

CHITIN HYDROGEL PREPARED AT VARIOUS TEMPERATURE OF WATER VAPOR-INDUCED PHASE INVERSION

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Chitin solutions were obtained by dissolving chitin extracted from crab shell waste in *N,N*-dimethylacetamide (DMAc) solvent, in the presence of 5, 7 and 10% lithium chloride (LiCl). Then, the concentrated chitin solution was subjected to water vapor-induced phase inversion to prepare the chitin hydrogel. The properties of the resultant chitin hydrogel having various LiCl concentrations were determined after coagulating at different temperatures of the water vapor, namely, 5, 20 and 60 °C. The viscosity of the chitin solutions decreased when the temperature increased, suggesting the formation of the hydrogel could be accelerated by applying heat. After the chitin solution was coagulated, the resultant hydrogels prepared at higher temperature of water vapor provided better properties than those of the others. When the concentration of LiCl in the hydrogel was 7%, the water content values decreased from 407 to 359%, and the tensile strength increased from 447 to 737 kPa as the temperature was adjusted from 5 to 60 °C, respectively. As for viscoelasticity, the storage modulus of the chitin hydrogels indicated that the increment in the LiCl concentration to 7% and the water-vapor temperature of 60 °C could form a tighter structure of the hydrogels. Considering the green preparation route of the chitin hydrogels and their obtained characteristics, they can be considered as promising materials for applications where such properties required, such as in the biomedical and agricultural sectors.

Keywords: bio-polymer, chitin, hydrogel, phase inversion

INTRODUCTION

Phase inversion, a process by which a polymer is transformed from a liquid or soluble state to a solid state, is the most versatile technique to prepare polymeric membranes. The concept of phase inversion covers a range of different techniques, such as immersion precipitation, thermally induced phase separation, vapor-phase precipitation and precipitation by controlled evaporation.¹ So far, phase inversion has been proven successful in preparing hydrogels, which are hydrophilic materials, containing an excess amount of water inside their three-dimensional structure.² Especially biopolymer-based hydrogels obtained by the phase inversion process have demonstrated good properties, such as biocompatibility, high strength, and flexibility in applications.³⁻⁷

Owing to the burgeoning interest in environmental issues, there is now greater emphasis on green routes for preparing inexpen-

sive and environmentally benign materials from biomass-generated polymers. Biomass can play a pivotal role in the production of high-quality carbon-neutral fuels, as well as provide regenerable feedstock for various industries. Biomass-based feedstocks can include a variety of residues from agriculture, forestry, wood processing and food processing industries, as well as municipal solid wastes and other industrial wastes.

As a by-product of the seafood processing industry, crustacean shells are a well-known biomass material. In developing countries, waste shells are often just dumped in landfills or into the sea. Chitin is the second most abundant polysaccharide, widely available as a structural component of crustacean shells. The chemical structure of chitin contains β -(1 \rightarrow 4)-*N*-acetyl-D-glucosamine units (Fig. 1). Chitin is well-known due to its high reactivity, owing to the presence of

functional groups of hydroxyls and amines, as well as by its poor solubility in most common solvents.⁸ Normally, the chemical structure of chitin is stabilized by its strong hydrogen bonding networks including inter- and intra-molecular bonds.⁹ However, because of its poor solubility, the applications of chitin are still limited.

Interestingly, the lithium salt dissolved in *N,N*-dimethylacetamide (DMAc) has been investigated as a green solvent system to obtain chitin solutions at room temperature.^{5,6} It has been reported that the concentration of LiCl in DMAc solution and the content of chitin have strong impacts on the properties of the resulting chitin hydrogels prepared by the water vapor phase inversion process at 20 °C.¹⁰ In addition, the effect of the coagulation water bath temperature on the variation in morphology and other characteristics of chitin hydrogels, as well as on the chemical formation of chitin fibers, has been studied.¹¹ In this latter case, the hydrogels were prepared by phase inversion via direct immersion precipitation. To the best of our knowledge, no studies have clearly defined the effects of water vapor temperature during the water vapor phase inversion process on the physical properties of chitin hydrogels. Therefore, the aim of the present research has been to fill this gap, by examining the formation of chitin hydrogels prepared by the water vapor phase inversion process, with different LiCl contents and chitin concentrations, and the effects of the water vapor temperature during the process on the basic physical properties of the resultant chitin hydrogels.

EXPERIMENTAL

Materials

Crab shells were collected from snow crabs (genus *Chionoecetes*) in Teradomari, Niigata, Japan. All chemicals used in the preparation of chitin hydrogels were of analytical grade. The chemicals were used without any further purification: hydrochloric acid (HCl), sodium hydroxide (NaOH), potassium hydroxide (KOH), *N,N*-dimethylacetamide (DMAc), lithium chloride (LiCl) and ethanol, which were

products of Nacalai Tesque, Inc. (Tokyo, Japan). Prior to the use of DMAc, the solvent was stored with KOH for over 5 days and LiCl was dried in a vacuum oven at 80 °C in 24 h to remove traces of moisture.

Method

Preparation of chitin extracted from crab shells

Considering the increasing interest in the utilization of renewable feedstocks, chitin was chemically extracted from crab shell waste, following a previously described method, with modifications.¹⁰ About 10 g of crab shells were treated with 300 mL of 1N HCl aqueous solution for 24 h at room temperature to remove mineral compounds from the crustacean shells. The acidic-treated crab shells were then immersed in 300 mL of 1N NaOH at 90 °C for 5 h to eliminate the protein matter. To remove pigments, 300 mL of ethanol was added into the pretreated crab shells at 60 °C for 6 h. After each step, the solid shells were filtrated and washed in an excess amount of water to reach neutral pH. The purified chitin from crab shells was dried in an oven at 80 °C for 24 h. As shown in Figure 2 (a), the reddish crab shells turned into white fragments of chitin, with an obtained yield of 42%.

Preparation of chitin hydrogels with different LiCl content and chitin concentrations at various water vapor temperatures

The chemically treated chitin was dissolved in DMAc solution, with the addition of varied LiCl contents – at 5, 7 and 10%. After that, the concentrated mixtures were constantly stirred until a viscous solution was obtained. During this process, the adjustment of the chitin concentration in the solution was performed at 2%. Then, these solutions were centrifuged at 9000 rpm for 1 h to remove undissolved segments. For the preparation of the chitin hydrogels, about 10 g of each chitin solution was poured into glass dishes with a diameter of 91 mm, which were then placed in a container filled with 40 mL of deionized water; the temperature of the water was set to 5, 20 and 60 °C. After 24 h in which the phase inversion process took place, the chitin hydrogels prepared with different LiCl/DMAc solutions and water-vapor temperatures were removed from the dishes and washed in an excess amount of water to remove any traces of solvent before any measurements.

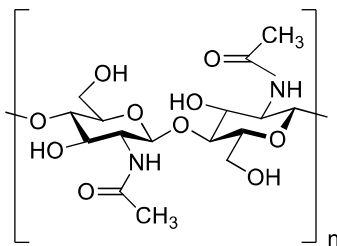


Figure 1: Chemical structure of chitin

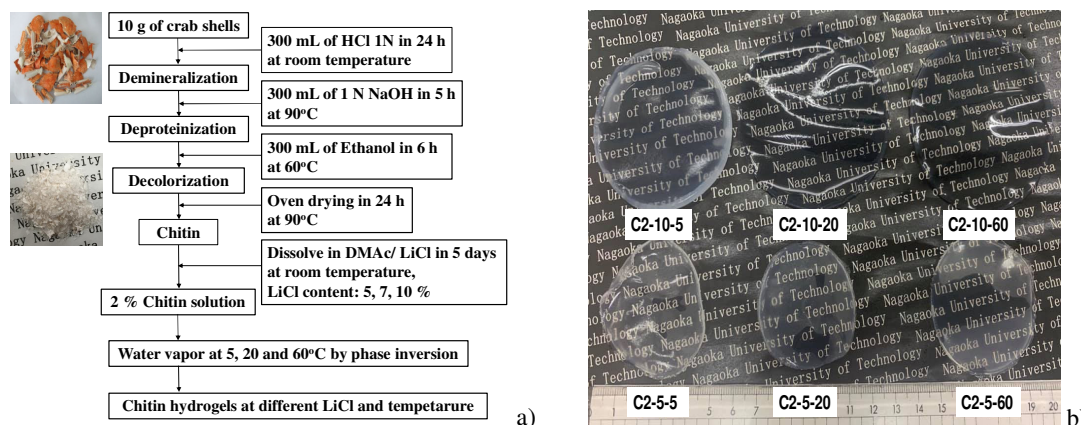


Figure 2: (a) Flowchart of chitin hydrogel preparation process, (b) Appearance of 2% chitin hydrogels prepared with 5% and 10% LiCl content at 5, 20 and 60 °C water-vapor temperature

The samples were denoted as C2-(LiCl content)-(temperature). For example, the chitin hydrogel prepared with 5% LiCl in DMAc solution and coagulated after processing at 60 °C water-vapor temperature was named as C2-5-60. The appearances of the extracted chitin and chitin hydrogel prepared with 5% LiCl/DMAc and 2% chitin concentration at different water-vapor temperature are presented in Figure 2 (b).

Evaluation of chitin solutions and hydrogels

The viscosity of the chitin solutions obtained at different LiCl contents in the DMAc solution was measured as a function of temperature, in the range from 5 to 60 °C, by dropping about 3 mL of each chitin solution on the metal plate of a Physica MCR 301 rheometer (Anton Paar). The shear rate was constant at 1/s.

The values of water content (WC) values of the hydrogels were measured at room temperature by immersing 30 mm x 10 mm strips of the dry hydrogels in distilled water. After a soaking period of 24 h, to ensure equilibrium sorption, the hydrogels were removed from the water, quickly wiped with tissue paper and finally weighed. The value of WC was calculated for each sample using the equation:

$$WC(\%) = (m - m_0) \times 100 / m_0 \quad (1)$$

where m_0 is the dry weight and m is the weight of the hydrogels after 24-h immersion in distilled water.

The mechanical properties of the resulting hydrogels were evaluated in terms of tensile strength and viscoelasticity. In tensile strength evaluation, the test was carried out using an LTS-500N-S20 testing machine (Minebea, Japan), with an operating head load of 500 N at 23 °C and 50% RH. The wet hydrogel specimens (40 mm x 10 mm) were placed between the grips of the testing machine. The initial length was 20 mm, and the speed of testing was 2 mm min⁻¹ till the

sample was broken. The values of tensile strength and elongation were calculated using the following equations:

$$\text{Tensile strength (N/mm}^2\text{)} = \text{Maximum load} / \text{cross-sectional area} \quad (2)$$

$$\text{Elongation (\%)} = (\text{Elongation at rupture} / \text{initial gauge length}) \times 100 \quad (3)$$

The viscoelasticity of the wet hydrogels prepared at different water vapor temperature, from 5 to 60 °C, was assessed at 20 °C to obtain the storage (G') and loss modulus (G'') of the samples as a function of the strain (%) from 0.01 to 100%, at the frequency of 1 Hz, using the Physica MCR 301 rheometer (Anton Paar).

RESULTS AND DISCUSSION

Viscosity of the chitin solution prepared at different LiCl contents and temperature

The temperature of the water vapor may play an extremely important role in the formation of the chitin hydrogels, as it can accelerate the coagulation process of the obtained materials during the phase inversion process. As can be seen in Figure 3, the concentrated chitin solution prepared with 2% chitin content showed higher values of viscosity, with increasing LiCl contents from 5 to 10%. For example, the viscosity observed at 30 °C of C2-5, C2-7 and C2-10 was 12900, 146267 and 16067 cP, respectively. It has been proved that a high amount of LiCl in DMAc is able to form the macro-cation (DMAc-LiCl-DMAc-Cl⁻), which then acts as a cross-linker in the chitin solution. Therefore, the resultant viscosity was enhanced while the LiCl concentration in the 2% chitin solution was changed from 5 to 10%. Similar results have been obtained in other studies.^{4,10} However, when the applied temperature was also increased from 5 to

60 °C, the relative viscosity decreased, and this phenomenon was observed in the case of all the samples. When the chitin solution was prepared with 7% LiCl in the mixture, the viscosity measured at 5 °C was 24400 cP, while lower values were seen at 20 and 60 °C – namely, 18067 and 4946 cP, respectively. This phenomenon indicates that higher temperature may reduce the adhesive forces between polymer molecules, which accelerated the evaporation of the solutions to fasten the formation of chitin hydrogels.¹¹ Consequently, similar values of viscosity of chitin solutions prepared at different LiCl contents were recorded at 60 °C due to the coagulation of the chitin gels.

Appearance of chitin hydrogel prepared with various LiCl contents and water-vapor temperature

Images of the chitin hydrogels prepared at various LiCl contents and water-vapor temperature are presented in Figure 2 (b). It could be observed that the round shape of the hydrogel was somehow deformed in the case of the samples prepared at 5 °C water-vapor temperature. The edges and the whole surface of the hydrogels were not flat, as seen in the case of the samples prepared at 20 and 60 °C. It may be explained by the fact that the low temperature caused shrinking of the hydrogel samples. Hence, the shape was not well-maintained. Due to the occurrence of the macro-cation at high concentration of LiCl (10%) in the chitin solution, the diameter of the C2-10 hydrogels was larger than those of C2-5 and C2-7, with no influence of the water-vapor temperature.¹⁰ This may be due to

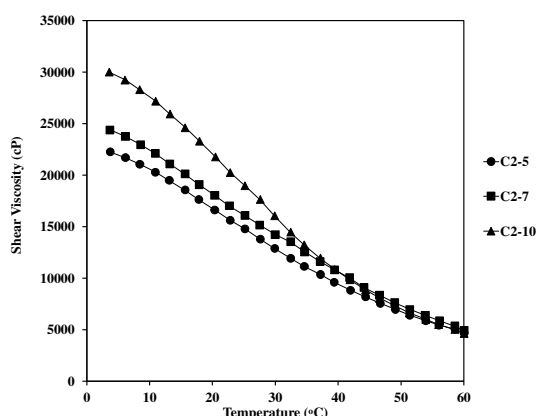


Figure 3: Viscosity of chitin solutions prepared at different LiCl contents and temperature

the interaction between free water molecules and the Cl⁻ ion in the macro-cation at 10% LiCl contents. However, the transparency of C2-10-5 was lower than that of the other samples prepared with the same amount of LiCl and chitin concentration, owing to the lack of solvent exchange during the phase inversion process. These results suggest that the physical characteristics of the chitin hydrogels can be affected by the studied parameters.

Physical properties of chitin hydrogel prepared with various LiCl contents and water-vapor temperature

The hydrophilicity of materials is one of the most important indicators of hydrogel properties. Figure 4 shows the water content (WC) of the chitin hydrogel achieved with different LiCl contents and at various water-vapor temperature. It has been reported that, after the water-washing process, the increment in LiCl amount in the chitin solution could lead to a reduction in the WC of biopolymer-based hydrogels.^{4,10} As a result, the WC of C2-5-5 was 412% and that of C2-10-5 was 343%. The amount of WC in the hydrogel could decrease when it was prepared at 60 °C water-vapor temperature. There was no doubt that the WC values of C2-5-60 and C2-10-60 were lower than those of the hydrogels fabricated at 5 °C. This result may suggest that a denser structure of the prepared hydrogels was obtained when the higher water-vapor temperature was applied during the phase inversion process. As seen, when the water-vapor temperature was varied from 5 to 60 °C, the WC values of C2-7 decreased from 407 to 359%.

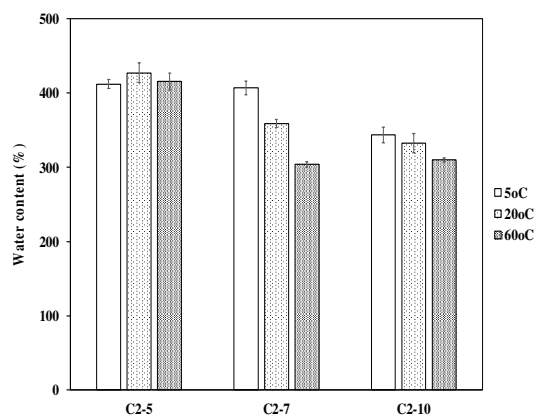


Figure 4: Water content of 2% chitin hydrogels prepared at different LiCl contents and temperature

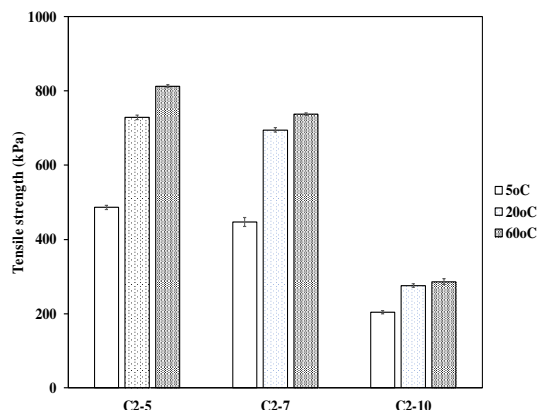


Figure 5: Tensile strength of 2% chitin hydrogels prepared at different LiCl contents and temperature

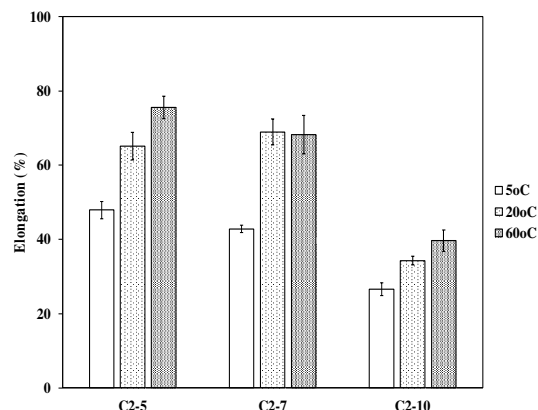


Figure 6: Elongation of 2% chitin hydrogels prepared at different LiCl contents and temperature

Table 1
Characteristics of the chitin hydrogel prepared with various LiCl content

Sample	Water content (%)	Tensile strength (kPa)	Elongation (%)
C2-5-5	412 ± 6	486 ± 5	48 ± 2
C2-5-20	427 ± 13	729 ± 6	65 ± 4
C2-5-60	415 ± 11	812 ± 4	76 ± 3
C2-7-5	407 ± 9	447 ± 12	43 ± 1
C2-7-20	359 ± 6	695 ± 6	69 ± 4
C2-7-60	359 ± 4	737 ± 4	68 ± 5
C2-10-5	343 ± 11	205 ± 4	27 ± 2
C2-10-20	332 ± 13	276 ± 5	34 ± 1
C2-10-60	310 ± 3	286 ± 8	40 ± 3

Figure 5 illustrates the tensile strength of the 2% chitin hydrogels prepared with 5, 7 and 10% LiCl content under different water-vapor temperature. The results indicate that the tensile strength was dependent on the applied water-vapor temperature. As observed, the hydrogel obtained at 60 °C water-vapor temperature during the solvent exchange process performed better in terms of mechanical properties than the others. For example, the C2 chitin hydrogel with 5% LiCl amount generated higher tensile strength – of 486 kPa at 5 °C and of 812 kPa at 60 °C. This evidence was supported by the WC values of C2-5-5 and C2-5-60, as listed in Table 1, suggesting the related hydrogel's structure may be denser, leading to a reduction in its water absorption ability. However, C2-10 was found to exhibit the weakest mechanical strength – of 205 kPa when prepared at 5 °C water-vapor temperature. Increasing the temperature up to 60 °C was able to enhance the tensile strength value of C2-10-60 to 286 kPa. In addition, the elongation results were attributed to the dose-dependence of LiCl/DMAc in chitin solutions (Fig. 6). Higher LiCl concentrations in the DMAc/chitin solution

could reduce the flexibility of the hydrogels. The enhancement in the water-vapor temperature led to an increase in the elongation values of the prepared chitin hydrogels. For instance, the elongation at break was 76% for C2-5-60 and 48% for C2-5-5. Thus, by way of conclusion regarding the physical properties, it can be stated that higher water-vapor temperature during the formation of chitin hydrogels provided better mechanical strength, with a reduction in hydrophilicity.

The basic physical properties of the chitin hydrogels prepared with different LiCl contents at various water-vapor temperature were summarized in Table 1.

To further study the effect of water-vapor temperature during the phase inversion process on the mechanical properties of the chitin hydrogels, viscoelasticity measurement was performed at 20 °C as a function of strain from 0.01 to 100%. As could be seen in Figure 7, when the temperature of the applied water vapor was increased from 5 to 60 °C, the storage modulus (G') gradually enhanced, with the presence of up to 7% LiCl content in the DMAc solution. At 1% of strain,

the G' increased from 64.2 kPa for C2-5-5 to 215.1 kPa for C2-5-60. When the LiCl content used was of 7%, the G' reached 87.9 kPa and 392.5 kPa for the chitin hydrogels prepared at 5 and 60 °C water-vapor temperature, respectively. However, when the strain exceeded 1%, the C2-5 hydrogels showed higher values of G' than those of C2-7, the lowest being recorded for C2-10.

When the water vapor was applied at 60 °C and the strain was 1.47%, G' values of 59.4 kPa and 53.1 kPa for C2-5 and C2-7, respectively, were recorded. The viscoelasticity results indicated that higher water vapor temperature during the preparation led to higher hardness of the chitin hydrogel structure.

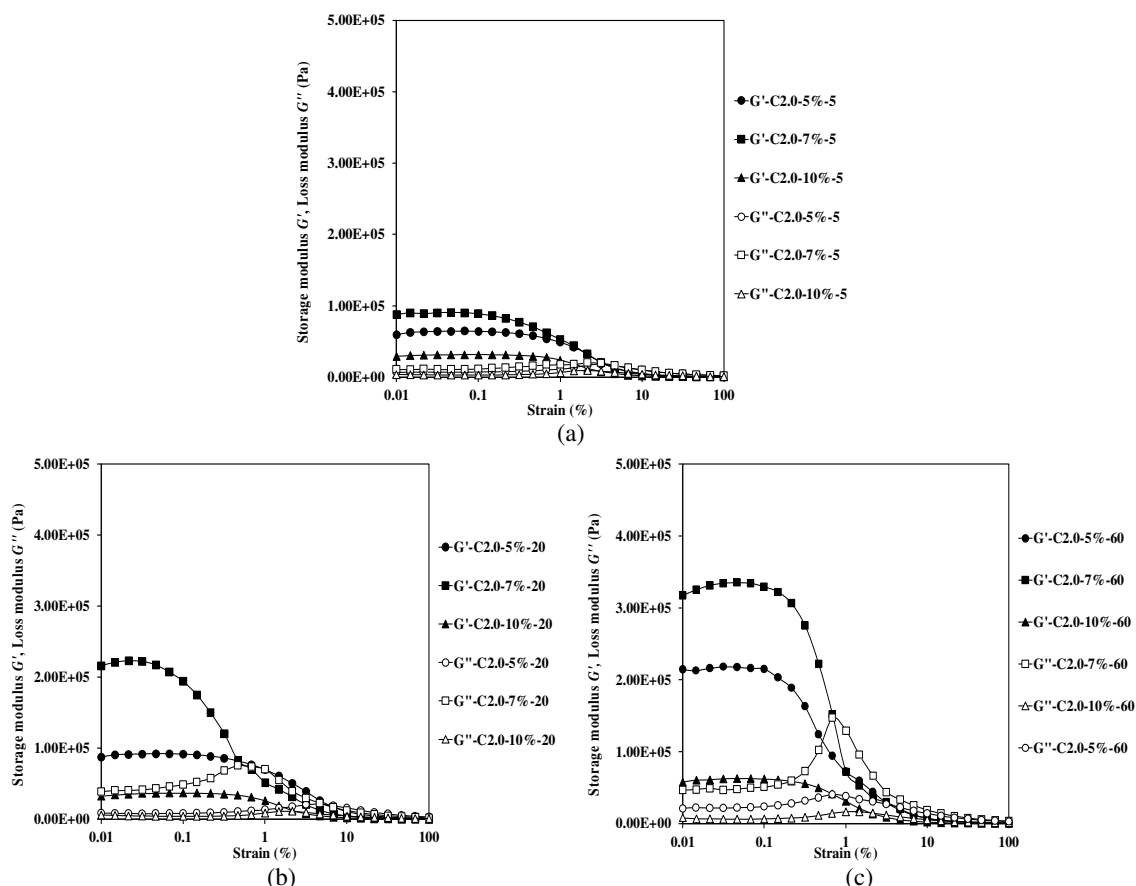


Figure 7: Viscoelasticity of chitin hydrogels prepared at different LiCl contents and temperature: (a) 5 °C, (b) 20 °C and (c) 60 °C

CONCLUSION

In the present study, the preparation of chitin hydrogels with different LiCl contents was successfully conducted by the water-vapor induced phase inversion process, while varying the temperature of the water vapor from 5 to 60 °C. The findings showed that better mechanical properties were achieved at higher water-vapor temperature applied during the formation of the chitin hydrogels prepared with up to 7% LiCl concentration. The reduction of the water content values and the enhancement in the storage modulus found in the viscoelasticity test indicated

a denser structure of the resultant chitin hydrogels prepared at 60 °C water-vapor temperature.

Owing to their excellent properties, the prepared chitin hydrogels can be considered promising for various applications. Since a green route is used for their preparation and satisfying properties of the hydrogels have been obtained, these biopolymer-based hydrogels can have potential for the development of biomedical materials, due to their good strength, as well as their known biocompatibility and biodegradability. Moreover, considering their high swelling ratio, they can be utilized as a moisture supporter in seed-germination

applications, to reduce the negative effects of soil exploitation.

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