

STATE OF WATER IN CITRATE CROSSLINKED CHITOSAN MEMBRANE

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Abstract

The ionically crosslinked membranes were prepared of chitosan (Ch) and trisodium citrate (Ch/CIT). The characteristics of the state of water in crosslinked and uncrosslinked chitosan membranes with different water content was performed by differential scanning calorimetry (DSC). The kinetics of water swelling in Ch and Ch/CIT membranes were also studied. Conclusions concerning effect of ionic crosslinking on the state of water in chitosan hydrogel membranes were drawn out. Results shows that freezable and non-freezable water exist in analysed Ch and Ch/CIT membranes, while there are variations in the amount of non-freezing bound water in these polymers.

Key words: *chitosan membrane, trisodium citrate, state of water, DSC method.*

1. Introduction

Biopolymers are a promising new class of low cost membrane materials. A particular interest attracts chitosan, a linear polysaccharide usually obtained by alkaline deacetylation of crustacean chitin from crab and shrimp shell wastes. Chitosan (Ch), the fully or partially deacetylated form of chitin, consists of two residues: N-acetylglucosamine (2-acetamido-2-deoxy- β -D-glucopyranose) and glucosamine (2-amino-2-deoxy- β -D-glucopyranose) (*Figure 1*) [1]. Due to its excellent properties, such as biocompatibility, biodegradability, non-toxicity, chemical and thermal stability, capability to formation of gels and films, etc., chitosan has been widely studied as a promising source of membrane materials for different applications in medicine and pharmacy and in various industrial areas. However, chitosan materials are soluble in acidic solutions and possess a rather low mechanical stability. Great efforts are therefore attempted to reduce solubility of chitosan in aqueous solvents at low pH and to improve its resistance to chemical degradation or long-term biological degradation such as blending, multilayer casting, adding inorganic reinforcements and crosslinking. Crosslinking is one of the most effective methods to improve the properties characterized above. Recently, we have reported the preparation of modified chitosan membranes using such low molecular ionic crosslinking compounds as trisodium citrate, sulfuric acid and pentasodium tripolyphosphate [2, 3]. We have studied the effect of ionic crosslinking agent type on swelling behavior of these modified membranes in solutions of different pH [2]. In the present paper the characteristics of the state of water in chitosan membrane crosslinked with trisodium citrate (Ch/CIT) and uncrosslinked chitosan membrane (Ch) was performed by differential scanning calorimetry (DSC). Detailed characterization of the types of water that exist within the hydrogel materials seems to be very important both to understand their dynamic and equilibrium swelling behaviour as well as to analyse solute transport and other diffusive properties of such systems.

2. Materials and methods

2.1. Materials

Commercially available chitosan (Ch) from crab shells of medium molecular weight, was purchased from Sigma Aldrich (Germany). Trisodium citrate (NaCIT), acetic acid and hydrochloric acid were analytical grade and were purchased from POCh (Gliwice, Poland). Potassium bromide (KBr) for spectroscopy was purchased from Merck (Germany).

2.2. Chitosan characterization

Degree of deacetylation, DDA, of chitosan was determined by potentiometric titration method and molecular mass was obtained by viscometry, as described earlier [2]. Degree of deacetylation was equal to $75.72 \pm 3.82\%$ and viscosity average molecular mass, M_v , was equal to 730 kDa.

2.3. Membrane preparation

Pure chitosan membranes (Ch) were prepared by solution casting and solvent evaporation technique. 1 wt.% (w/v) chitosan solution, prepared by dissolving chitosan powder in 2% (w/v) acetic acid, was filtered, left over night, degassed, cast as film on a clean glass plate and evaporated to dryness in an oven at 37 °C, then further dried under vacuum at

60 °C. Dry membranes were immersed in 2 M sodium hydroxide solution for 5 min to remove residual acid, thoroughly washed with deionized water and air-dried. The obtained film was further dried under vacuum at 60 °C.

Chitosan/trisodium citrate (Ch/CIT) membranes were obtained by immersing pure chitosan membranes in aqueous 5.0% (w/v) sodium citrate solution for 24 hours. Following crosslinking conditions were applied: $T_{\text{crosslink.}} = 4\text{ °C}$, pH = 5.0 (initial solution acidified with HCl). After crosslinking membranes were repeatedly washed with deionized water, thoroughly dried in air and under vacuum at 60 °C.

2.4. FTIR spectroscopy analysis

FTIR spectra of Ch and Ch/CIT membrane samples as well as NaCIT in KBr disc form were recorded on Perkin-Elmer 2000 FTIR spectrometer from 400 to 4000 cm^{-1} with a resolution 4 cm^{-1} and 100 scans.

2.5. Kinetics swelling experiments

In order to study the swelling behaviour, the dry membrane samples (approximately 15 mg) were immersed in water at 37 °C on definite time period and the weight of the swollen samples was determined after carefully drying their surface with a filter paper. The degree of swelling (S) for each membrane sample at time t was calculated according to the formula:

$$S = (W_t - W_0)/W_0$$

where: W_0 - weight of the dry sample in g, W_t - weight of the swollen sample in g at time t .

2.6. Differential scanning calorimetry (DSC) measurements

A Polymer Laboratories Ltd. (Epsom, United Kingdom) differential scanning calorimeter equipped with a liquid nitrogen cooling accessory was used. The temperature scale of the DSC cell was calibrated using water. Modified and unmodified chitosan membranes for DCS measurements were dried at 60 °C under reduced pressure for several days to obtain completely dry films.

To characterize water state in membranes dry polymer sample (about 5 mg) was weighed in an aluminium pan designed for volatile samples, a known amount of water was added by a micro-syringe, the pan was sealed hermetically, equilibrated for 24 hours at room temperature and weighed. Then it was cooled to -140 °C at a rate of 10 °C/min, under constant purging of nitrogen at 2,5 mL/min, and allowed to stay at that temperature for 10 min before heating to 70 °C at a rate of 10 °C/min was initiated. After the DSC measurement the pan was weighed in order to check that it had been properly sealed and that no water had evaporated.

The total water content, W_c , of each sample was defined as $W_c(\text{g/g}) = (\text{water, g})/(\text{dry polymer, g})$. The amount of water able to crystallize (freezable water), W_f , was calculated after integration of the melting endotherm, using distilled water as a reference and assuming both melting enthalpies for freezing free water (W_{ff}) and freezing bound water

(W_{fb}) to be the same as that of bulk water ($\Delta H_0=334 \text{ J/g}$). The amount of freezable water was calculated from the following equation:

$$W_f = \Delta H_m / H_0$$

where ΔH_m is the melting enthalpy for freezable water in hydrogel membrane obtained from the DSC thermogram in J/g and ΔH_0 is the melting enthalpy of pure water ($\Delta H_0 = 334 \text{ J/g}$).

The total amount of non-freezing bound water, W_{nf} , was obtained from the difference between the amount of sorbed water, W_c , and the total amount of freezable water W_f :

$$W_{nf} = W_c - W_f = W_c - (W_{ff} + W_{fb})$$

where W_{ff} is the amount of free water.

3. Results and discussion

3.1. Spectral characterization of membrane structure

FTIR spectra of unmodified chitosan membrane (Ch), modified chitosan membrane (Ch/CIT) and trisodium citrate (NaCIT) are shown in **Figure 2**. As can be seen, after treating of Ch with NaCIT solution absorption bands at 1656 cm^{-1} (C=O stretching in amide group, amide I vibration [4, 5]) and 1598 cm^{-1} ($-\text{NH}_2$ bending in non-acetylated 2-aminoglucose primary amine [4, 5]) disappear. Moreover, new absorption bands at 1634 cm^{-1} and 1380 cm^{-1} appear, they can be attributed to N-H deformation vibration in NH_3^+ ion and C-O bending vibration in COO^- ion, respectively [4, 6].

Spectral changes presented above, as we discussed earlier [2, 7] indicate the crosslinking reaction between the protonated amino groups of chitosan and carboxylate group of trisodium citrate, according to the scheme presented in **Figure 1**.

3.2. Swelling behaviour studies

Swelling behaviour of uncrosslinked chitosan membrane and ionically crosslinked chitosan membrane is shown in Fig.3. As can be seen, unmodified chitosan membrane swollen in water underwent disintegration, but citrate crosslinked membrane was water-resistant.

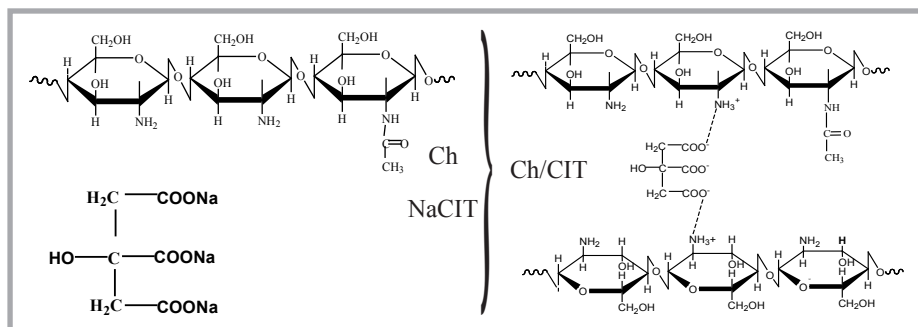


Figure 1. Preparation of ionically crosslinked chitosan membrane.

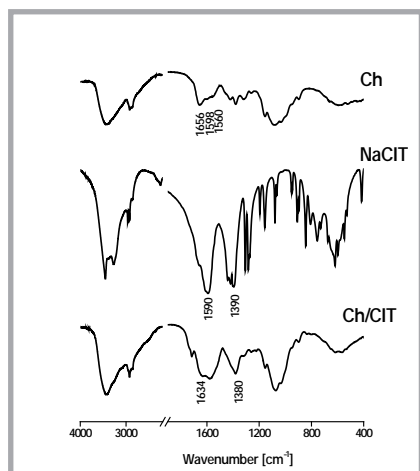


Figure 2. FTIR spectra of Ch, NaCIT and Ch/CIT.

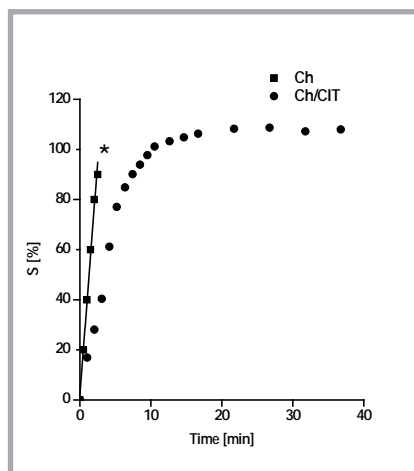


Figure 3. Dynamic swelling behaviour of Ch and Ch/CIT membranes in distilled water (* - moment of membrane disintegration).

Moreover, the water was sorbed by Ch/CIT at high rate in the beginning and then the rate slowed down to reach equilibrium after approximately 15 minutes. It could be also noted that the initial rate of water sorption was lower for Ch/CIT sample than for Ch.

3.3. State of water in uncrosslinked and crosslinked chitosan membranes

There is a variety of techniques for the study of water binding in polymers. Differential scanning calorimetry (DSC) is in many ways the most convenient and remarkable method. Three energetically distinct states of water have been identified in water swollen systems. They are defined as follows:

- i) *free water*, which undergoes similar thermal transitions to that of bulk water,
- ii) *freezing bound water*, which undergoes a thermal phase transition at a temperature shifted with respect to that of bulk water and
- iii) *non-freezing water*, which is the tightly bound to the polymer and does not exhibit a first order transition over the range of temperatures normally associated with bulk water.

Figure 4 and **Figure 5** show the DSC melting thermograms of frozen water in Ch and Ch/CIT membranes with various water content W_c . Both for all analysed Ch-water as well as for Ch/CIT-water systems no peaks are observed at $T \approx 0$ °C below certain water content. The absence of endothermic peaks above a water content threshold indicates that this water is non-freezing bound type. For each membrane with the increase of water content the broad endothermic peak appears, corresponding to melting of freezing water. Melting of water sorbed in all the samples starts at a temperature lower than that of pure water (the DSC heating curve of pure water is shown in **Figures 3 - 4** by the broken line). The water melting below 0 °C is regarded as freezable bound or intermediate water, but water melting at 0 °C is free water. Moreover, for all studied Ch-water and Ch/CIT-water

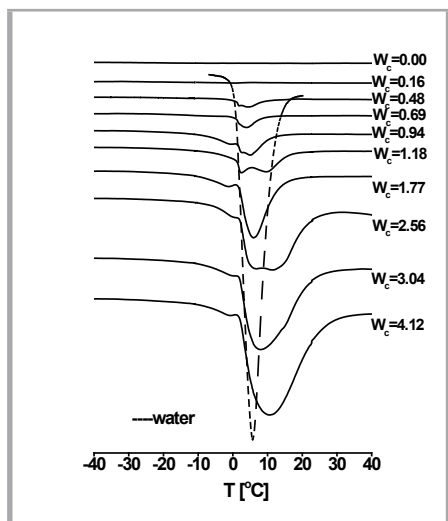


Figure 4. DSC heating thermograms of Ch membranes with different water content.

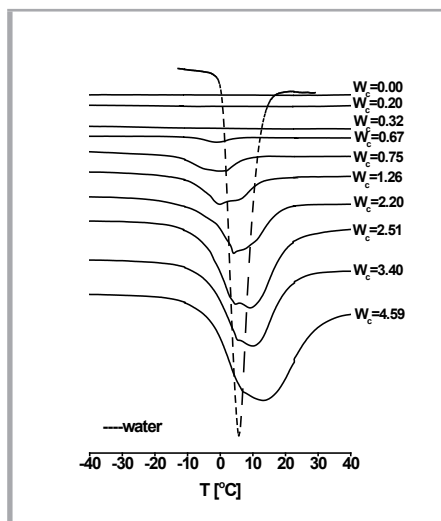


Figure 5. DSC heating thermograms of Ch/CIT membranes with different water content.

systems these endothermic peaks are poorly resolved, broad and structured. The observed DSC endotherms for samples with rather high water content are broad and asymmetric and no distinct peak splitting is observed, but for lower water content multi-peaks with distinct submaxima can be seen.

Figure 6 shows a variation of the total melting enthalpy of freezing water per gram of polymer ΔH_m versus the water content W_c for Ch and Ch/CIT membranes. The slope of the linear plot represents the “average apparent” value of the melting enthalpy associated with the freezing water (ΔH_m). The intersection with the horizontal axis corresponds to the

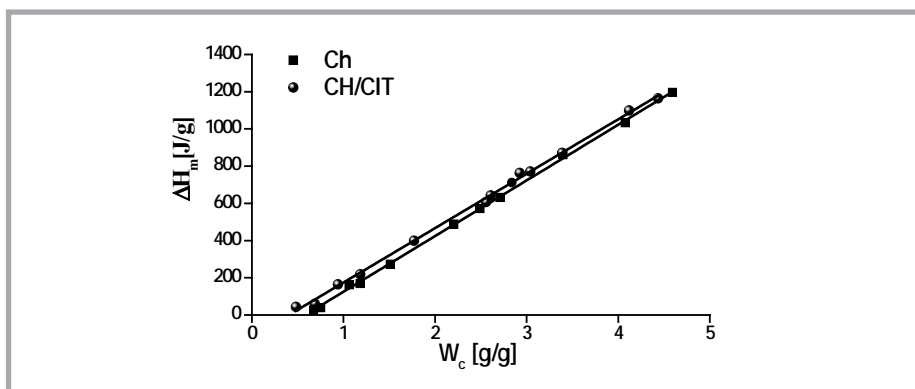
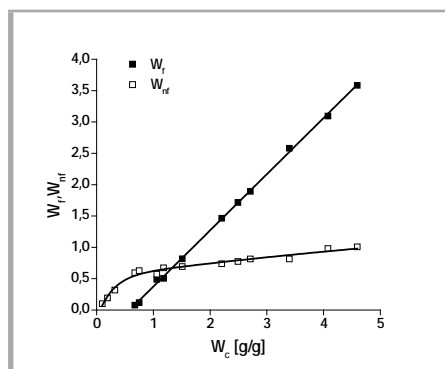
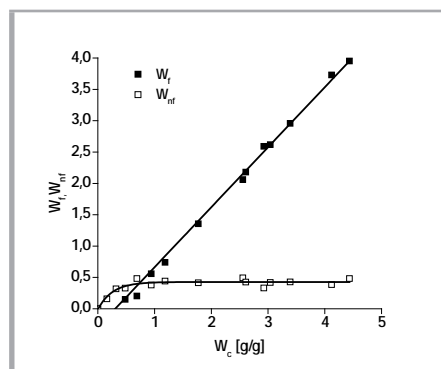


Figure 6. Relationship between enthalpy of water melting and water content.

Table 1. Values of ΔH_m and $W_{nf, max}$ for Ch-water and Ch/CIT-water systems.

Polymer sample	ΔH_m , J/g	$W_{nf, max}$	Linear regression equation	R ²
Ch	291.94	0.58	$\Delta H_m = 291.9 W_c - 169.8$	0.9985
Ch/CIT	331.20	0.40	$\Delta H_m = 331.2 W_c - 133.8$	0.9993

**Figure 7.** The amounts of freezing and non-freezing water in Ch membranes.**Figure 8.** The amounts of freezing and non-freezing water in Ch/CIT membranes.

maximum amount of non-freezing water ($W_{nf, max}$) in the hydrogel membrane, defined as the maximum amount of water present in the polymer, which is not associated with any endothermic peak [7, 8]. The curves presented in **Figure 6** were estimated by linear functions. Characteristics of both linear regressions and calculated values of ΔH_m and $W_{nf, max}$ for Ch-water and Ch/CIT-water systems are presented in **Table 1**.

Figure 7 and **Figure 8** show the effect of total water content in the membrane on the amount of non-freezable and freezable water. For all studied membranes the W_f value decreases with the water content decrease, while W_{nf} is practically constant until the freezable water vanishes. In the region in which $W_f = 0$, W_{nf} decreases with decrease in W_c .

4. Conclusions

The results reported above can be summarized as follows:

1. FTIR analysis confirmed the formation of crosslinked chitosan membrane with ionic bonds between protonated amino groups of chitosan and carboxylate groups of trisodium citrate.
2. Addition of trisodium citrate to chitosan and formation of ionic crosslinks influenced the state of water in chitosan hydrogel membrane. There were observed, both in uncrosslinked as well as in ionically crosslinked chitosan membranes, non-freezing water and freezing water, but their amounts were different.
3. The amount of non-freezing water in ionically crosslinked chitosan membrane was notable lower than in uncrosslinked one.

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