked with glutaraldehyde

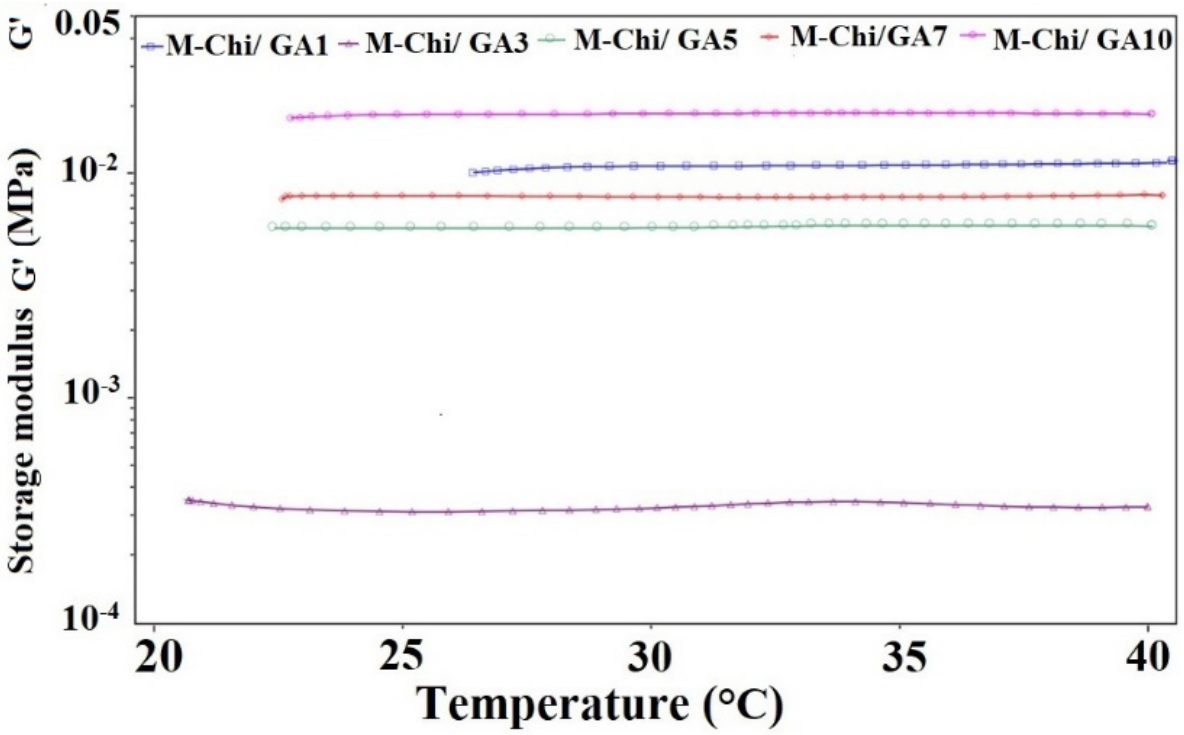

Figure 9. Storage modulus (G′) of medium molecular chitosan hydrogels crosslinked with glutaraldehyde

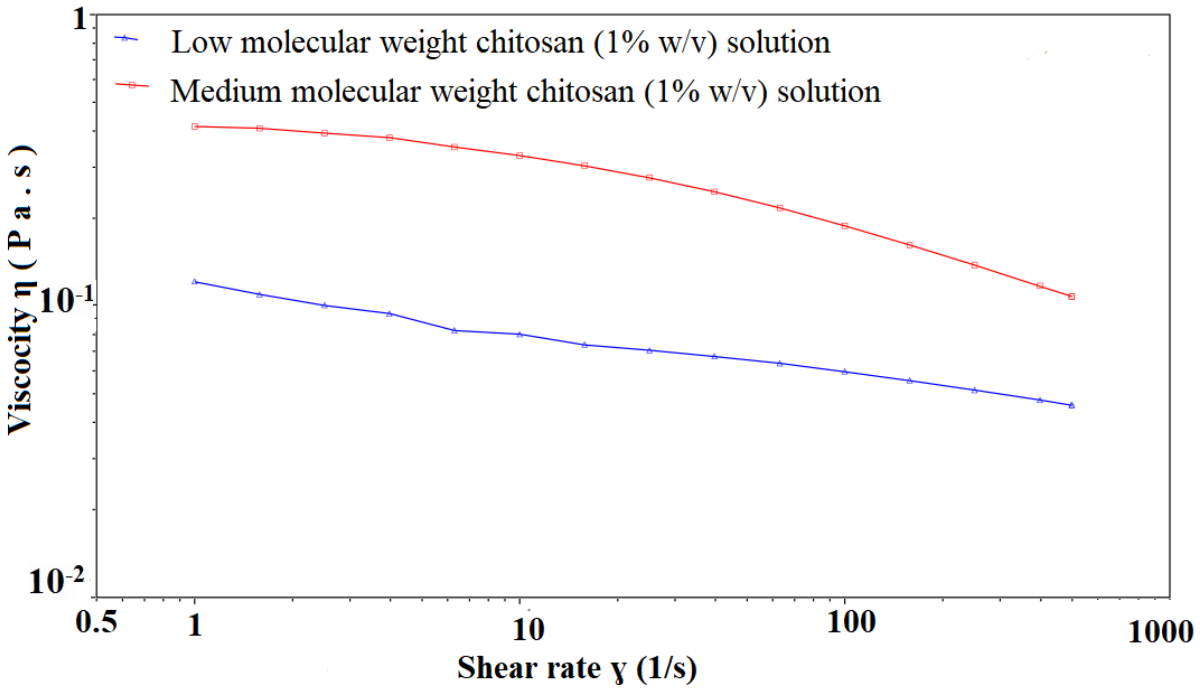

Figure 10. Viscosity of low and medium molecular weight chitosan solutions

Figure 10 displays the rheogram of chitosan solutions obtained using a Discovery Hybrid Rheometer-1 (DHR-1) at a temperature of 25 °C and shear rates ranging from 0.5 to 1000 1/s. It was observed that the viscosity of the chitosan solutions prepared with medium molecular weight chitosan (1% w/v) was higher than the chitosan solutions prepared with low molecular weight chitosan (1% w/v) due to the higher molecular weight effect. The viscosity value of chitosan solutions is affected by many factors, such as molecular weight, deacetylation degree, and ionic strength. These values may vary depending on the chitosan concentration in the solution as well as the acid environment in which the solution is prepared [24, 38]. The viscosity values of commercially used low molecular weight chitosan (%1 wt.) vary between 20-300 centipoise (cps), and the viscosity values of medium molecular weight chitosan (%1 wt.) vary between 200-800 cps in a 1% acetic acid solution at 25 °C [39]. Although high molecular weight chitosan (%1 wt.) has viscosity values of 2000 cps and above, they are not preferred in medical applications as the high viscous solutions to be obtained will cause processing difficulties. In addition, we employ other equipment to measure the viscosity of chitosan solutions. We also used a Brookfield Viscometer to measure the viscosity of low and medium molecular weight chitosan solutions in our study. The values we got were 101.5 and 333.5 cps, sincerely.

IV. CONCLUSIONS

The chitosan hydrogels, cross-linked with glutaraldehyde at different concentrations ranging from 1% to 10%, had their highest swelling values after 24 hours. Hydrogels made with both Low and Medium molecular weight chitosan exhibited greater swelling values when containing 1% glutaraldehyde compared to the other hydrogels. The study found that higher concentrations of cross-linkers led to a notable decrease in the water absorption percentages of hydrogels. The re-swelling properties of both hydrogel groups were found to be highly stable

throughout 5 cycles. The loss in swelling qualities was significantly reduced. These hydrogels are prepared chemically cross-linked to remain stable and resistant to re-swelling. The rehydration capability of hydrogels made with medium molecular weight chitosan was more consistent compared to hydrogels made with low molecular weight chitosan.

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