Journal of Applied Polymer Science

Water State in Chemically and Physically Crosslinked Chitosan Membranes

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ABSTRACT: Chemically and physically crosslinked chitosan membranes were prepared by treating chitosan (Ch) with glutaraldehyde (GA) and sulfuric acid (SA). FTIR and XRD results were employed to confirm the formation of covalent and ionic crosslinks between Ch, GA, and SA. The states of water in non-crosslinked and covalently and ionically crosslinked chitosan membranes containing different amount of water were investigated by low temperature differential scanning calorimetry measurements. The equilibrium swelling in water was examined gravimetrically. Two types of water were found in the polymer samples, i.e., freezing water and non-freezing water. The effect of crosslinking process on water state and water uptake was analyzed. The water uptake decreased after chitosan crosslinking with GA, but significantly increased after later crosslinking with SA. The amount of non-freezing water was generally smaller in crosslinked membranes. An impact of molecular and supermolecular structure on water uptake and state of water in non-crosslinked and crosslinked chitosan membranes was discussed. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 130: 1707–1715, 2013

KEYWORDS: polysaccharides; membranes; crosslinking; differential scanning calorimetry; swelling

Received 11 October 2012; accepted 3 April 2013; Published online 3 May 2013
DOI: 10.1002/app.39357

INTRODUCTION

Hydrogels are three-dimensional and hydrophilic polymer networks capable of swelling in water or biological fluids and retaining a large amount of fluids in the swollen state (usually more than 20% of the total weight).[1] A variety of natural, modified natural, and synthetic polymers are used to form hydrogels, including chitosan.

Chitosan is the most important derivative of chitin, a polysaccharide found in the exoskeleton of shellfish like shrimp and crab. It results from deacetylation of chitin under alkaline conditions or by enzymatic hydrolysis in the presence of chitin deacetylase.[2,3] Both chitin and chitosan are linear polysaccharides and are chemically defined as copolymers consisting of varying amounts of β-(1→4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc) and 2-amino-2-deoxy-β-D-glucopyranose (GlcN); (Figure 1). Chitosan is one of the most promising polymers for the preparation of membranes for various uses: for instance for pervaporation, ultrafiltration, reverse osmosis, gas separation, purification processes, or drug delivery due to its high hydrophilicity, favorable permselectivity of water, excellent chemical-resistant properties, and good film-forming character.[4,5] However, chitosan membranes highly swell in water (especially in acidic solutions) and the swollen membranes usually lose their permselectivity and mechanical stability. To improve mechanical and chemical resistance and water permselectivity of chitosan membranes, they are modified by different methods, including blending, multilayer casting, the addition of inorganic reinforcements, and bulk and surface crosslinking.[4,5]

In our previous study, we have reported the synthesis of the doubly crosslinked membranes comprising of chitosan (Ch) chemically crosslinked with glutaraldehyde (GA) and physically crosslinked with different ionic crosslinking agents, such as sodium citrate, sodium tripolyphosphate, and sulfuric acid (SA) and data on their equilibrium swelling in buffer solutions of different pH.[6] In this article, we focus on the swelling of Ch/GA/SA membranes in water and the state of water in chitosan membranes with different water content.

The nature of water in hydrogel membranes is the most important in understanding their equilibrium and dynamic swelling behavior as well as in analyzing a solute transport and other diffusive properties of such systems.[7–10]

Much research on the water-swollen polymers by means of differential scanning calorimetry (DSC), NMR spectrometry, FTIR spectroscopy, and other techniques has demonstrated that the state of water in polymer/water systems is different from that of bulk water.[11–15] DSC is in many ways the most convenient and informative method.[14] Water sorbed by polymers can be classified into three main categories: free water, freezable bound water, and non-freezable bound water.[8,15] Literature data[11,12,16–18] and our

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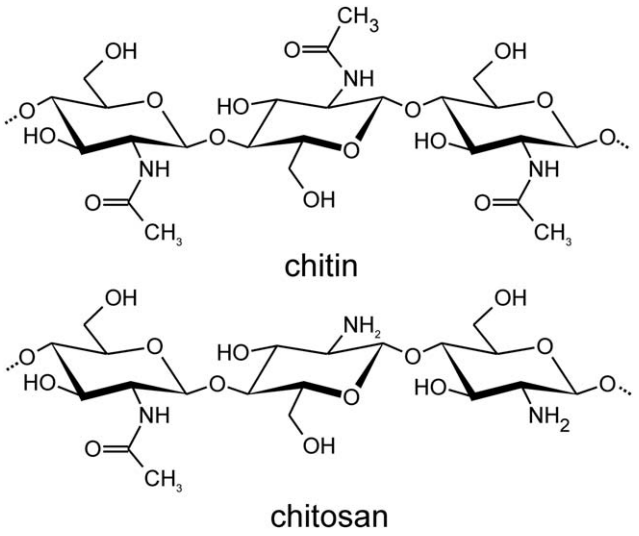

Figure 1. Chemical structures of chitin and chitosan.

previous studies[19,20] have indicated that the water structure in the hydrogel is sensitive to the nature of the polymer network.

Although many studies have been done on the total water uptake of ionic polymers, different states of water in the total water uptake are still necessary to investigate. In particular, it is not clear how the crosslinking of polymer affects the non-freezing water content. Therefore, we have put our attention especially on non-freezing water in doubly crosslinked chitosan membranes.

As it was stated by us and others, modification of chitosan by the addition of different crosslinking agents directly affects its molecular and supermolecular structure. Thus, it could be expected that the covalent and ionic crosslinking of chitosan by glutaraldehyde and sulfuric acid should influence the water properties in chitosan membrane. To investigate the contributions of the covalent and ionic crosslinking to water structure both non-crosslinked chitosan membrane as well as chitosan membranes crosslinked by glutaraldehyde or glutaraldehyde and sulfuric acid were prepared and analyzed. The characterization of the state of water in chitosan membranes was performed by DSC. The amount of non-freezing and freezing bound water in membranes with different water content was determined.

EXPERIMENTAL

Materials

Commercially available chitosan (Ch) from crab shells of medium molecular weight and glutaraldehyde (GA, 25 wt % solution in water) of analytical grade were purchased from Sigma-Aldrich (Germany). Acetic acid, sulfuric acid, and sodium hydroxide were analytical grade and were purchased from POCh (Poland). Potassium bromide for spectroscopy was purchased from Merck (Germany).

Chitosan Characterization

Degree of deacetylation (DDA) of chitosan was determined by potentiometric titration method.[21] The chitosan sample was dissolved in a known excess of 0.02*M* HCl and the obtained solution was titrated with 0.1*M* NaOH. DDA was calculated using eq. (1):

$$\mathrm{DDA} = \left(\frac{M_{\mathrm{GlcNAc}} \cdot c_{\mathrm{NaOH}} \cdot \Delta V}{m_{\mathrm{Ch}} + (M_{\mathrm{GlcNAc}} - M_{\mathrm{GlcN}}) \cdot c_{\mathrm{NaOH}} \cdot \Delta V}\right) \cdot 100 = \left(\frac{203 \cdot c_{\mathrm{NaOH}} \cdot \Delta V}{m_{\mathrm{Ch}} + 42 \cdot c_{\mathrm{NaOH}} \cdot \Delta V}\right) \cdot 100 \quad (1)$$

where M_{GlcNAc} and M_{GlcN} are molecular weights of GlcNAc and GlcN units ($\mathrm{g \cdot mol^{-1}}$), respectively, c_{NaOH} is molarity of NaOH solution, ΔV is the difference of the volume of NaOH solution between inflection points of the titration curve, and m_{Ch} is mass of the chitosan sample. Degree of deacetylation was equal to 75.72 ± 3.82%.

Viscosity average molecular weight, M_v, was determined by viscosity analysis of chitosan solutions according to Il′ina and Varlamov.[22] Relative viscosities of polymer samples in 0.2*M* NaAc/2 wt % HAc (v/v = 1/1) were measured in triplicate using an Ubbelohde capillary viscometer at 25.0 ± 0.1°C. The intrinsic viscosity was determined by Huggins and Kraemer plots. M_v of chitosan was calculated using the classical Mark–Houwink equation [eq. (2)]:

$$[\eta] = KM_v^a \quad (2)$$

where $[\eta]$ is the intrinsic viscosity of the polymer solution, K and a are constants for given solute–solvent system and temperature ($K = 1.38 \cdot 10^{-4}$, $a = 0.85$). Viscosity average molecular weight was equal to (730 ± 16) kDa.

Membrane Preparation

Pure chitosan membranes were prepared by solution casting and solvent evaporation technique. Chitosan solution of 1% (w/v), prepared by dissolving chitosan powder in 2% (w/v) acetic acid, was filtered, left over night, degassed, cast as film on a clean glass Petri dish, evaporated to dryness in an oven at 37°C and then further dried under vacuum at 60°C for 24 h. The membrane thickness was controlled by pouring a definite amount of chitosan solution on Petri dish with the same surface area. Dry membranes were immersed in 2*M* sodium hydroxide solution for 5 min to remove the residual acid, thoroughly washed with deionized water and air-dried.

Two-component chitosan/glutaraldehyde (Ch/GA) membranes were prepared in two steps, as described elsewhere.[6] 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 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 membranes were washed repeatedly with double distilled water, immersed in 2*M* sodium hydroxide solution for 5 min and again washed repeatedly with water and dried in air. Two-component Ch/SA membrane (prepared specially for FTIR analysis) and three-component chitosan/glutaraldehyde/sulfuric acid (Ch/GA/SA) membranes were prepared by immersing pure Ch or Ch/GA membranes in 0.5*M* sulfuric acid solution at room temperature for 60 min and 24 h, respectively. These modified membranes, designated as Ch/SA (60 min), Ch/SA (24 h), Ch/GA/SA (60 min), and Ch/GA/SA (24 h) were thoroughly washed with

deionized water and dried at air. Then, all unmodified and modified chitosan membranes were dried under vacuum at 60°C for 24 h.

FTIR Spectroscopy Analysis

FTIR spectra of unmodified and modified chitosan 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. The polymer samples were ground into a fine powder using liquid nitrogen and thoroughly dried under vacuum at 60°C before milling with anhydrous KBr.

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 Cu $K\alpha$ radiation and scanned from $2\Theta = 4°$ to 40° at a step size of 0.02°.

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 h, which was checked to be sufficient to reach an equilibrium state. Then membrane was taken out, wiped quickly with filter paper and weighed. EWC, expressed as the percentage of water in the membrane at equilibrium, was calculated using the following equation:

$$\mathrm{EWC} = \frac{W_s - W_d}{W_d} \times 100\% \quad (3)$$

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

Differential Scanning Calorimetry (DSC) Measurements

The state of water in chitosan hydrogel membranes was analyzed by DSC measurements, as described elsewhere.[19] A Polymer Laboratories (Epsom, UK) 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 h 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, was determined by direct 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^{-1}). The amount of freezable water was calculated from the following equation:

$$W_f = W_{ff} + W_{fb} = \frac{\Delta H_m}{\Delta H_0} \quad (4)$$

where ΔH_m 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 nonfreezing 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:

$$W_{nf} = W_c - W_f \quad (5)$$

In these equations, the amounts of freezing water and nonfreezing bound water are defined as weights relative to the weight of dry polymer.

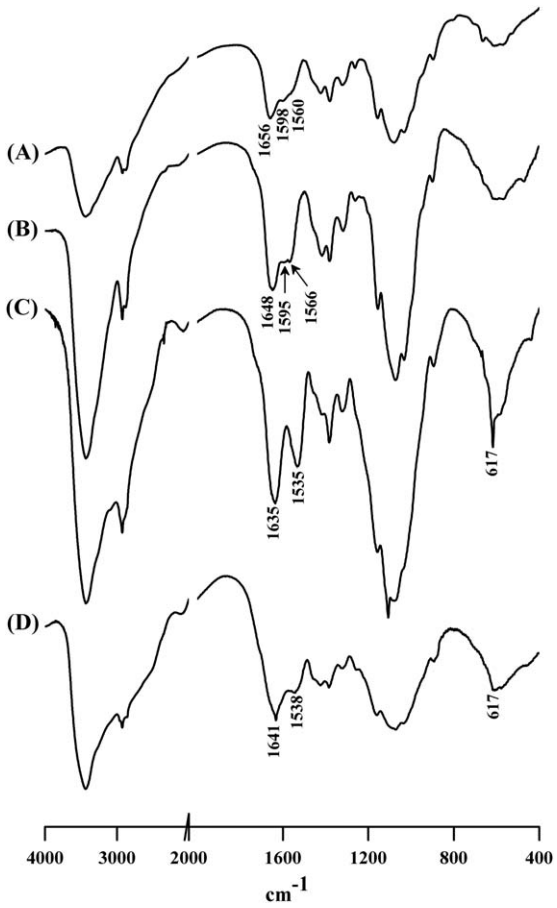

Figure 2. FTIR spectra of (A) Ch, (B) Ch/GA, (C) Ch/SA (24 h), and (D) Ch/GA/SA (24 h).

RESULTS AND DISCUSSION

Membrane Characterization by FTIR and X-ray Spectroscopy

Figure 2 shows the FTIR spectra of the non-crosslinked and crosslinked chitosan. After the addition of crosslinking agents, several changes can be observed in the FTIR spectrum of chitosan. Absorption bands situated at 1656 cm^{-1} (C=O stretching in amide group, amide I vibration), 1598 cm^{-1} (-NH_2 bending

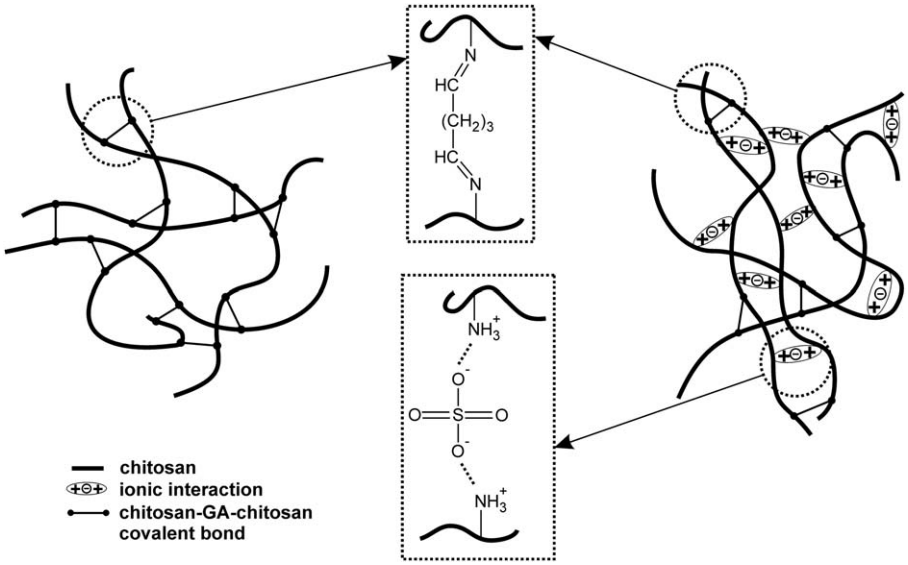

Figure 3. Schematic representation of chemical structure of crosslinked chitosan membranes.

in non-acetylated 2-aminoglucose primary amine), and 1560 cm^{-1} (N–H bending in amide group, amide II vibration)[23,24] change their positions. After the crosslinking with GA, the peak at 1656 cm^{-1} shifts to the lower wavenumber, i.e., to 1648 cm^{-1}. Moreover, in the region of about 1600–1560 cm^{-1} it can be observed a broad band with submaxima at about 1595 and 1566 cm^{-1}.

The band at 1648 cm^{-1} is most probably composed of amide I band of chitosan and the C=N stretching band of Schiff's base. C=N peak, depending on the compound being reacted, can appear between 1620 and 1660 cm^{-1}.[25] No band is observed at ~1715 cm^{-1}, related to the free aldehyde group.[26] Thus, it can be concluded that under experimental reaction conditions GA completely reacted with chitosan and imines (Schiff bases) were formed. The formation of crosslinks between Ch and GA was also confirmed visually by the change of membrane color from transparent to deep yellow.[27] The broad band in the spectrum of Ch/GA with two submaxima at about 1595 and 1566 cm^{-1} seems to be composed of two peaks assigned to -NH_2 bending vibration in non-acetylated 2-aminoglucose primary amine group and N–H bending vibration in amide group, respectively.

In the FITR spectra of Ch/SA and Ch/GA/SA in the frequency range of 1650–1500 cm^{-1}, two main bands can be seen: the first at 1635 cm^{-1} (Ch/SA) or 1641 cm^{-1} (Ch/GA/SA) and the second at 1535 cm^{-1} (Ch/SA) or 1538 cm^{-1} (Ch/GA/SA). It can be supposed that these bands represent envelope of several bands, because both amine and protonated amine groups, as well as acetamide and imine groups absorb in these frequency regions. Protonated amines show an asymmetric and symmetric N–H deformation vibrations in the 1625–1560 cm^{-1} and 1550–1505 cm^{-1} range, respectively.[26] The absorption bands at 1635 and 1535 cm^{-1} in the spectrum of Ch/SA and the bands at 1641 and 1538 cm^{-1} in the spectrum of Ch/GA/SA derive mainly from the asymmetric and symmetric N–H deformation vibrations in protonated amines, but the initial amide-I, amide-II, and imine bands are possibly overlapped by these vibrations. The new band observed at 617 cm^{-1} in FTIR spectra of Ch/SA and Ch/GA/SA can be attributed to S–O bending vibration in SO_4^{2-} ions.[26]

The spectral changes observed in the FTIR spectra of chitosan membrane treated with glutaraldehyde and/or sulfuric acid confirm the presence of GA and SA in modified chitosan membrane and indicate the formation of covalent and ionic crosslinks between chitosan and crosslinking agents, as shown in Figure 3. The preparation of composite membranes by crosslinking of chitosan or chitosan blends with glutaraldehyde or sulfuric acid and their spectral characteristics were reported earlier by several researchers.[28–30] The results of all these studies agree with our statement on the formation of imine linkages –N=CH– and Coulombic interactions between sulfate anions and –NH_3^+ cations of chitosan.

To characterize the influence of crosslinking process on chitosan crystallinity the wide X-ray diffraction patterns of non-crosslinked and crosslinked membranes were compared (Figure 4). X-ray pattern of Ch 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.[31] In the case of crosslinked membranes, these 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 or glutaraldehyde and sulfuric acid, but the crosslinked membranes retained their semicrystalline morphology.

Swelling of Non-Crosslinked and Crosslinked Chitosan Membranes

The equilibrium water content (EWC) for unmodified and modified chitosan membranes is shown in Figure 5. Values of EWC change in the following order: Ch/GA/SA (60 min) > C/GA/SA (24 h) > Ch > Ch/GA. As discussed earlier by

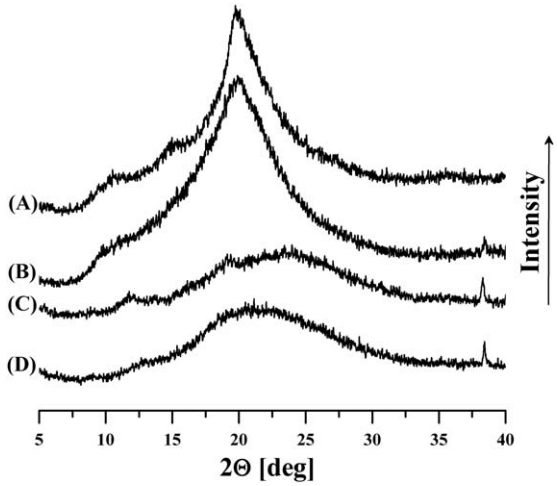

Figure 4. Wide angle X-ray diffractograms of (A) Ch, (B) Ch/GA, (C) Ch/GA/SA (60 min), and (D) Ch/GA/SA (24 h).

Peppas et al.,[32] the equilibrium swelling of ionic hydrogels is a function of the network structure, crosslinking degree, hydrophilicity, and degree of ionization of the functional groups. The hydrogels studied by us differ both in their molecular structure (hydrophilic/hydrophobic properties) as well as in degree of crosslinking and the content of ionizable groups. Thus, there could be expected some differences in their swelling ability.

We have drawn conclusion on the degree of ionic crosslinking and the content of ionizable groups in Ch/GA/SA (60 min) and Ch/GA/SA (24 h) membranes from FTIR and elemental analysis data. We have used the A_{1641}/A_{1380} ratio, where A_{1641} and A_{1380} are the peak areas of the bands assigned to $-NH_3^+$ ion and CH_3- groups, respectively, to monitor the changes of the degree of protonation of amino groups during the process of chitosan membrane treating with sulfuric acid, as proposed earlier by Cui et al.[30] The band at 1380 cm^{-1} was used as internal standard. Results of this analysis indicated that both degree of protonation of amino groups as well as the sulfur content (and thus sulfate groups content) increased with the sulfuric acid treatment time. Degree of protonation showed very similar dependence on crosslinking time to that observed for sulfate groups content. These results confirm that protonation (binding of sulfuric acid to active sites) is related to adsorption of sulfuric acid by the Ch/GA membrane. The sulfur content in Ch/GA/SA (60 min) and Ch/GA/SA (24 h) was equal to 7.49% wt and 7.59% wt, respectively, and therefore degree of crosslinking of those membranes was similar.

Analysis of the EWC values for Ch and Ch/GA hydrogels indicates that covalent crosslinking lowers the swelling of Ch membrane. Addition of GA affects the chemical structure of chitosan membrane and thus its hydrophobic/hydrophilic properties. Unmodified chitosan membrane is hydrophilic because polysaccharide chains contain three different polar groups, namely primary amine ($-NH_2$), hydroxyl (–OH), and ether (C–O–C) groups that can form hydrogen bonds with water. As it results from our calculations presented elsewhere,[6] degree of ionization of chitosan at doubly distilled water (at pH $\approx$ 6.5) is very small and therefore chitosan membrane in water solution practically does not contain hydrophilic $-NH_3^+$ groups. The crosslinking of chitosan by GA reduces the content of $-NH_2$ groups and thus decreases its hydrophilicity and EWC. Beppu et al.[29] have also shown that the chemical modification with glutaraldehyde turns chitosan more hydrophobic. Moreover, covalent crosslinks in the structure of the studied chitosan hydrogels hinder the mobility of the polymer chains. These factors cause decrease of swelling. Higher crosslinked hydrogels have a tighter structure and swell less compared to the same hydrogels with lower crosslinking degree.[32]

Value of EWC for Ch/GA membrane markedly increases when it is additionally crosslinked with sulfuric acid. We suppose that it results from differences in molecular and supermolecular structure of covalently and simultaneously covalently and ionically crosslinked polymers. The doubly crosslinked membrane has a tighter structure and should swell less compared to non-crosslinked and singly crosslinked hydrogels. However, when doubly crosslinked Ch/GA/SA polymer is formed then the number of hydrophilic groups of chitosan that interact with water molecules decreases and simultaneously new $-SO_4^{2-}\cdots NH_3^+-$ groups appear. Thus, hydrophilicity of Ch/GA/SA membrane differs both from non-crosslinked as well as covalently crosslinked chitosan membrane. Moreover, after covalent and ionic crosslinking the crystallinity of chitosan decreases (Figure 4) and the portion of amorphous regions increases. Thus, the structure is more accessible for water molecules.

State of Water in Non-Crosslinked and Crosslinked Chitosan Membranes

To further elucidate the swelling behavior of non-crosslinked and crosslinked chitosan membranes the state of water, i.e., the free water and bound water contents, were determined by DSC experiments. As mentioned above, water sorbed by polymers is generally classified into three main categories: free water, freezing bound water, and non-freezing bound water.[8,15,33] Free water (also referred to bulk, frozen, freezable, or freezing) consists of water molecules that have a structure similar to that of bulk water. This water possesses the same physical

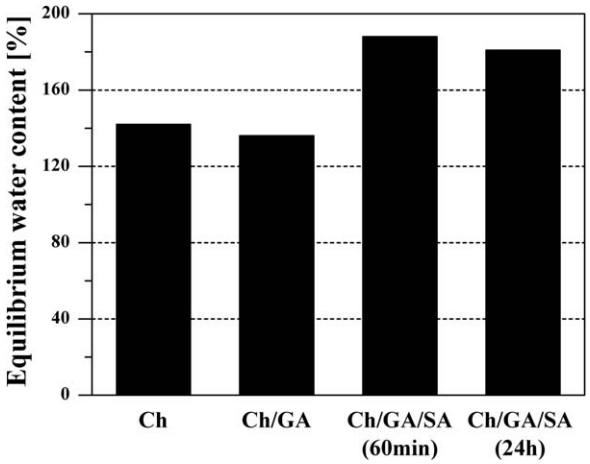

Figure 5. Equilibrium water content of non-crosslinked and crosslinked chitosan membranes.

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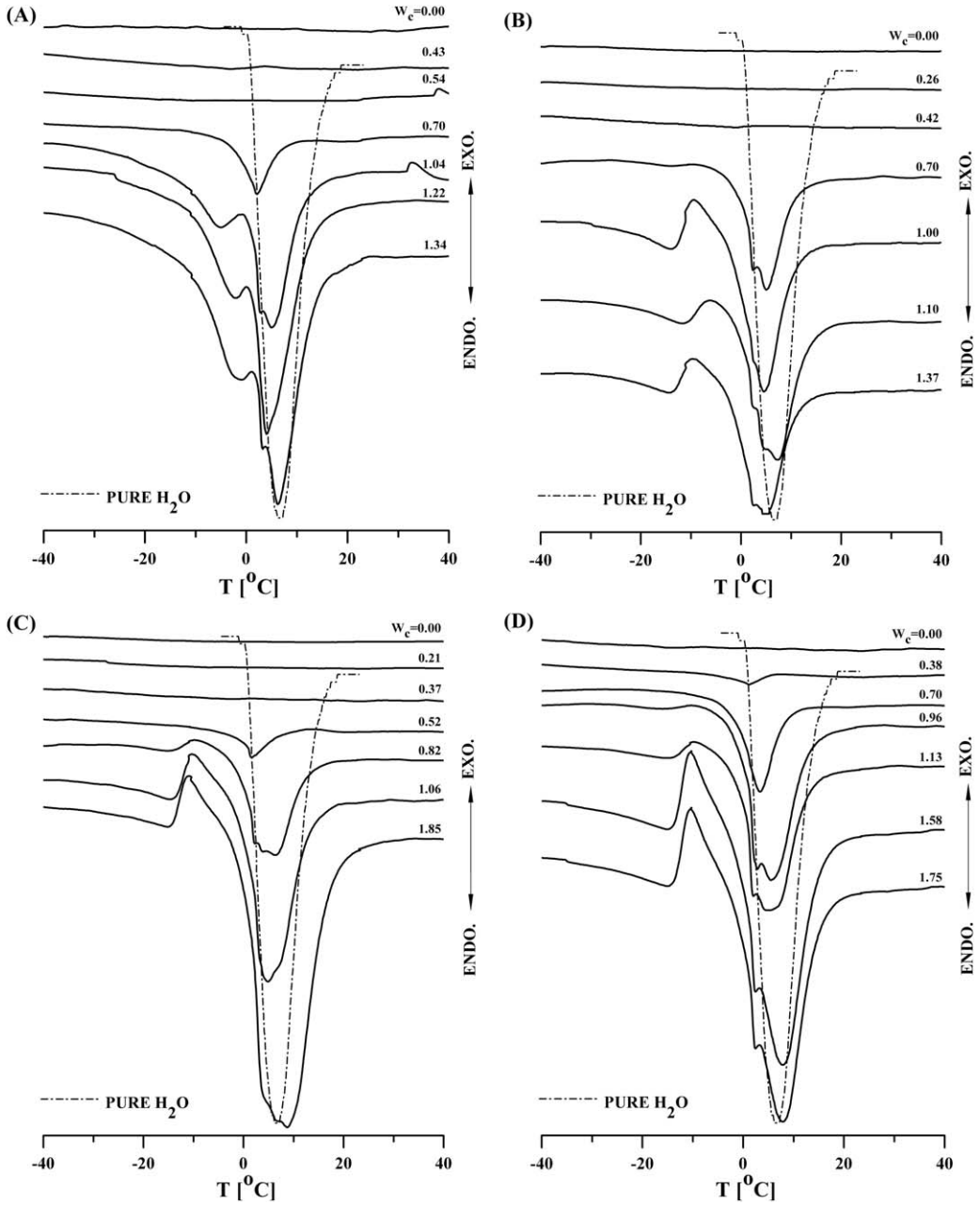

Figure 6. DSC heating curves for (A) Ch, (B) Ch/GA, (C) Ch/GA/SA (60 min), (D) and Ch/GA/SA (24 h) membranes with different water content.

characteristics as bulk water, including the freezing enthalpy of pure water and the normal freezing temperature close to 0°C. Freezing bound water (also called freezable bound water, loosely bound water, or intermediate water) is defined as water that has a phase transition temperature less than 0°C. It is regarded to interact with a polymer in an intermediate way that is stronger than freezing water but weaker than non-freezing water. This water may originate from impure water clusters, water molecules confined in small pores, or involvement in physical interaction with the polymer backbone matrix.[33] Non-freezing bound water (also called non-freezable or unfrozen water) is tightly bound to the polymer and has no detectable phase transition in the temperature range from -73°C to 0°C.[14] The mechanism of formation of unfrozen water in polymers is not explained.[34] It is generally accepted that unfrozen bound water is formed by the hydrogen bonds between water molecules and polar groups in the polymer.

When hydrated membranes are cooled to –140°C ("DSC experiment" section) the free water and freezing bound water freeze but the non-freezing bound water remains in the non-frozen state. When the frozen polymer sample is again heated in a DSC cell, the heat required to melt the frozen water is measured. Figure 6 shows the DSC melting thermograms of frozen water in Ch, Ch/GA, and Ch/GA/SA membranes with different water content. One common finding from these data is

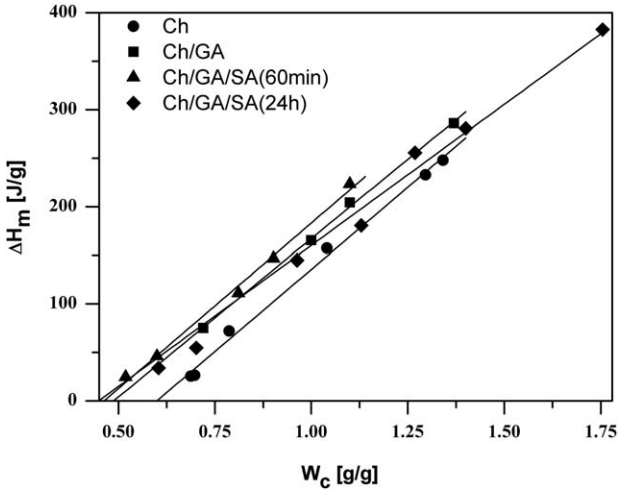

Figure 7. Relation between enthalpy of water melting and water content in non-crosslinked and crosslinked chitosan membranes.

that all sorbed water was in a non-freezable bound state below a critical value.

At low water content, no water fusion peaks were observed in the scanned temperature range [Figure 6(A–D)]. The absence of endothermic peaks below a water content threshold indicates that this water is non-freezing bound type. For each studied membrane at definite water content, the broad, structured endothermic peak appears that corresponds to the melting of freezable water. Analogous poorly resolved multipeaks with distinct submaxima and composed of several endothermic peaks were observed by us earlier for polyelectrolyte complexes composed of chitosan and sodium alginate[19] and by others for different polymers.[33,35,36] Sometimes only asymmetric DSC freezing peaks in hydrated polymers were observed. For example, Mochizuki et al. have recently observed asymmetric shape of the fusion peaks for poly-HEMA and concluded on the existence of at least two types of water.[35] It can be suggested that within the broad transitional envelope there are several metastable states of water due to the water interacting and being bound with different binding sites of the polysaccharide structure and/or the freezable water exists in different environments.

Our results indicate that DSC could be used successfully to quantify the non-freezing water (tightly bound) and freezing water (loosely bound and free water) separately in chitosan membranes. However, it was difficult to quantify the loosely bound and free water separately only using DSC method. This is due to the fact that the melting endotherms of free and loosely bound water overlapped with each other.

The area under the DSC peak represents the change in enthalpy associated with the melting of freezable 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 freezable water (ΔH_m) and the intercept with the horizontal axis corresponds to the maximum amount of non-freezable water ($W_{nf,max}$) in the hydrogel, defined as the maximum amount of water present in the polymer, which is not associated with any endothermic peak.[37] The curves presented in Figure 7 were estimated by linear function. Values ΔH_m and $W_{nf,max}$ are presented in Table I. The heat of melting of freezable water in analyzed membranes changes from 334 J g^{-1} for Ch membrane to 313 J g^{-1} for Ch/GA/SA (24 h). Similar values of ΔH_m were observed earlier by us for chitosan/sodium alginate complex membrane[19] and by others for different non-ionic polymers, ionic polymers, and ionic complexes.[18,38–40]

The results presented in Table I show that the value of $W_{nf,max}$ decreases after crosslinking of chitosan membrane with GA or GA and SA. Moreover, for doubly crosslinked membranes, $W_{nf,max}$ is decreasing function of EWC. Similar data were reported earlier for another ionic polymer. DSC studies of water state in copolymers with sulfonate and ammonium ionic groups (polymer gels containing different amounts of *p*-sodium styrene sulfonate and (vinylbenzyl)trimethylammonium chloride),[41] in carboxymethyl chitosan-based polyampholytes,[42] and in crosslinked sulfonated poly(phthalazinone ether sulfone ketone)[36] have shown that crosslinking decreases equilibrium swelling but increases the non-freezable water content of the gels.

The decreasing amount of non-freezing bound water after polymer crosslinking suggests that fewer water molecules are strongly bound to the polar sites of chitosan. It is well known that many factors may influence the state of water and/or the amount of the non-freezing bound water in hydrated membrane, such as molecular structure of polymers, amount and distribution of ionic groups, and supermolecular structure of polymers.[11,18,36,38–40] Thus, it can be supposed that the differences observed by us in $W_{nf,max}$ result from differences in molecular and supermolecular structure (degree of crystallinity) of unmodified and modified chitosan membranes. As we discussed above, addition of GA or GA and SA affects the chemical structure of chitosan membrane and thus its hydrophobic/hydrophilic properties as well as its crystallinity.

The crosslinking of chitosan with GA reduces the content of hydrophilic $-NH_2$ groups and decreases slightly its crystallinity. This consequently leads to decrease in the strongly bound water molecules, i.e., the decrease in the number of non-freezable water molecules in Ch/GA membrane. Similar effect of GA on the content of non-freezing water was observed earlier for chitosan membrane[43] and PVA membrane.[44] Ionic crosslinking of Ch/GA with SA leads to the additional decreasing of $-NH_2$ groups and simultaneous appearing of new $-SO_4^{2-}\cdots NH_3^{+}-$ groups and to large decrease of crystallinity degree. It was

Table I. Values of ΔH_m and $W_{nf,max}$ for Non-Crosslinked and Crosslinked Chitosan Membranes

Membrane	ΔH_m (J/g)	$W_{nf,max}$ ($g_{water}/g_{polymer}$)
Ch	334	0.60
Ch/GA	333	0.50
Ch/GA/SA (60 min)	326	0.45
Ch/GA/SA (24 h)	313	0.51

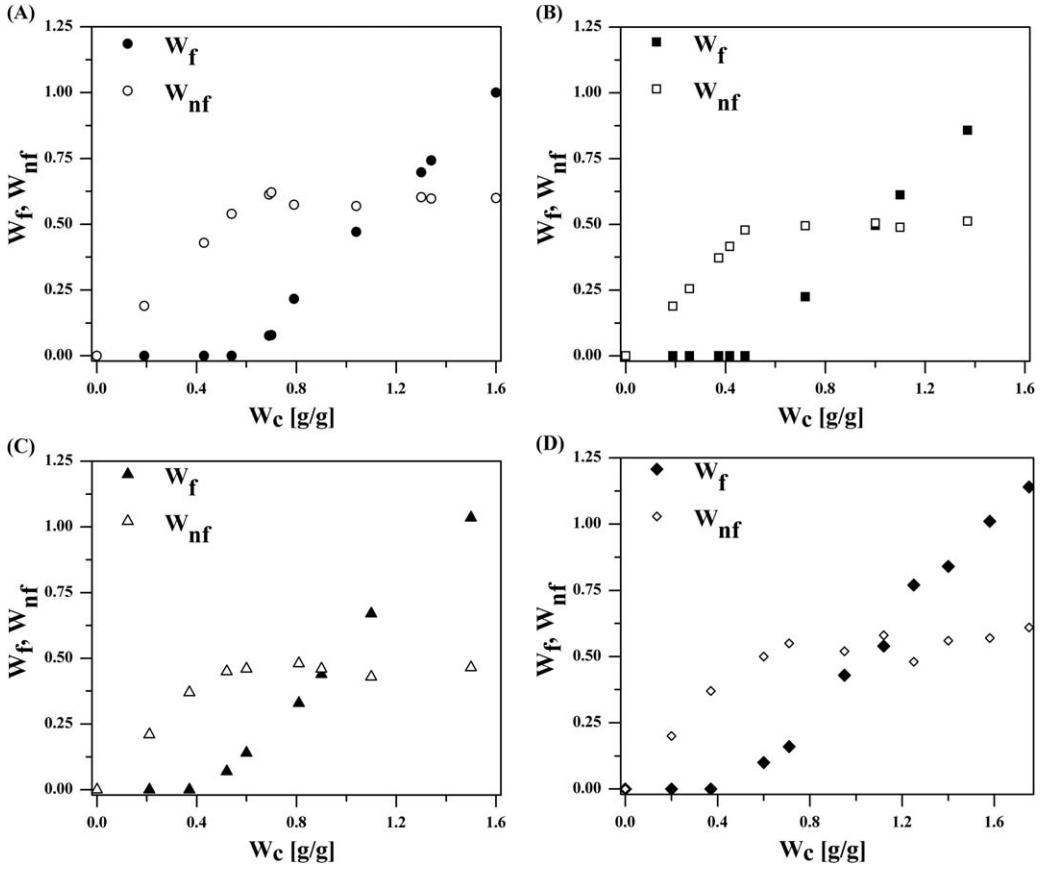

Figure 8. The amounts of freezing water (W_f) and non-freezing water (W_{nf}) in (A) Ch, (B) Ch/GA, (C) Ch/GA/SA (60 min), and (D) Ch/GA/SA (24 h) membranes with different water content.

observed by others that non-freezing water content decreases after the formation of interpolymer complex,[18,39] but it increases with decrease of polymer sample crystallinity.[15,45] Thus, values of $W_{nf,max}$ for doubly crosslinked membranes are result of these two opposite effects. Crosslinking with GA or GA and SA causes a decrease of $W_{nf,max}$, but decrease of polymer crystallinity causes an increase of $W_{nf,max}$.

To get more information on water state in non-crosslinked and crosslinked chitosan membranes an effect of total water content, W_c, on freezable, W_f, and non-freezable water content, W_{nf}, in membranes were analyzed. Figure 8 shows the curves $W_f = f(W_c)$ and $W_{nf} = f(W_c)$ for studied membranes. It can be observed that when the water content is below the critical threshold, W_{nf} increases linearly with W_c. At higher values of water content, W_{nf} is practically constant. Analogous relation between W_{nf} and W_c was observed earlier by us for chitosan/sodium alginate complex membrane[19] as well as by Ping et al.[11] and Guan et al.[12] for different hydrophilic polymers. Moreover, for all analyzed membranes, the W_f value decreases when the water content decreases.

CONCLUSIONS

Modified chitosan hydrogel membranes were prepared using glutaraldehyde and sulfuric acid as crosslinking agents. FTIR spectroscopy of non-crosslinked and crosslinked chitosan membranes confirmed the formation of covalent crosslinking between chitosan and glutaraldehyde or simultaneously covalent and ionic crosslinking between chitosan, glutaraldehyde, and sulfuric acid.

It was found that crosslinking influenced the supermolecular structure of chitosan membrane, it decreased its crystallinity. Analysis of the EWC values for Ch, Ch/GA, and Ch/GA/SA hydrogels indicated that crosslinking of chitosan membrane with GA lowered its EWC, but crosslinking of Ch with GA and then with SA led to markedly increasing its EWC. Moreover, crosslinking of chitosan membrane influenced also the state of water. Both in non-crosslinked as well in singly and doubly crosslinked chitosan membranes, there were observed freezing and non-freezing water, but their amounts were different. Value of $W_{nf,max}$, defined as the maximum amount of non-freezing water, which is not associated with any endothermic peak, was generally smaller for crosslinked membrane than uncrosslinked one. It was the result (the sum) of the two opposite effects: crosslinking of chitosan with GA or with GA and SA and decreasing of polymer crystallinity. For all membranes, the freezable water content increased linearly with water uptake and non-freezable water content remained constant beyond critical value $W_{nf,max}$. The DSC results revealed that the formation of non-freezable water in polymer membrane was determined both by the molecular structure as well as its supermolecular structure.

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