STATE OF WATER IN NONCROSSLINKED AND CROSSLINKED HYDROGEL CHITOSAN MEMBRANES – DSC STUDIES

Jadwiga Ostrowska-Czubenko, Milena Pieróg, Magdalena Gierszewska-Drużyńska

*Chair of Physical Chemistry and Physicochemistry of Polymers,
Faculty of Chemistry
Nicolaus Copernicus University
ul. Gagarina 7, 87-100 Toruń, Poland
E-mail: jocz@chem.uni.torun.pl*

Abstract

Modified chitosan hydrogel membranes were prepared using glutaraldehyde (GA) and sodium citrate (NaCIT) as crosslinking agents. Molecular and supermolecular structure analyses of unmodified and modified chitosan membranes have been conducted by FTIR and X-ray spectroscopy. FTIR results showed covalent and ionic crosslinks formation between chitosan (Ch) and GA or simultaneously Ch, GA and NaCIT. The state of water in noncrosslinked and crosslinked chitosan membranes were analysed by differential scanning spectroscopy (DSC). Three types of water in hydrogel membranes were found: non-freezing bound water, freezing bound water and freezing free water, while there were variations in the amount of non-freezing bound water in these polymers. The effect of ionic crosslinking on water state, mainly on the non-freezing water content, was discussed.

Key words: *chitosan membranes, crosslinking, DSC, states of water, glutaraldehyde, trisodium citrate.*

1. Introduction

In recent years hydrogel membranes formed from natural polymers arouse a big interest. A special attention has been given to such membrane material as chitosan. Recently, chitosan is widely used as membrane material for ultrafiltration, reverse osmosis, pervaporation and another membrane processes.

It is well known, that equilibrium water content as well as state of water influence on properties of hydrogel membranes. Generally, the state of water in hydrogels is categorized into three different types. The experimentally determined separate states of water can be defined as follows [1, 2]: (i) *free water* - water that is not intimately bound to the polymer chain and behaves like bulk water, i.e. undergoes thermal transition at temperature analogous to bulk water (at 0 ºC), (ii) *freezable bound water* - water that is weakly bound to the polymer chain or interacts weakly with nonfreezing water and undergoes a thermal phase transition at a temperature lower than 0 ºC and (iii) *bound water* (*non-freezing water*) – water tightly bound to the polymer, which does not exhibit a first order transition over the temperature range from –70 to 0 ºC [1]. Two first types of water mentioned above are so-called freezing water. There is a variety of techniques for the study of water binding in polymers. Differential scanning calorimetry (DSC), used by us to characterize water state in chitosan hydrogel membranes, is in many ways the most convenient and informative method [3].

In our previous studies the ionically crosslinked membranes (Ch/CIT) were prepared of chitosan (Ch) and trisodium citrate (NaCIT) and the characteristics of the state of water in these membranes with different water content was performed by DSC [4]. The present work deals with a DSC study of the state of water in doubly crosslinked Ch/GA/CIT membranes that were obtained by covalent crosslinking of chitosan with glutaraldehyde (GA) and next by ionic crosslinking with NaCIT.

2. Materials and methods

2.1. Materials

Medium molecular weight chitosan (M_v=730 kDa determined by viscometry [5], degree of deacetylation DDA=76.3% determined by potentiometric titration method [6]) and glutaraldehyde (GA, 25 wt.% solution in water) were analytical grade and were purchased from Sigma-Aldrich (Germany). Trisodium citrate (NaCIT), sodium hydroxide and acetic acid were analytical grade and were purchased from POCh (Poland). Potassium bromide for spectroscopy was purchased from Merck (Germany).

2.2. Membrane preparation

Pure chitosan membranes (Ch) were prepared by casting and solvent evaporation technique*, as described in detail elsewhere [4]. Two-component chitosan/glutaraldehyde (Ch/GA) membranes were prepared as follows: first, 1% (w/v) chitosan solution in 2% (w/v) acetic acid and 0.25% (w/v) glutaraldehyde solution in water were mixed and stirred at room temperature for at least 6 hrs to obtain a homogeneous solution and then the solution was cast as a film on a clean glass plate and evaporated to dryness at 37 °C. The content of glutaraldehyde in casting solution was 2.5 wt.%. Finally, the prepared mem-

branes were washed repeatedly with bidistilled water, immersed in 2M sodium hydroxide solution for 5 min and again washed repeatedly with water and dried in air. Three-component chitosan/glutaraldehyde/sodium citrate (Ch/GA/CIT) membranes were prepared by immersing two-component Ch/GA membranes in aqueous NaCIT solution for 24 hrs. The following crosslinking conditions were applied: concentration of NaCIT solution c = 5% (w/v), temperature $T_{crosslink.}$ = 4 °C, pH of NaCIT solution pH = 5.0 (initial NaCIT solution acidified with HCl). After crosslinking the membranes were repeatedly washed with water, thoroughly dried in air and then under vacuum at 60 ºC for several days to obtain completely dry films.

2.3. FTIR spectroscopy analysis

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

2.4. Wide angle X-ray diffraction studies

Wide angle X-ray diffraction patterns of unmodified and modified chitosan membranes were measured by an X-ray diffractometer (X-Pert Pro Systems, Philips, Netherlands). X-ray diffraction was performed on powdered samples by exposing them to CuK$_\alpha$ radiation and scanned from 2Θ = 4° to 40° at a step size of 0.02°.

2.5. Differential scanning calorimetry (DSC) measurements

The state of water in chitosan hydrogel membranes was analysed by DSC measurements, as described elsewhere [4, 7]. A Polymer Laboratories Ltd. (Epsom, United Kingdom) differential scanning calorimeter equipped with a liquid nitrogen cooling accessory was used to monitor both bound as well as free water in membranes. The temperature scale of the DSC cell was calibrated using water. Dry membrane sample (about 5 mg) was weighed in an aluminium pan designed for volatile samples and a known amount of water was added by a micro-syringe. The pan was sealed hermetically to prevent water loss during DSC scanning, equilibrated for 24 hours at room temperature and then weighed. After that the pan was first cooled from room temperature to –140 °C at a rate of 10 °C/min, under constant purging of nitrogen at 2.5 mL/min, allowed to stay at that temperature for 10 min and then heated at the same rate up to 70 °C. 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 phase transition of water in the hydrogel membrane during heating was recorded as the endothermic peak, which was later integrated using DSC software.

The amount of water able to crystallize (freezable water), W_f, defined as W_f (g/g) = (water, g)/(dry polymer, g) was calculated after integration of the melting endotherm, using double 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 (ΔH_0 = 334 J/g). The amount of freezable water was calculated from the following equation:

$$W_f = \Delta H_m / \Delta H_0$$

where ΔH_m (J/g) is the melting enthalpy for freezable water in hydrogel membrane obtained from the DSC thermogram and ΔH_0 is the melting enthalpy of pure water. 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 freezing free water.

2.6. Swelling measurements

Equilibrium water content (*EWC*) of the membrane was measured by the gravimetric method. The preweighed, completely dried membrane sample was immersed in water at temperature 37 °C for 24 hrs, which was determined to be sufficient to reach an equilibrium state. Then membrane was taken out, wiped quickly with filter paper and weighed. EWC was calculated using the following equation:

$$EWC = \frac{W_s - W_d}{W_d} \cdot 100\%$$

where W_s is the weight of the swollen membrane and W_d is the weight of the dried membrane.

3. Results and discussion

3.1. Membrane characterization by FTIR and X-ray spectroscopy

Figure 1 shows FTIR spectra of hydrogel chitosan membrane before and after chemical and physical modification. In the spectrum of noncrosslinked chitosan absorption bands situated at 1656 cm^{-1} (C=O stretching in amide group, amide I vibration), 1598 cm^{-1} (-NH$_2$ bending in non-acetylated 2-aminoglucose primary amine) and 1560 cm^{-1} (N–H bending in amide group, amide II vibration) can be seen [8 - 10]. Some changes can be observed after chitosan modification - crosslinking with glutaraldehyde. The peak at 1656 cm^{-1} shifts to the lower wavenumber, i.e. to 1641 cm^{-1}. This band is most probably composed of amide I band of chitosan, that as we discussed above appears at 1656 cm^{-1}, and the C=N stretching band of Schiff's base, that according to literature appears at the frequency range 1620-1660 cm^{-1} [11]. Moreover, it is not observed any band at ~1715 cm^{-1}, related to the free aldehyde group [12]. Two main crosslinking mechanisms, involving formation of Schiff's base structures or Michael-type adducts, have been proposed for the reaction of chitosan and glutaraldehyde [13]. Our findings seem to indicate that under experimental reaction conditions GA completely reacted with chitosan and imines (Schiff bases) were formed, as presented in *Figure 2*. The formation of crosslinks between Ch and GA was also confirmed visually by the change of membrane color from transparent to deep yellow [14]. Comparison of the FITR spectra of Ch/GA and Ch/GA/CIT indicates that these spectra are quite similar in the frequency range of 1650-1500 cm^{-1}. In both mentioned spectra two main bands can be seen: the first at 1641 cm^{-1} (Ch/GA) or 1637 cm^{-1} (Ch/GA/CIT) and the second at 1573 cm[-1](Ch/GA) or 1584 cm^{-1} (Ch/GA/CIT). Moreover, some differences in relative intensity of these bands can be observed. It can be supposed that these bands represent envelope of several bands, because both protonated amine groups as well as amine, acetamide and imine groups absorb in these frequency region. Protonated amines show an antisymmet-
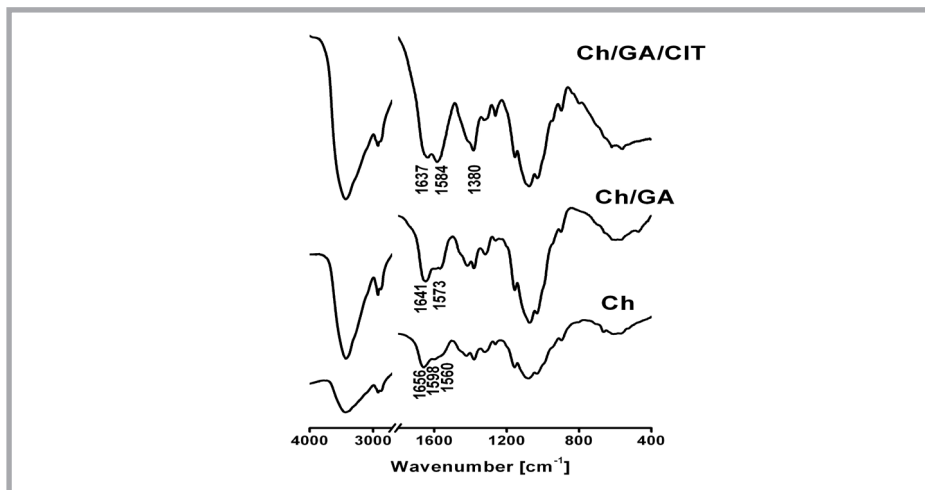
Figure 1. *FTIR spectra of noncrosslinked and crosslinked chitosan membranes.*
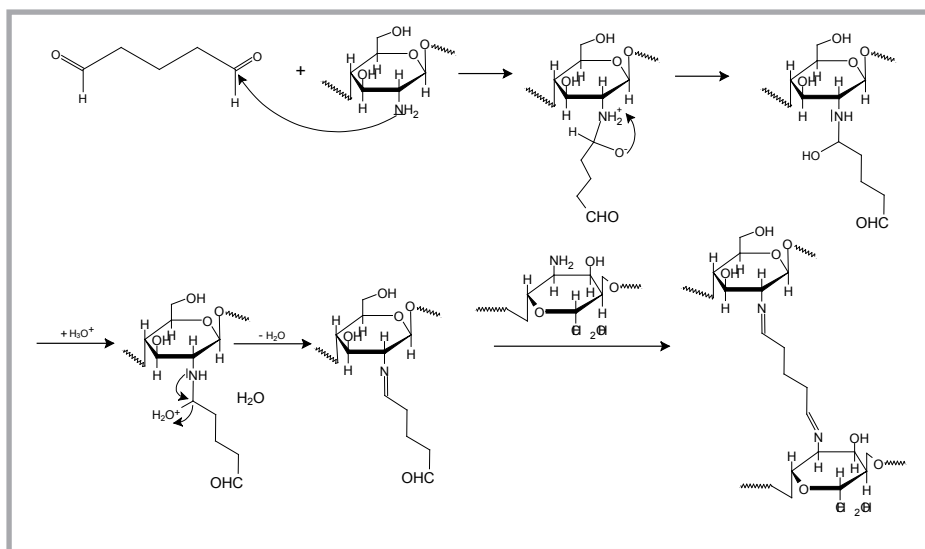

Figure 2. *Reaction mechanism between amino groups of chitosan and carbonyl groups of glutaraldehyde for the formation of Schiff base.*

ric and symmetric N-H deformation vibrations in the 1625-1560 cm^{-1} and 1550-1505 cm^{-1} range, respectively [12]. In conclusion we can say that the absorption band at 1637 cm^{-1} and 1560 cm^{-1} in the spectrum of Ch/GA/CIT derive mainly from the antisymmetric N–H deformation vibrations in protonated amines, but the initial amide-I, amide-II and imine bands are possibly overlapped by these vibrations. In the spectrum of Ch/CIT membrane, presented and discussed elsewhere [4], analogous peaks were observed at 1634 cm^{-1} and
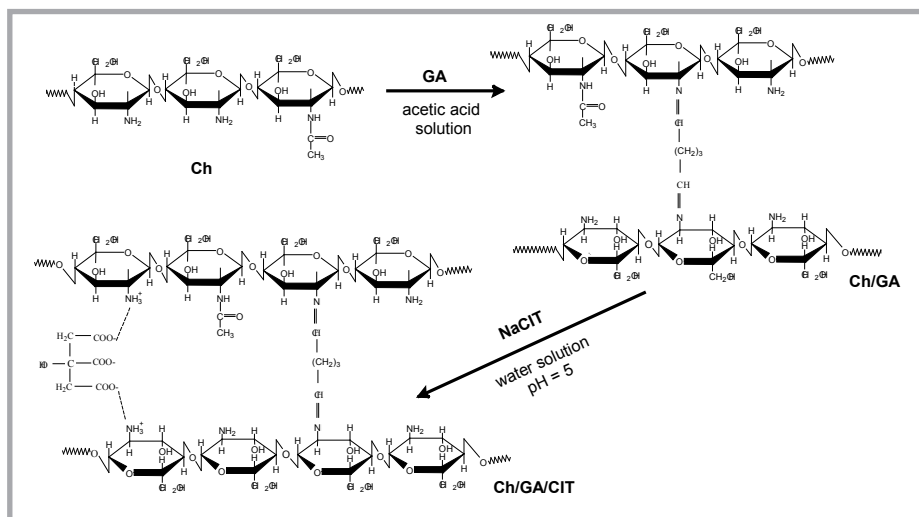
Figure 3. *Synthesis of Ch/GA and Ch/GA/CIT membranes.*

1584 cm^{-1}. Moreover, in the spectrum of Ch/GA/CIT membrane the characteristic band at 1380 cm^{-1} is observed. It corresponds to C-O symmetric vibrations in COO^{-} ions [12].

The spectral changes in the FTIR spectra of chitosan membrane treated with glutaraldehyde and sodium citrate indicate the formation of covalent and ionic crosslinks between chitosan and crosslinking agents, as presented in **Figure 3**.

To confirm the influence of modification on crystallinity of chitosan membrane the wide X-ray diffraction patterns of unmodified and modified membranes were compared (**Figure 4**). X-ray pattern of unmodified chitosan shows three major crystalline peaks: the two weaker peaks at $2\theta \approx 10°$ and $2\theta \approx 15°$ and the strongest one at $2\theta \approx 20°$, characteristic for the crystalline forms I, II and anhydrous form [15, 16]. The same reflections can be also observed on WAXS diffraction patterns of Ch/GA/CIT and Ch/GA membranes, but in the case of Ch/GA membrane the peak at $2\theta \approx 15°$ practically disappeared. Moreover, in the case of crosslinked membranes crystalline peaks, mainly the peak at $2\theta \approx 20°$, became wider and weaker. These results seem to indicate that crystallinity of the chitosan decreased after its crosslinking with glutaraldehyde and sodium citrate, but the crosslinked membranes retained their semicrystalline morphology.

3.2. Membrane swelling and state of water

The equilibrium degrees of swelling (EWC) for noncrosslinked and crosslinked chitosan membranes are presented in **Figure 5**. Values of EWC decrease in the following order: Ch > Ch/GA > Ch/CIT > Ch/GA/CIT. The equilibrium swelling of hydrogels is a result of the balance of osmotic forces determined by their affinity to the solvent and the network elasticity. Swelling ability of the studied chitosan membranes depends simultaneously on several parameters, such as hydrophilicity of the whole network, crosslinking degree
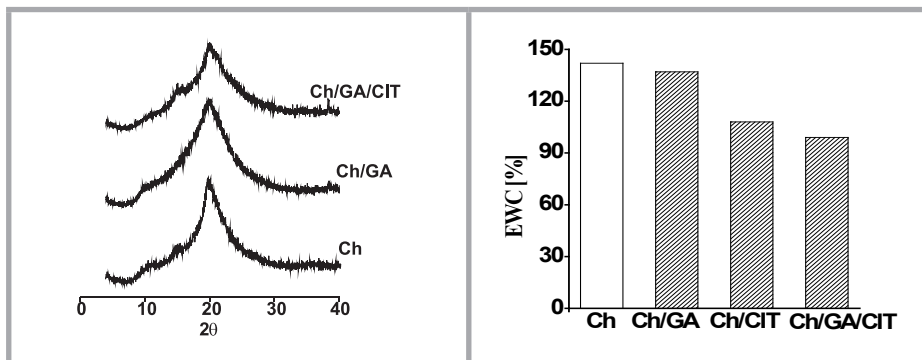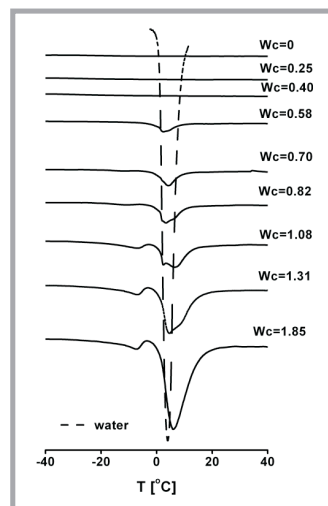
Figure 4. *X-ray diffraction patterns of unmodified and modified chitosan membranes.*
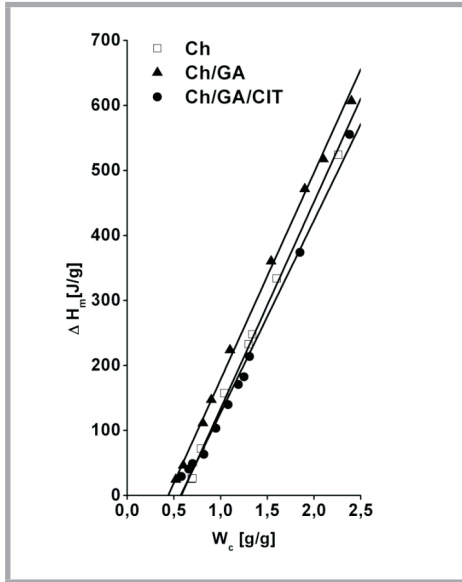
Figure 5. *Values of EWC for noncrosslinked and crosslinked chitosan membranes (data for Ch/CIT taken from the earlier work [4]).*

and polymer crystallinity. These parameters were different for Ch, Ch/GA and Ch/GA/CIT membranes.

Figure 6 shows the DSC melting thermograms of frozen water in Ch/GA/CIT membrane with various water content W_c. The other two studied membranes (Ch and Ch/GA) showed very similar melting behaviour of water to Ch/GA/CIT. For all analysed polymer-water systems no peaks are observed at T ≈ 0 °C below certain water content. For example, no peak is observed for Ch/GA/CIT membrane with $W_c = 0.25$ ($g_{water}/g_{polymer}$), $W_c = 0.40$ ($g_{water}/g_{polymer}$) (***Figure 6***). The absence of endothermic peaks above a water content threshold indicates that this water is non-freezing bound type. Moreover, for each studied polymer at define water content the broad endothermic peak appears, corresponding to the melting of freezable water. Such peak appears on thermogram of Ch/GA/CIT with water content $W_c = 0.58$ ($g_{water}/g_{polymer}$). In all the samples melting of water starts at temperature lower than that of pure water (the DSC heating curve of pure water is shown in ***Figure 6*** by the dashed line). Generally, for all studied hydrogel membranes of higher water content the endothermic peaks are broad and structured. For some water content

Figure 6. *DSC heating curves for Ch/GA/CIT membrane with different water content.*

multipeak with two well-defined submaxima can be observed. For example, more than one transition for Ch/GA/CIT with water content of 1.08 ($g_{water}/g_{polymer}$) is evident. This phenomenon can be attributed to the presence of at least two types of freezable water [1]: (i) freezing free water, which undergoes similar thermal transition to that of bulk water and (ii) freezing bound water, which undergoes a thermal phase transition at temperature shifted to lower temperatures with respect to that of bulk water. The formation of different states of

---

Figure 7. *Dependence of ΔHm per unit sample weight on water content.*
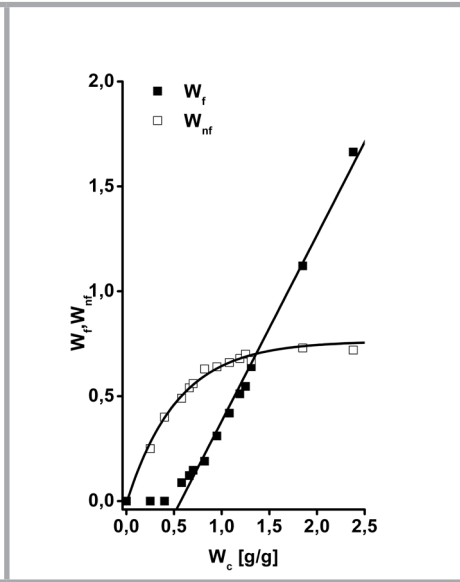
Figure 8. *The amounts of freezing water (Wf) and non-freezing water (Wnf) in Ch/GA/ CIT membrane with different water content.*

water within a polymeric network takes place in the following order: non-freezing, freezing bound and freezing free water.

The area under the DSC peak represents the change in enthalpy associated with the melting of freezing water (free water and freezable bound water). *Figure 7* presents a graph of the enthalpy of melting of freezing water per gram of polymer versus the water content W_c. The slope of the linear plot represents the "average apparent" value of the melting enthalpy associated with the freezing water (ΔH_m). The intercept with the horizontal axis corresponds to the maximum amount of non-freezing water ($W_{nf,max}$) in the hydrogel membrane. $W_{nf,max}$ is defined as the maximum amount of water present in the membrane, which is not associated with any endothermic peak [1].

The procedure described above gave ΔH_m=317 J/g and $W_{nf,max}$=0.65 g/g for Ch membrane, ΔH_m = 326 J/g, $W_{nf,max}$ = 0.47 g/g for Ch/GA membrane and ΔH_m = 296 J/g, $W_{nf,max}$ = 0.73 g/g for Ch/GA/CIT membrane. According to literature data [17, 18] bound water content in different polymer-water systems depends on both chemical as well as higher-order structure of a polymer. Thus, it can be supposed that differences in values of $W_{nf,max}$ for chitosan membranes result both from the changes in hydrophilicity or hydrophobicity as well as crystallinity of noncrosslinked and crosslinked membranes.

To get more information on water state in hydrogel membranes effect of total water content on freezable and non-freezable water content in membranes was analysed.

Figure 8 shows the dependencies of freezable water content W_f and non-freezable water content W_{nf} on total water content, W_c, for the Ch/GA/CIT membrane. Analogous curves were obtained for Ch and Ch/GA membranes. For all membranes W_f increased linearly with increasing W_c, while W_{nf} increased with increasing W_c until it reached a characteristic value and then remained constant.

4. Concluding remarks

Modified chitosan hydrogel membranes were prepared using glutaraldehyde (GA) and sodium citrate (NaCIT) as crosslinking agents. Fourier transform infrared spectroscopy of unmodified and modified chitosan membranes confirmed the formation of covalent and ionic crosslinking between chitosan (Ch) and GA or simultaneously covalent and ionic crosslinking between Ch, GA and NaCIT. It was found that crosslinking influenced both molecular and supermolecular structure of membranes as well as swelling properties and state of water in studied membranes. Equilibrium water content decreased in the following order: Ch > Ch/GA > Ch/GA/CIT. DSC studies showed the presence of both freezing and nonfreezing water in noncrosslinked and crosslinked chitosan membranes. The formation of different states of water within a polymeric network took place in the following order: non-freezing, freezing bound and freezing free water. For all membranes the freezable water content increased linearly with the water uptake and the non-freezable water content remained constant beyond critical value (ranging from 0.47 to 0.65 g/g dry membrane).

5. References

1. *Higuchi A, Komiyama J, Iijima T; (1984) The states of water in gel cellophane membranes. Polym Bull 11, 203-208.*
2. *Hodge RM, Edward GH, Simon GP; (1996) Polymer 37, 1371-1376.*
3. *Corkhill PH, Jolly AM, Ng ChO, Tighe BJ; (1987) Synthetic hydrogels: 1. Hydroxyalkyl acrylate and methacrylate copolymers – water binding studies. Polymer 28, 1758-1766.*
4. Ostrowska-Czubenko J, Pieróg M; *(2010)* State of water in citrate crosslinked chitosan membrane. *In: Jaworska MM (ed), Progress on Chemistry and Application of Chitin and its Derivatives. Vol. XV, Polish Chitin Society, Łódź, 33-40.*
5. *Il´ina AV, Varlamov VP; (2004) Hydrolysis of chitosan in lactic acid. Appl Biochem Microbiol 40, 300-303.*
6. *Muzzarelli RAA, Rocchetti R, Stanic V, Weckx M; (1997) Methods for the determination of the degree of acetylation of chitin and chitosan. In: Muzzarelli RAA, Peter MG (eds), Chitin Handbook, Atec Edizioni, Grottammare, 109–119.*
7. *Ostrowska-Czubenko J, Gierszewska-Drużyńska M; (2009) Effect of ionic crosslinking on the water state in hydrogel chitosan membranes. Carbohydr Polym 77, 590-598.*
8. *Pawlak A, Mucha M; (2003) Thermogravimetric and FTIR studies of chitosan blends. Thermochim Acta 396, 153-166.*
9. *Modrzejewska Z, Dorabialska M, Zarzycki R, Wojtasz–Pająk A; (2009) The mechanism of sorption of Ag+ ions on chitosan microgranules: IR and NMR studies. In: Jaworska MM (ed), Progress on Chemistry and Application of Chitin and its Derivatives. Vol. XIV, Polish Chitin Society, Łódź, 49-64.*
10. *Pearson FG, Marchessault RH, Liang CY; (1960) Infrared spectra or crystalline polysaccharides. V. Chitin. J Polym Sci 43, 101-116.*
11. Knaul J, Hudson SM, Creber KAM; *(1999) Crosslinking of chitosan fibres with dialdehydes: proposal of a new reaction mechanism. J Polym Sci Part B: Polym Phys 38, 1079-1094.*

12. *Rao CNR; (1963) Chemical Application of Infrared Spectroscopy. New York, London: Academic Press.*

13. *Schiffman JD, Schauer CL; (2007) Cross-linking chitosan nanofibers.* Biomacromolecules 8*, 594-601.*

14. *Monteiro OAC, Airoldi C; (1999) Some studies of crosslinking chitosan – glutaraldehyde interaction in homogeneous system. Int J Biol Macromol 26, 119-128.*

15. *Ogawa K, Yui T; (1993) Crystallinity of partially N-acetylated chitosans. Biosci Biotech Biochem 57, 1466-1469.*

16. *Ogawa K, Hirano S, Miyanishi T, Yui T, Watanabe T; (1984) A new polymorph of chitosan. Macromolecules 17, 973-975.*

17. *Qu X, Wirsén A, Albertsson A-C; (2000) Novel pH-sensitive chitosan hydrogels: swelling behavior and states of water. Polymer 41, 4589-4598.*

18. *Hatakeyama H, Hatakeyama T; (1998) Interaction between water and hydrophilic polymers. Thermochim Acta 308, 3-22.*