

Polymers **2014**, *6*, 1178-1186; doi:10.3390/polym6041178

OPEN ACCESS

polymers

ISSN 2073-4360

www.mdpi.com/journal/polymers

Article

Chitosan Membranes Exhibiting Shape Memory Capability by the Action of Controlled Hydration

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Received: 28 February 2014; in revised form: 31 March 2014 / Accepted: 10 April 2014 /

Published: 17 April 2014

Abstract: Chitosan membranes can undergo a glass transition at room temperature triggered by hydration. The mechanical properties of the membranes were followed by a tension test and dynamic mechanical analysis (DMA), with the sample in wet conditions after being immersed in varying compositions of water/ethanol mixtures. Results show that with the increasing of water content, the Young's and storage modulus decrease systematically. For water contents of *ca.* 35 vol%, chitosan (CHT) exhibits a glass transition, showing an elastomeric plateau in the elastic modulus above this hydration level and the occurrence of a peak in the loss factor. Due to the semi-crystalline nature of CHT, membranes of this biomaterial present a shape memory capability induced by water uptake. By fixation of the permanent shape by further covalent cross-linking, the membranes can have different permanent shapes appropriate for different applications, including in the biomedical area.

Keywords: shape memory; glass transition dynamics; biopolymers; biomaterials; smart systems

1. Introduction

Shape memory polymers (SMPs) represent a technologically important class of stimuli-responsive materials for which the response lies in the shape change [1].

Recent studies have shown that environmental conditions, such as the humidity, can substantially influence the conformational mobility of macromolecular chains and, thus, the shape memory properties of some polymers [2–4]. These findings motivated the development of the concept of a moisture-triggered shape memory effect. Water- or solvent-driven shape recovery effects have been observed in hydrophilic SMPs having glass transitions as the switching transition. This type of polymers absorb water, and this affects their mechanical and physical properties [5,6]. By disrupting intramolecular hydrogen bonds and acting as a plasticizer, water reduces the glass transition temperature, hence effectively allowing for room temperature actuation [7].

Chitosan (CHT) is a partially N-deacetylated derivative of chitin [8]. Considerable attention has been given to this polymer, due to its advantages, like low cost, large-scale availability, antimicrobial activity, non-toxicity, biodegradability and biocompatibility [8,9]. CHT can be processed into devices with a variety of shapes using different processing techniques to be used in tissue engineering and other biomedical applications [10,11]. Previous studies showed that CHT can undergo a glass transition by the action of hydration [12,13]. Therefore, we hypothesize that the occurrence of a T_g in CHT activated by hydration variation could be used in the production of devices with shape memory, which could find interest in the biomedical field. Previous studies have presented chitosan membranes as a shape memory device for stent applications [14]. However, to our knowledge, a detailed mechanical characterization of the process and the phenomenological explanation of the origin of this effect were never reported.

In this study, we report a detailed investigation of the influence of water content on the viscoelastic properties of the CHT membranes. To control this parameter, the tests were conducted in water/ethanol mixtures with distinct compositions. We hypothesize that glass transition triggered by controlling water absorption could be linked to the shape memory phenomenon observed in CHT membranes prepared by solvent casting.

2. Materials and Methods

2.1. Materials

Chitosan of medium molecular weight ($M_w = 190,000$ – $310,000$, 75%–85% degree of deacetylation, viscosity 200–800 cps) was purchased from Sigma Aldrich (St. Louis, MO, USA). Before being used, CHT was purified by the reprecipitation method. CHT powder was dissolved at a concentration of 1% (w/v) in 2% (v/v) aqueous acetic acid and precipitated with a NaOH solution (final pH ~8). The CHT flakes were washed with distilled water until neutralization and dehydrated with ethanol. Finally, the CHT flakes were frozen and lyophilized. Genipin was a product of Wako Chemicals (Richmond, VA, USA). All other chemicals were reagent grade and were used as received.

2.2 Methods

2.2.1. Preparation of Chitosan Membranes

Chitosan (CHT) membranes were prepared by solvent casting. First, chitosan was dissolved at 1 wt% in 1 wt% aqueous acetic acid. The solutions were cast in Petri dishes and allowed to dry at room temperature. After drying, chitosan membranes were peeled off and neutralized in a 0.1 M NaOH solution for about 10 min, washed thoroughly with distilled water and dried again. The obtained films had a uniform thickness of *ca.* 60 μm . The prepared chitosan-based films were cut into strips with a length of 100 mm and a width of 10 mm. To obtain the genipin-crosslinked CHT membranes, the prepared, pure CHT membranes were cut into strips and were wound onto a mandrel (the membranes were fixed on the mandrel by an adhesive tape) and then chemically fixed in a 3% aqueous genipin solution at 37 °C for 6 h.

2.2.2. Tension Tests

The ultimate tensile strength (UTS) and tensile modulus of the membranes were determined using an Instron 4505 Universal Machine tensile testing machine (Instron Corporation, Norwood, MA, USA) in tensile mode. The tests were performed at room temperature at a rate of 5 $\text{mm}\cdot\text{min}^{-1}$. Each tensile test was performed with the membranes in a wet condition after being immersed in water/ethanol mixtures with distinct compositions for 3 h before the test and were fixed with sandpaper, to prevent slipping. Tests were performed on a single membrane with a gauge length of 10 mm. For each condition, a minimum of five specimens were tested.

2.2.3. Dynamic Mechanical Analysis (DMA)

All of the viscoelastic measurements were performed using a TRITEC2000B Dynamic Mechanical Analysis (DMA) from Triton Technology (Grantham, UK), equipped with the tensile mode. The measurements were carried out at room temperature (*ca.* 20 °C). The distance between the clamps was 10 mm, and the chitosan membrane samples were cut with about a 2 mm width (measured accurately for each sample). Chitosan membranes were tested at a constant frequency (1 Hz) following the changes in the storage modulus (E') and loss factor ($\tan \delta$) as a function of the water content. The dried samples of known geometry were first clamped in the DMA apparatus and immersed in a bath of ethanol ($V_{\text{EtOH}} = 400 \text{ mL}$) and kept under the testing constant strain amplitude (30 μm) for 30 min. After this preconditioning step, E' reached an equilibrium value. Finally, the water was pumped into the reservoir at a constant flow rate ($Q = 7.5 \text{ mL/min}$), providing a time (t)-dependent change in the content of water, described by the following equation:

$$\text{water (vol\%)} = \frac{Qt}{Qt + V_{\text{EtOH}}} \quad (1)$$

Note that in these measurements, E' should be taken as an apparent value, as during the introduction of water, the geometry of the sample continuously changes, due to swelling, and the calculation of this parameter used the initial geometry of the sample. Triplicates were performed.

3. Results and Discussion

3.1. Mechanical Properties

Homogeneous membranes with a thickness of about 60 μm were produced using the solvent casting methodology described above. Chitosan absorbs water, and this affects its mechanical and physical properties. Tensile testing under distinct water/ethanol mixtures was conducted to evaluate the mechanical properties of CHT membranes (see Figure 1 and Table 1). The combined effect of solvent and non-solvent in miscible liquid pairs is expected to be rather useful to control the swelling ratio within polymer networks and to observe the mechanical modifications induced by hydration.

Representative stress-strain curves from tensile tests obtained for CHT membranes in a wet condition after being immersed in water/ethanol mixtures with distinct compositions are presented in Figure 1. The corresponding tensile properties of the membranes are summarized in Table 1. With increasing water content, the ultimate tensile strength decreased, but the strain at break tends to increase.

Figure 1. Representative stress-strain curves of chitosan (CHT) membranes at distinct hydration levels.

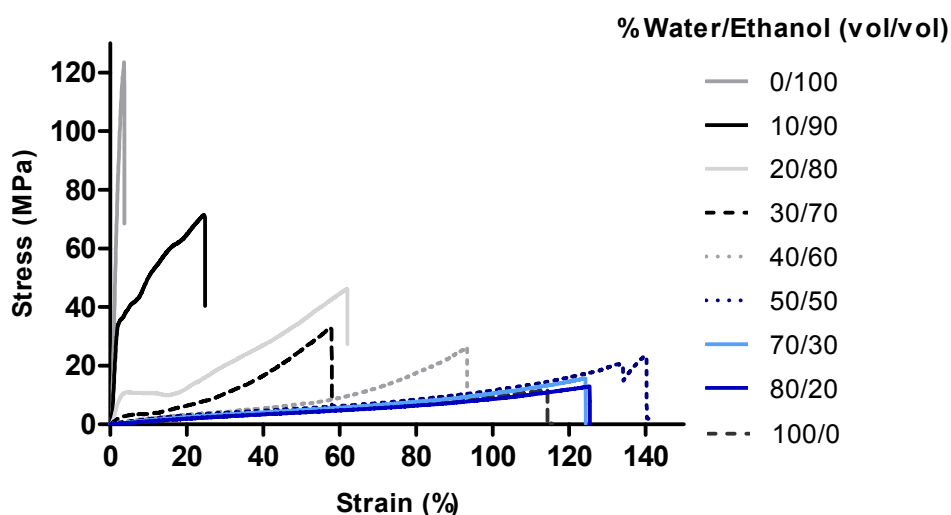


Table 1. Mechanical properties of CHT membranes under distinct hydration levels.

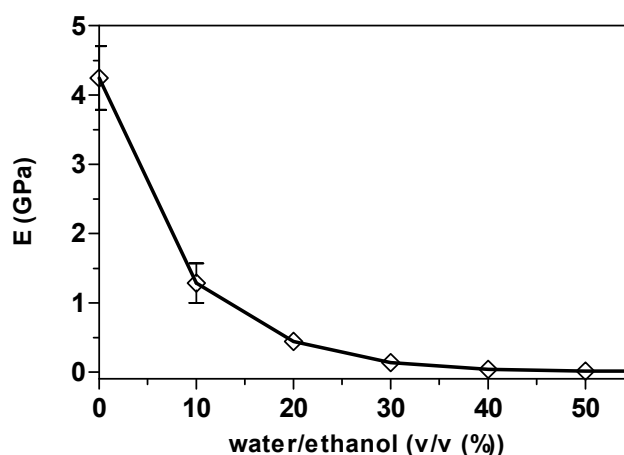
% water/ethanol (vol/vol) (n = 5)	0	10	20	30	40	50	70	80	100
Young's modulus (GPa)	4.246 ± 0.459	1.282 ± 0.284	0.441 ± 0.089	0.137 ± 0.013	0.037 ± 0.012	0.011 ± 0.002	0.013 ± 0.003	0.0091 ± 0.0010	0.008 ± 0.0013
Ultimate tensile strength (MPa)	81.54 ± 24.23	70.24 ± 1.70	35.11 ± 8.28	32.54 ± 5.84	30.57 ± 6.75	20.43 ± 1.92	14.54 ± 3.10	12.61 ± 1.02	11.82 ± 0.44
Elongation at break (%)	3.66 ± 0.51	22.94 ± 2.07	52.05 ± 5.99	56.42 ± 6.32	79.25 ± 7.15	158.30 ± 4.93	122.16 ± 4.99	125.02 ± 8.16	112.16 ± 10.61

The Young's modulus (*E*) of chitosan membranes upon immersion in water/ethanol mixtures was also assessed via the tensile tests, obtained from the slope of the stress-strain curve. Figure 2 shows an inverse relationship between *E* versus water content. This decreasing happens because water acts as a

very good plasticizer, interfering with hydrogen bonds between the chitosan macromolecular chains. The chains acquire greater mobility, and the free volume increases, leading to a decrease in the glass transition temperature and the stiffness [15]. In others studies, which compared wet with dry conditions, the stiffness of similar CHT membranes systematically decrease with increasing hydration [16]. However, to the best of our knowledge, the mechanical characterization of CHT membranes performed under such controlled conditions has never been reported before.

The membranes exhibit a water-dependent Young's modulus from 4.3 GPa (pure ethanol) to 0.01 GPa (pure water). For water contents above 35 vol%, all the samples show a plateau in the elastic modulus, suggesting a rubber-like behavior where the mobile chains in the amorphous regions are sustained by the crystalline domains of chitosan. Such results provide strong indications that chitosan can undergo a glass transition by the action of hydration with the transition for water values around 35 vol%.

Figure 2. The variation of Young's modulus (E) with water/ethanol mixtures for CHT membranes.



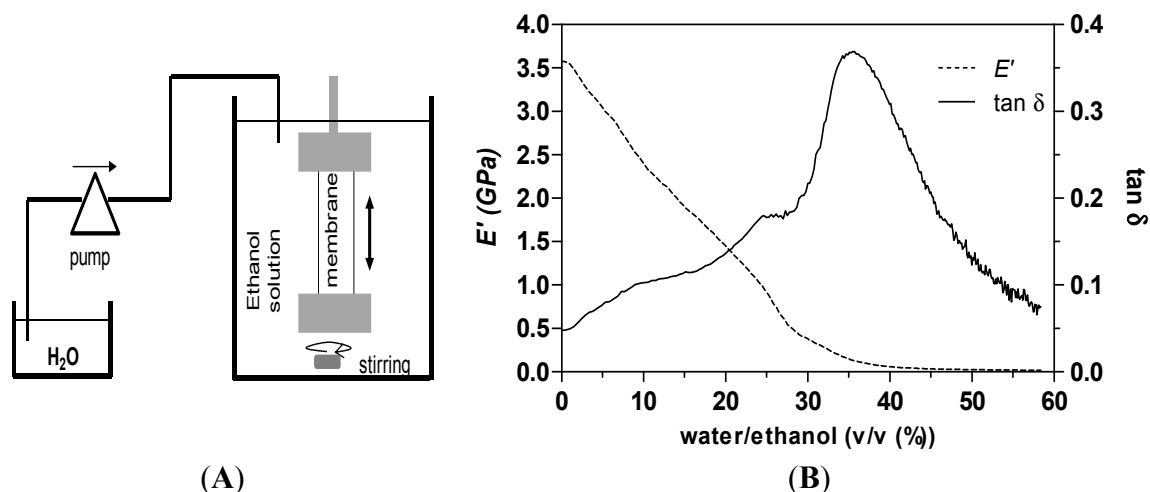
3.2 Dynamic Mechanical Analysis (DMA)

The influence of water content on the viscoelastic properties of CHT membranes was also studied by DMA. The CHT membrane was clamped in the DMA apparatus and immersed in an ethanol bath (see Figure 3A) during a period of 30 min. After such a period, water was introduced in the reservoir at a constant flow rate, changing gradually the composition of the mixture. The variations of E' and $\tan \delta$ as a function of ethanol/water mixture in the bath are described in Figure 3B. For 0 vol% of water, pure ethanol, values of E' of 0.35 GPa were found, which are very similar to the data found in dry chitosan [12], indicating that, at this composition of ethanol, CHT is in a glassy state. In a previous work, Caridade *et al.* [13] reported that no absorption was detected for 100 vol% ethanol in swelling experiments. When water started to be introduced in the ethanol bath, the values of E' decreased continuously. For example, in 40 vol% of water content, the values of E' presented a reduction during the wetting process, also consistent with previous results obtained in wet chitosan [12]. Such results offer relevant information for applications, where the material may be found in different high hydration levels. Figure 3B also presents the variations of $\tan \delta$ once the damping component could also have implications on the solid-state rheology performance of such membranes. From Figure 3B, $\tan \delta$ values oscillated between 0.05 and 3.5, indicating that water has a profound impact on the capability of the material to absorb mechanical energy. For high ethanol content, the values of $\tan \delta$ are

lower once chitosan is in a glassy state possessing long relaxation times. As the content of water increased in the mixture, $\tan \delta$ presented a well-defined peak around 35 vol% of ethanol, reaching a maximum value of 0.35. This peak around 35 vol% is in accordance with the water content above which the Young's modulus reaches a plateau, mentioned before (Figure 2). This peak is attributed to the glass transition (T_g) of chitosan, whereby it passes from a glass to rubber-like state transition that is induced by the entrance of water in the chitosan matrix [13]. Such water content can be seen as an external variable that may control the molecular mobility of the polymeric structure.

Other works also reported the T_g in chitosan, but the results are far from being in agreement. For example, Mucha and Pawlak suggest that the T_g of pre-heated chitosan can be found in the temperature range of 140–160 °C [17]; however, similar DSC experiments did not present any clear change in the heat capacity in this temperature range [18]. Sakurai *et al.* used DSC and DMA and reported a T_g for chitosan at 203 °C [19]. Other values of T_g for chitosan were reported by Cheung *et al.*, around 103 °C as measured by Differential Scanning Calorimetry (DSC) [16], and by Toffey and Glasses, that suggested that it could range from 60 to 90 °C, as measured by DMA [18]. In the present work, such a controversial glass transition process observed in dry conditions is clearly seen at lower temperatures by increasing in a controlled manner the hydration environment of the CHT membranes.

Figure 3. (A) Schematic representation of the Dynamic Mechanical Analysis (DMA) experiment performed where the chitosan membrane was clamped in the DMA apparatus and immersed in an ethanol bath. After a certain period of time, water was added to the ethanol bath in a controlled manner to vary the water/ethanol composition of the bath. (B) Apparent storage modulus (E') and loss factor ($\tan \delta$) at room temperature measured with samples immersed in water/ethanol mixtures as a function of water content.



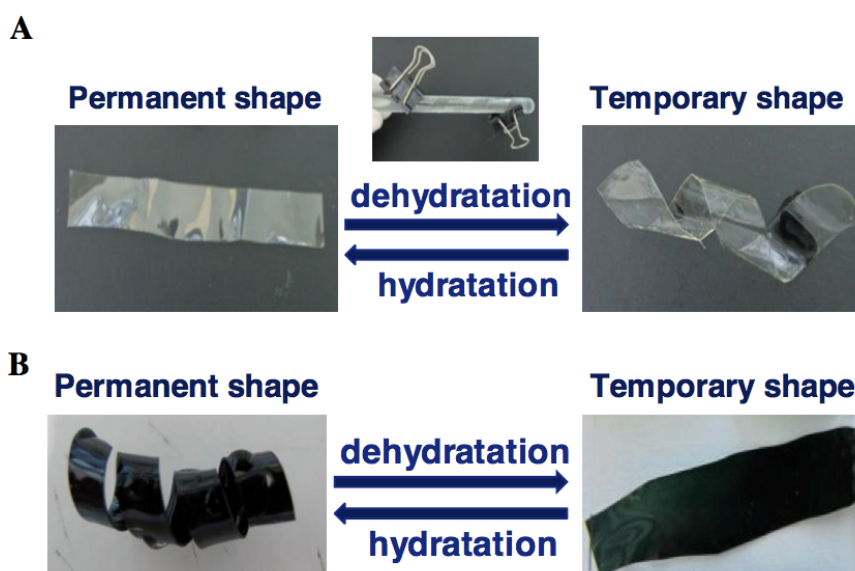
3.3. Shape Memory Induced by Hydration

The effect of water/ethanol mixtures in the mechanical properties of CHT membranes can be observed by the shape memory capability as a result of the glass transition induced by hydration. As can be seen in Figure 4A, the membrane with a flat permanent shape was obtained by solvent casting; the membrane can be deformed upon hydration, and by fixing this geometry through dehydration, a permanent shape can be obtained. Such dehydration can be performed, for example, by drying the

sample in air, but to accelerate the process, we soaked the deformed membrane in pure ethanol. With dehydration with higher contents of ethanol, the vitreous-like nature of the amorphous component of chitosan allows the fixation of the temporary shape of the scaffold. In Figure 4A, the temporary shape is obtained by winding the membranes onto a mandrel followed by dehydration. This shape is retained until the membrane is hydrated in mixtures with a water content higher than 35 vol%. The presence of water disrupts inter-molecular hydrogen bonds, permitting the large-scale segmental mobility of the chitosan chains upon the occurrence of the glass transition and, thus, the recovery of the permanent shape of a pre-deformed scaffold. For values higher than 35 vol%, the chitosan undergoes a glass transition and recovers the permanent shape. In a practical point of view, the recovery of the shape could take place, for example, upon the implantation of the CHT device inside any hydrated region of the body.

For pure CHT membranes, the permanent shape corresponds to the shape obtained during the processing. The limitation of the permanent shape can be solved by the fixation of the membrane by a crosslinking agent, such as genipin, resulting in different permanent shapes. The initial chitosan membrane is hydrated, deformed and crosslinked in a genipin solution, in order to obtain a new permanent shape. The new membranes possess the ability to memorize their permanent shapes, because of the covalent crosslinks formed in the chitosan stripes during fixation with genipin. Moreover, the mechanical properties may also be enhanced through covalent crosslinking [20]. Figure 4B shows a membrane with a helicoidal shape as a permanent shape where the maintenance of the structural integrity is provided by crosslinking. With deformation upon hydration, followed by dehydration, the crosslinked membrane could acquire a new temporary shape; in the example in Figure 4B, this corresponds to the extended flat configuration, which could recover the permanent helicoidal shape by hydration in water or other aqueous-based solution. By fixing the permanent shape by covalent crosslinking, the membranes can have different permanent shapes appropriate for different applications. For example, the helicoidal shape can be envisaged in applications of CHT for stents with a shape memory capability.

Figure 4. (A) Photographs of the permanent and temporary shapes of the pure CHT membranes. (B) Photographs of the permanent and temporary shapes of the genipin crosslinked CHT membranes.



4. Conclusions

Chitosan membranes present different mechanical properties when immersed in distinct water/ethanol solutions. With the increasing of the water content, the Young's and storage modulus decrease systematically. For water contents of 35 vol%, CHT exhibits the occurrence of a glass transition, showing a plateau in the elastic modulus above 35 vol% and a peak in the loss factor curve in this hydration region. CHT membranes present a shape memory capability induced by hydration. The limitation of the permanent shape can be solved by fixation of the membrane by a crosslinking agent, such as genipin, resulting in different permanent shapes.

Acknowledgments

This work was supported by the Portuguese Foundation for Science and Technology Foundation (FCT) through project PTDC/FIS/115048/2009.

Author Contributions

Cristina O. Correia performed most of the experiments and wrote part of the manuscript; Sofia G. Caridade performed the DMA experiments and wrote part of the manuscript; João F. Mano conceived the initial hypothesis and the design of the experiments, supervised the overall experimental work, and contributed to the treatment/discussion of the results and in the writing of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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