Carbohydrate Polymers 77 (2009) 590–598

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Effect of ionic crosslinking on the water state in hydrogel chitosan membranes

Jadwiga Ostrowska-Czubenko *, Magdalena Gierszewska-Drużyńska

Faculty of Chemistry, Nicolaus Copernicus University, Gagarina 7, 87-100 Toruń, Poland

ARTICLE INFO

Article history:
Received 11 July 2008
Accepted 29 January 2009
Available online 11 February 2009

Keywords:
Chitosan
Alginate
Polyelectrolyte complex
Hydrogels
DSC

ABSTRACT

The polyion complex membrane (PEC) composed of chitosan (Ch) and sodium alginate (NaAlg) designated for the separation of water/organic mixtures by pervaporation and/or direct methanol fuel cell technology was synthesized and analysed by FTIR, DSC, DTG and X-ray diffraction. The polyion complex formation between Ch (cationic polyelectrolyte) and NaAlg (anionic polyelectrolyte) was confirmed by Fourier transform infrared (FTIR) spectroscopy, thermogravimetric analysis (TG) and differential scanning calorimetry (DSC). The state of water in pure polyelectrolytes (PE) and PEC was studied by DSC. Results show that freezable and non-freezable water exist in analysed Ch, NaAlg and Ch/NaAlg hydrogels, while there are variations in the amount of non-freezing bound water in PE/water and PEC/water systems. Both ionic crosslinking as well as physical structure influence the state of water, and especially the non-freezable water content, in ionic hydrogel membranes.

1. Introduction

Generally, 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). Three-dimensional networks are usually formed by chemical or physical crosslinking of hydrophilic polymer chains. In chemical gels polymer chains are connected by covalent bonds, but in physical gels they are held together by molecular entanglements, noncovalent bonds, such as van der Waals interactions, ionic interactions, hydrogen bonding, hydrophobic interactions, traces of crystallinity and multiple helices (Blanco, Olmo, & Teijon, 2002; Kishida & Ikada, 2001). A variety of natural, modified natural and synthetic polymers are used to form hydrogels, including chitosan and alginate. Alginate and chitosan are naturally occurring polysaccharides.

Alginates constitute a family of linear unbranched anionic polysaccharides, mainly extracted from three species of brown algae, that are composed of (1 → 4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues (Fig. 1A). The uronic acid residues are arranged in pattern of homopolymeric M blocks, homopolymeric G blocks and heteropolymeric sequentially alternating MG blocks along the chain (Fig. 1B) (Draget, Smidsrød, & Skjåk-Bræk, 2002). Due to their physical, chemical and biological properties alginates have been used in food processing, medicine and pharmacy, textile and paper industries, etc.

Chitosan is a cationic linear polysaccharide usually obtained by alkaline deacetylation of crustacean chitin from crab and shrimp shell wastes. Both chitin and chitosan are linear polysaccharides and are chemically defined as copolymers consisting of two residues: *N*-acetylglucosamine (2-acetamido-2-deoxy-β-D-glucopyranose) and glucosamine (2-amino-2-deoxy-β-glucopyranose) (Fig. 1C) (Peter, 2002). The difference between chitin and chitosan lies in the degree of deacetylation. The typical commercial chitosan has approximately 85% deacetylation. Due to their excellent properties, such as biocompatibility, biodegradability, non-toxicity, adsorption properties, etc., chitosan has found applications in medicine and pharmacy, as food additives, antimicrobial agent, in paper and textile industry, in environmental remediation and other industrial areas (Berger, Reist, Mayer, Felt, & Gurny, 2004; Berger, Reist, Mayer, Felt, Peppas, et al., 2004; Kim, Lee, & Lee, 2007).

Chitosan, as a cationic polyelectrolyte, is able to form polyelectrolyte complexes (PECs) by reaction with various natural and synthetic anionic polyelectrolytes. Polyelectrolyte complexes of chitosan with other polysaccharides, DNA, proteins and different synthetic anionic polyelectrolytes were investigated. These complexes are generally water insoluble and form hydrogels. A great number of PECs have been studied and characterized due to their wide variety of applications in medicine, pharmacy (especially as drug delivery systems), technology and other fields (Berger, Reist, Mayer, Felt, & Gurny, 2004; Berger, Reist, Mayer, Felt, Peppas, et al., 2004). Recently, PECs have attracted interest as membranes for different end uses. Microcapsules with PEC membranes has been intensively studied. Much attention has been also focused on the application of the polyelectrolyte complex membranes to the separation of water/organic or organic/organic mixtures by pervaporation and as an electrolyte for

---

* Corresponding author. Tel.: +48 56 6114524; fax: +48 56 6542477.
E-mail address: jocz@chem.uni.torun.pl (J. Ostrowska-Czubenko).

0144-8617/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2009.01.036

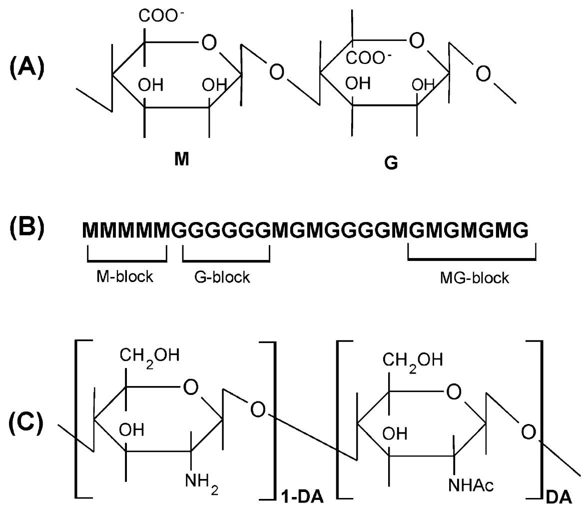

Fig. 1. Structural characteristics of alginates: β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues (A), blocks distribution (B) and chemical structure of chitosan (C) (DA-degree of acetylation).

methanol fuel cells (Kim et al., 2007; Smitha, Sridhar, & Khan, 2005).

Physical and chemical properties of hydrogels depend on their molecular and supramolecular structure, water content and state of water. The nature of water in ionic hydrogel membranes is of the most importance in understanding their dynamic and equilibrium swelling behaviour as well as in analysing solute transport and other diffusive properties of such systems. However until now, little has been done to analyse the water structure in polyelectrolyte complex hydrogels formed from ionic polysaccharides (Bhaskar, Ford, & Hollingsbee, 1998; Guan, Shao, & Yao, 1996). In our earlier work we have synthesized ionically crosslinked chitosan/sodium alginate membranes for the separation of water/alcohol mixtures by pervaporation (Gierszewska-Drużyńska & Ostrowska-Czubenko, 2007). We have observed three different types of water in chitosan/sodium alginate hydrogels. In the present paper a comparison of ionic hydrogels from pure (uncrosslinked) ionic polymers: chitosan and sodium alginate and ionically crosslinked polymers: chitosan/sodium alginate, with respect to the molecular interactions and state of sorbed water at different water content was carried out, using spectral and thermal methods. The aim of the study is to clarify the effect of ionic crosslinking on water state in hydrogel membranes, especially on the non-freezable water content.

2. Experimental

2.1. Materials

Commercially available polysaccharides: high molecular weight chitosan (Ch-HMW) and medium molecular weight chitosan (Ch-MMW) and sodium alginate (NaAlg) were purchased from Sigma–Aldrich (Germany). Chitosans selected for this study varied in their molecular weight (MW), but had similar degrees of deacetylation (DDA). Acetic acid (HAc), sodium acetate (NaAc), sodium hydroxide (NaOH), sodium chloride (NaCl) were analytical grade and were purchased from POCh (Poland). Potassium bromide (KBr) for spectroscopy was purchased from Merck (Germany).

2.2. Chitosan and sodium alginate characterization

Degree of deacetylation, DDA(=1 − DA), of chitosan was determined by potentiometric titration method (Muzzarelli, Rocchetti, Stanic, & Weckx, 1997). 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. The potentiometric titration was performed with a pH meter Elmetron CX 721 (Poland). Degree of deacetylation was equal to 75.72 ± 3.82% (Ch-MMW) and 79.53 ± 1.49% (Ch-HMW).

The viscosity average MW was determined by viscosity analysis of chitosan solutions according to Iĺina and Varlamov (2004) and of sodium alginate solutions according to Martinsen, Skjåk-Bræk, Smidsrød, Zanetti, and Paoletti (1991). Five polymer solutions of known concentrations of Ch-MMW, Ch-HMW and NaAlg were prepared. Relative viscosities of polymer samples in 0.2 M NaAc/2 wt.% HAc, v/v=1/1 (in the case of chitosan) and in 0.1 M NaCl (in the case of sodium alginate) were measured in triplicate using an Ubbelohde capillary viscometer at 25.0 ± 0.1 °C. The intrinsic viscosity was determined by both Huggins and Kraemer plots. Viscosity average MW of chitosan and sodium alginate was calculated using the classical Mark–Houwink equation:

$$[\eta] = K(\mathrm{MW})^a$$

where $[\eta]$ is the intrinsic viscosity of the polymer solution, K and a are constants for given solute–solvent system and temperature ($K = 1.38 \times 10^{-4}$, $a = 0.85$ for chitosan solutions and $K = 7.3 \times 10^{-5}$, $a = 0.92$ for sodium alginate solutions). Viscosity average MW of chitosan and alginate samples was equal to 730 kDa (Ch-MMW), 980 kDa (Ch-HMW) and 100 kDa (NaAlg).

2.3. Membrane preparation

Chitosan and chitosan/sodium alginate (Ch/NaAlg) membranes were prepared by solution casting and solvent evaporation technique. Chitosan solution of 1 wt.% was prepared as follows: 1 g of Ch-MMW (or Ch-HMW) was dispersed in 100 mL of 2 wt.% aqueous HAc solution and left over night. Viscous chitosan solution was filtered, degassed for 1 h in an vacuum oven, cast as a film on clean glass plate and evaporated to dryness in an oven at 37 °C, then further dried under vacuum at 60 °C to constant weight. Sodium alginate solution of 1 wt.% concentration was prepared by dissolving NaAlg in water and left to stand over night. To obtain chitosan/alginate PEC films Ch and NaAlg solutions were mixed in the weight ratio of 3:1 and the pH of the mixture was measured (polymer solutions mixture exhibits pH 3.5). PEC membranes were prepared analogously as chitosan films. The obtained PEC films were additionally thoroughly washed in deionised water and then dried similarly as chitosan films. Dried polymer films were stored in a desiccator over P_2O_5 at ambient temperature.

2.4. Fourier transform infrared (FTIR) spectroscopy measurements

FTIR spectra of chitosan, sodium alginate, chitosan–sodium alginate mixture and PEC samples 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. Frequencies were calibrated by the standard absorptions of polystyrene. Polymers were thoroughly powdered and powders dried under vacuum at 60 °C for several days before milling with anhydrous KBr.

2.5. X-ray diffraction studies

Wide angle X-ray diffraction patterns of chitosan, sodium alginate and chitosan/sodium alginate complex were measured by an X-ray diffractometer (X-Pert Pro Systems, Philips, The Netherlands). X-ray diffraction was performed on powdered samples by exposing them to CuKα radiation and scanned from $2\Theta = 4°$ to 40° at a step size of 0.02°.

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. Chitosan, sodium alginate and PEC membranes for DSC measurements were dried at 60 °C under reduced pressure for several days to obtain completely dry films.

DSC was used to detect interactions between chitosan and sodium alginate and to analyse the state of water in pure polymers and polymer complexes. To detect chitosan–sodium alginate interactions air-dried and/or thoroughly dried polymer samples (about 15 mg) were weighed in an aluminium pan designed for volatile samples and sealed. Samples were heated from room temperature to 400 °C at a heating rate of 10 °C/min. under constant purging of nitrogen at 4 mL/min. An empty cup was used as a reference and runs were performed at least duplicate. DSC measurements of chitosan were also performed in sealed and non-sealed pans. This experiment consisted of two separate series, one comprising the heating of the polymer sample in sealed pan from room temperature to 200 °C at a heating rate of 10 °C/min and the other – the heating of the sample in a non-sealed pan from room temperature to 200 °C, cooling it to room temperature and then immediately heating to 200 °C.

To characterize the water state in chitosan, sodium alginate and chitosan/sodium alginate complexes 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 pen was sealed hermetically, equilibrated for 24 h 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 (g/g) = (water, g)/(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 (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 is the measured water melting enthalpy.

This procedure for freezable water calculation does not take into account the difference in the melting enthalpies of ice of different crystal structures. There are nine polymorphic forms of ice: i.e. I, Ic, II, III, IV, V, VI, VII and VIII (Eisenberg & Kauzmann, 2005). The structures of ice IV to ice VIII are found only at very high pressure. Therefore, in our case the possible structures to be considered are ice I, Ic, II and III. However, even in the case where the difference in the melting enthalpies of ices is the largest (312 J/g for ice III and 334 J/g for ice I), the maximum error in the calculated amount of freezable water is expected to be only 6.6%.

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.

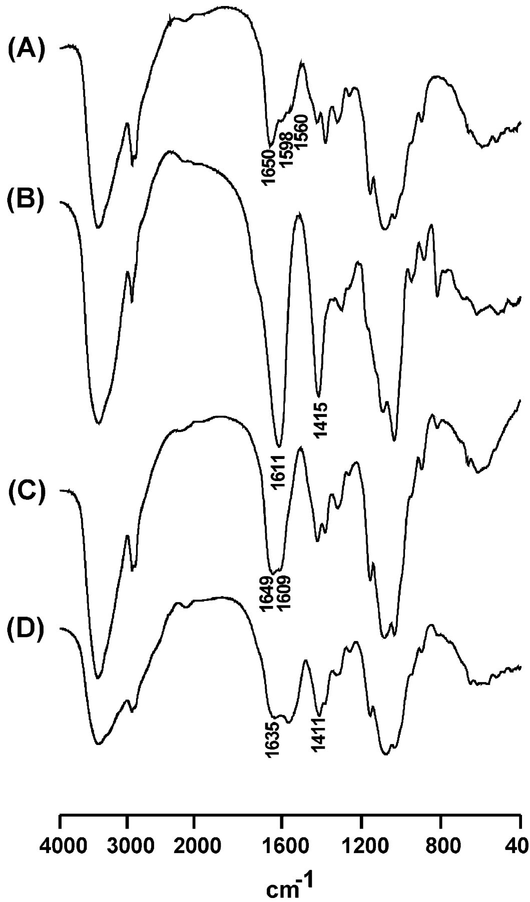

Fig. 2. FTIR spectra of Ch-MMW (A), NaAlg (B), Ch-MMW/NaAlg physical mixture (Ch:NaAlg mass ratio of 3:1) (C) and Ch-MMW/NaAlg polyelectrolyte complex (D).

2.7. Thermogravimetric studies

Thermogravimetric (TG) analysis and differential thermogravimetric (DTG) analysis were carried out with SDT 2960 Simultaneous TGA-DTA thermal analyzer system (TA Instruments, USA). All measurements were performed with a 5 mg samples in aluminium pans under dynamic nitrogen atmosphere from room temperature to 500 °C. The experiments were run at a scanning rate of 10 °C/min and gas flow of 100 mL/min.

3. Results and discussion

3.1. Polyion–polyion interactions in polyelectrolyte complex hydrogels

3.1.1. Fourier transform infrared spectroscopy

FTIR spectra of individual polymers: chitosan (Ch), sodium alginate (NaAlg), their mixture and polymer complex are shown in Fig. 2. The spectrum of Ch exhibits strong and broad band centered at about 3430 cm^{-1} with a shoulder at 3350 cm^{-1} that results from overlapping of the O–H and N–H stretching vibrations of functional groups engaged in hydrogen bonds (Rao, 1963). It is well known, that alcohol, amine, amide and ether functions present in the structure of chitosan can be involved in the formation of inter- and/or intramolecular hydrogen bonds and that the hydrogen bonding plays an important role in solid state and in the gelation process of chitosan (Peter, 2002). The chitosan spectrum also exhibits the distinctive absorption bands at 1650 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). Absorption bands at 1153 cm^{-1} (antisymmetric stretching of the C–O–C bridge), 1083 and 1031 cm^{-1} (skeletal vibrations involving the C–O stretching) are characteristic of chitosan saccharide structure (Pawlak & Mucha, 2003; Pearson, Marchessault, & Liang, 1960; Smitha et al., 2005). Bands at 2922, 2875, 1420, 1320 and 1260 cm^{-1} belong to symmetric and antisymmetric CH_2 vibrations of carbohydrate ring.

In the FTIR spectrum of NaAlg two strong absorption peaks at 1611 and 1415 cm^{-1} are seen. They can be assigned to the antisymmetric and symmetric stretching vibration of the carboxylate group, respectively (Rao, 1963; Sartori, Finch, Ralph, & Gilding, 1997). The spectrum of the NaAlg shows also a strong and broad band at 3424 cm^{-1} (O–H stretching), the weak band at 2930 cm^{-1} (aliphatic C–H stretching) and two strong bands at 1090 and 1034 cm^{-1} (C–O stretching).

The Ch/NaAlg complex shows a characteristic spectrum, different from those of the individual polymers. There can be observed some changes in the 3500–3150 cm^{-1} and 1800–1600 cm^{-1} regions. After PEC formation the band around 3500–3100 cm^{-1} decreases in intensity what indicates that some hydrogen bonds in chitosan structure are destroyed and new H-bonds between Ch and NaAlg are formed. The symmetric stretching vibration of the carboxylate anion shifts from 1415 to 1411 cm^{-1}. A new absorption band at 1635 cm^{-1} appears that can be attributed to the antisymmetric N–H deformation vibration in protonated amines and can be used to confirm the $–NH_3^+$ ion formation (Pearson et al., 1960; Rao, 1963). Absorption band in the region 1640–1620 cm^{-1} due to $–NH_3^+$ ion vibration has been observed earlier in spectra of chitosan film swollen in acid solution at pH 1 (Pearson et al., 1960) and in phosphate buffer at pH 1 (Knaul, Hudson, & Creber, 1999), chitosan/polyether interpenetrating gels swollen in acid solution at pH 1 (Yao, Peng, Goosen, Min, & He, 1993) and in polyelectrolyte complexes: chitosan/sodium alginate (Kim, Kim, Jegal, Lim, & Lee, 2002; Kim, Lim, Jegal, & Lee, 2000), chitosan/poly(-acrylic acid) (Wang, Li, Lu, & Wang, 1997) and chitosan/pectate (Yao, Tu, Cheng, Zhang, & Liu, 1997) and used to draw conclusion on $–NH_3^+$ ions formation. Thus, the above mentioned changes observed in the FTIR spectra of chitosan after reaction with sodium alginate seems to indicate the formation of PEC complexes with ionic bonds between carboxylate group of alginate and ammonium group of chitosan, according to the scheme presented in Fig. 3.

To confirm the formation of the polyion complexes a solubility test was performed. Membranes from protonated chitosan, pure sodium alginate and polyelectrolyte complex were immersed in water for 24 h. It was observed that membranes from pure NaAlg and Ch were highly soluble but PEC membrane did not dissolve in water.

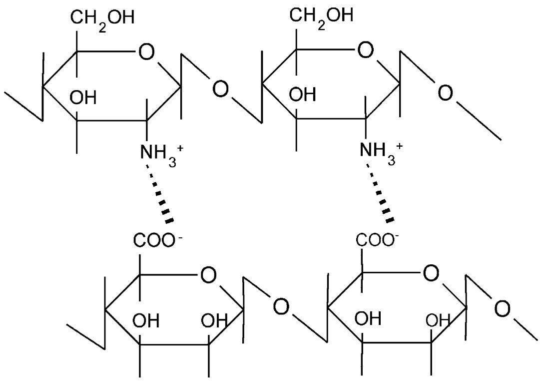

Fig. 3. Chemical structure of Ch/NaAlg complex.

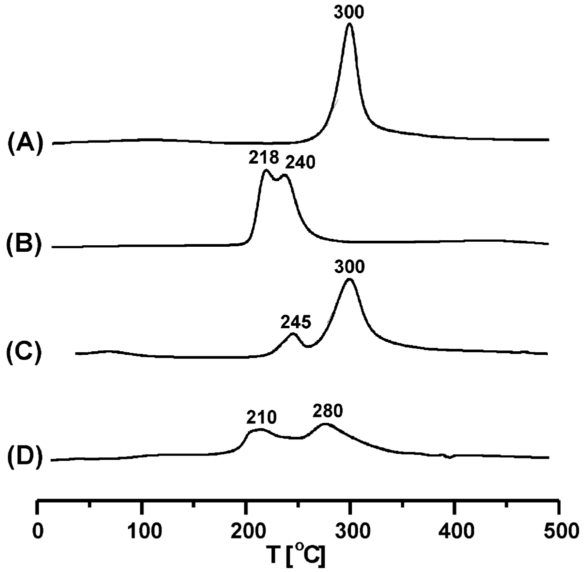

Fig. 4. DTG curves of Ch-HMW (A), NaAlg (B), Ch-HMW/NaAlg physical mixture (Ch:NaAlg mass ratio of 3:1) (C) and Ch-HMW/NaAlg complex (D).

The interaction between Ch and NaAlg according to the scheme presented in Fig. 3 is also confirmed by thermal analysis, described below.

3.1.2. Thermogravimetry and differential scanning calorimetry

Fig. 4 exhibits DTG curves for pure chitosan and sodium alginate, the physical mixture of chitosan and sodium alginate as well as chitosan/sodium alginate complex. It can be seen that thermal degradation of chitosan, sodium alginate and their physical mixture consists of two/three stages but thermal degradation of chitosan/sodium alginate hydrogel membrane is more complex. Thermogram of physical mixture, both TG (not shown in Fig. 4) and DTG curves, is different from that of polyelectrolyte complex. It appears to be combination of data obtained for pure polymers. Moreover, it can be seen that the peaks of the PEC are shifted from those of physical mixture.

For all polymers, dried at 60 °C under reduced pressure, the first stage starts below 100 °C and can be assigned to the loss of water which could not be removed completely on drying. As it is known, polysaccharides have a strong affinity for water and their hydration properties depend on their primary and supramolecular structures (Chaplin, 2008; Despond, Espuche, Cartier, & Domard, 2005; Kittur, Harish Prashanth, Udaya Sankar, & Tharanathan, 2002). Moreover, they release water at different temperatures, depending on the different interactions of water with polysaccharide chains. For example, three different kinds of interactions of water and polymer were identified in alginate hydrogels (Avella et al., 2007; Russo, Giuliani, Immirzi, Malinconico, & Romano, 2004). The first one is free water, that is released in the region 40–60 °C, the second one is water linked through hydrogen bonds, that is released in the region 80–120 °C and finally water more tightly linked through polar interactions with carboxylate groups that is released up to 160 °C. In our case on the DTG curves of Ch, Ch/NaAlg mixtures and Ch/NaAlg complexes only very small peaks (practically not evident on Fig. 4) with maximum at around 110–120 °C were observed. The peaks can be related to the water tightly bound to the functional groups of polymers that has not been removed on drying.

The second and third stage of polymers degradation correspond to the thermal decomposition of pure polyelectrolytes or polyelectrolyte complexes and vaporization and elimination of volatile

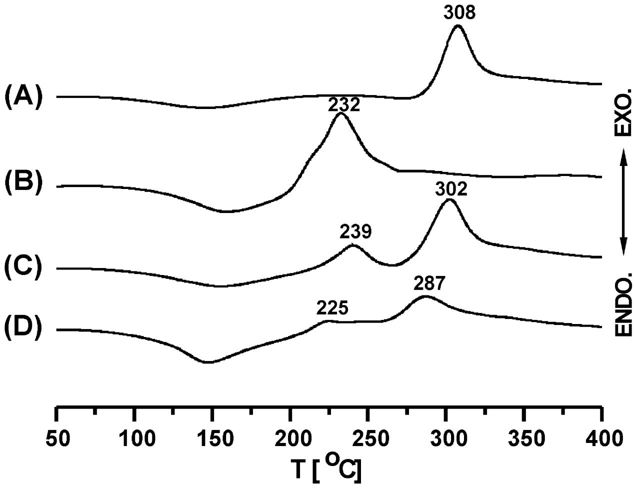

Fig. 5. DSC thermograms of Ch-HMW (A), NaAlg (B), Ch-HMW/NaAlg physical mixture (Ch:NaAlg mass ratio of 3:1) (C) and Ch-HMW/NaAlg complex (D).

products. For pure Ch the second stage starts at 240 °C and reaches a maximum at 300 °C, but for NaAlg it starts at 190 °C and reaches one maximum at 218 °C and the second one at 240 °C. According to literature data pyrolysis of chitosan starts by a random split of the glycosidic bonds, followed by a further decomposition to acetic, butyric and lower fatty acids (Neto et al., 2005). Sodium alginate decomposes by dehydration and degradation to Na_2CO_3 (Soares, Santos, Chierice, & Cavalheiro, 2004). Ch/NaAlg mixture (Fig. 4) reveals two stages (two peaks) of fast thermal degradation at 245 and 300 °C, characteristic for both components. Thermogram of chitosan/sodium alginate complex (both TG and DTG curves) is different from those of pure polymers and their mixture. The peak at about 300 °C typical for pure chitosan is not observed what seems to indicate on some interaction between chitosan and sodium alginate and may be considered as a proof of their complexation. This observation indicates that degradation of the PEC membrane occurred at different temperatures compared to the mixture. Similar results has been obtained earlier for chitosan/pectin (Ghaffari, Navaee, Oskoui, Bayati, & Rafiee-Tehrani, 2007) and chitosan/carboxymethylcellulose complexes (Rosca, Popa, Lisa, & Chitanu, 2005). Considering the temperature at which thermal degradation starts as a criterion of the thermal stability, the thermal stability increases according to the series: Ch/NaAlg < NaAlg < Ch. TG/DTG results are in good agreement with those obtained by DSC and characterized below.

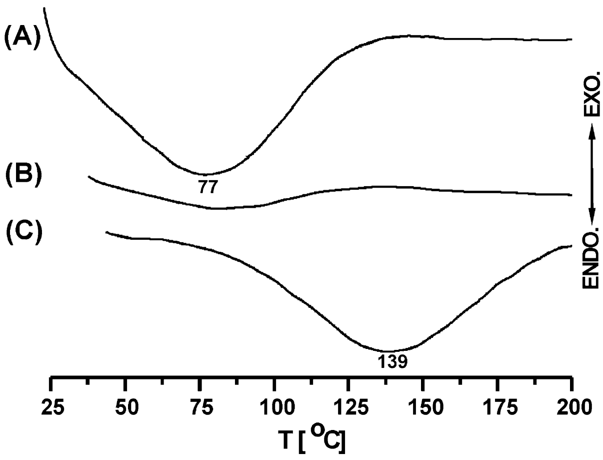

Fig. 6. DSC thermograms of air-dried Ch-HMW samples: first heating run, open pan (A), second heating run, open pan (B), first heating run, sealed pan (C).

DSC thermograms of pure polymers, their physical mixture and polyelectrolyte complex are shown in Fig. 5. DSC curves show a broad endothermic peak between 75 and 200 °C and exothermic peaks at temperature above 200 °C. The detected endotherm can be attributed, as discussed above, to the evaporation of water linked to the chitosan and/or alginate chains by different kinds of physical bonds (H-bonds, electrostatic interactions). Exothermic peaks result from degradation of polymers.

DSC thermogram of Ch/NaAlg physical mixture appeared to be combination of each polymer and is different from that of polyelectrolyte complex. It shows two exothermic peaks at 239 and 302 °C resulted from individual contribution of alginate and chitosan, respectively. In thermogram of Ch/NaAlg complex a broad exothermic peak at 287 °C appears that can be assigned to the formation of an ionic pairs $-COO^{-}\cdots{}^{+}NH_3-$ between carboxylate group of sodium alginate and ammonium group of chitosan. Similar broad exothermic peak, with intermediate peak maximum temperature compared to individual polyelectrolytes, was observed earlier by others for chitosan/alginate and chitosan/carboxycellulose hydrogels and related to polyelectrolyte complex formation (Ribeiro, Silva, Ferreira, & Veiga, 2005; Sankalia, Mashru, Sankalia, & Sutariya, 2007; Sarmento, Ferreira, Veiga, & Ribeiro, 2006).

Fig. 6 shows the transitions detected in the temperature range 25–200 °C for pure air-dried chitosan samples heated in open and sealed pans. As can be observed, heating of the sample up to 200 °C in the open pan, followed by an immediate second run in the same temperature range, yielded a thermogram with a reduced peak area. This result supports the conclusion that water evaporation occurred during the first run. Comparison of DSC results for chitosan samples heated in non-sealed and sealed pans indicates that they differentiate only in the water peak position. Maximum of this peak for chitosan heated in open pan lies at 77 °C and for polymer heated in sealed pan at 139 °C. These results are in accordance with DSC data for chitin, chitosan and modified chitosan presented by Kittur et al. (2002). These authors conclude that the temperature of that endothermic peak position should be related to pressure formed inside the sealed pans during evaporation of water.

3.2. State of water in pure polyelectrolytes and polyelectrolyte complex hydrogels

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 informative method (Corkhill, Jolly, Ng, & Tighe, 1987). Much research on the water-swollen polymers by means of 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 (Hatakeyama & Hatakeyama, 1998; McBrierty, Martin, & Karasz, 1999; Ping, Nguyen, Chen, Zhou, & Ding, 2001; Tamai, Tanaka, & Nakanishi, 1996). Three energetically distinct states of water have been identified in water-swollen systems. The experimentally determined separate states of water within the polymer can be defined as follows (Higuchi, Komiyama, & Iijima, 1984; Hodge, Edward, & Simon, 1996): (i) *free water*, which undergoes similar thermal transitions to that of bulk water, (ii) *freezable bound water*, which undergoes a thermal phase transition at a temperature shifted with respect to that of bulk water and (iii) *non-freezable 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.

Fig. 7 shows the DSC melting thermograms of frozen water in chitosan, sodium alginate and chitosan/sodium alginate complex

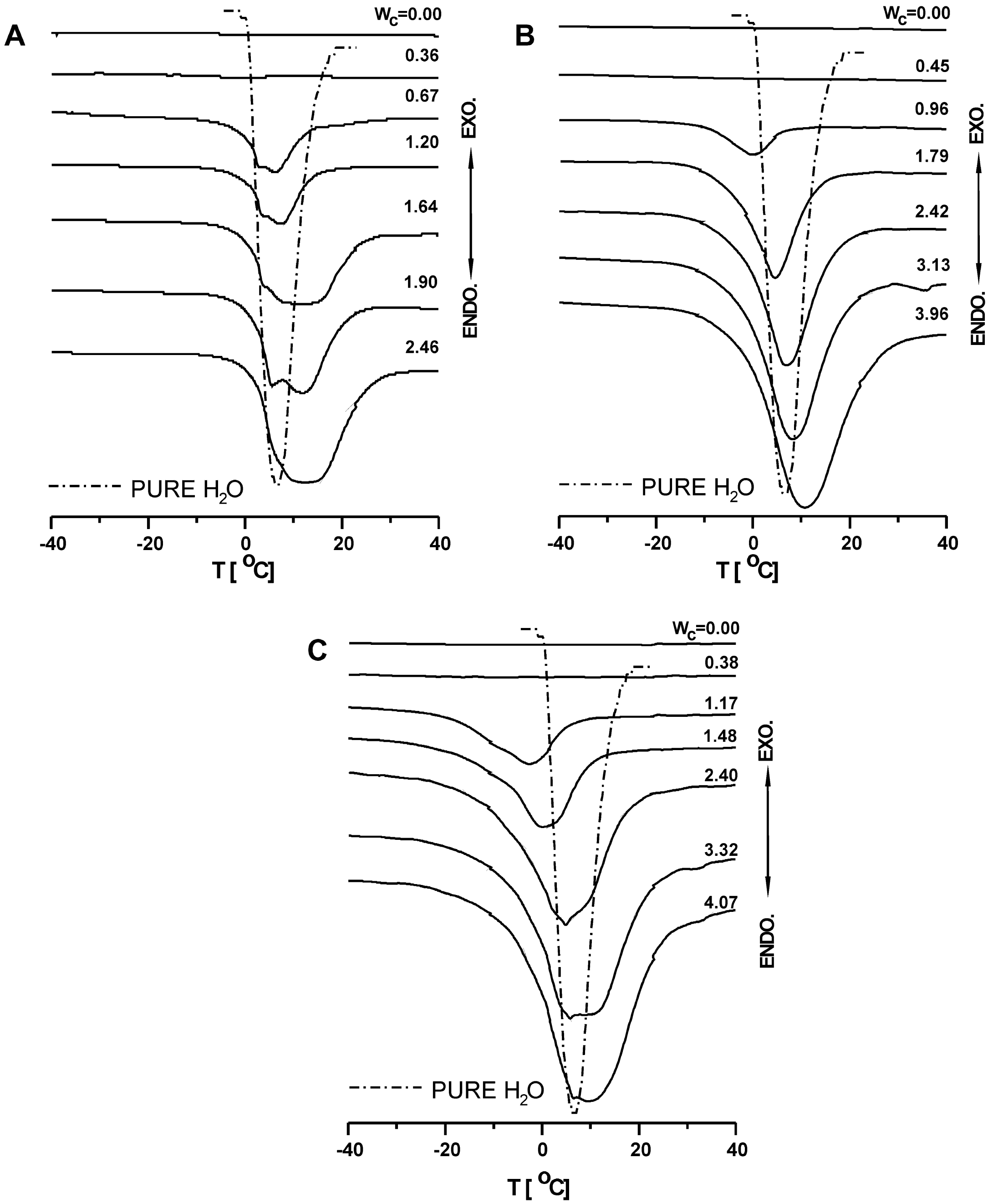

Fig. 7. DSC heating thermograms of Ch-HMW (A) NaAlg (B) and Ch-HMW/NaAlg samples (C) with different water content.

membranes at various water uptake W_c. For all polymer–water systems analysed no peaks are observed at $T \approx 0$ °C below a certain water content. The absence of endothermic peaks above a certain water content threshold indicates that this water is of the non-freezable bound type. It is well known that non-freezable bound water has no detectable phase transition over the temperature range from −73 to 0 °C, usually associated with the water freezing–melting transition (Higuchi et al., 1984).

For each studied polymer with the increase of water content the broad endothermic peak appears, corresponding to the melting of freezable water. This peak moves to higher temperatures with increase of water content. As can be seen from Fig. 7, 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 by the broken line). The water melting at 0 °C is free water and that melting below 0 °C is regarded as freezable bound or intermediate water.

Generally, for all studied hydrogels of higher water content the endothermic peaks are broad and structured. Uncrosslinked and ionically crosslinked chitosan–water systems exhibit poorly resolved multipeak with distinct submaxima (Fig. 7A and Fig. 7C) composed of several endothermic peaks. No attempt was made here to deconvolute the latter peaks. For example, more than one transition is evident for uncrosslinked Ch samples at water content of 0.67, 1.20, 1.64 and 1.90 g/g. 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. For higher water contents these individual transitions (peaks) are masked and only unsym-

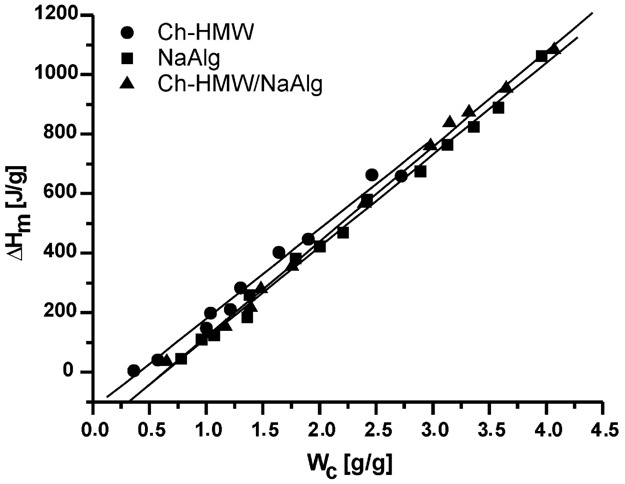

Fig. 8. Relation between enthalpy of water melting and water content.

Table 1
Values of ΔH_m and $W_{nf,max}$ for chitosan, sodium alginate and chitosan complexes.

Polymer sample (DDA, MW)	$W_{nf,max}$ $g_{water}/g_{polymer}$	ΔH_m (J/g)	Reference
Ch-HMW (80%, 980 kDa)	0.40	301	This work
Ch-MMW (76%, 730 kDa)	0.38	319	This work
Ch (83%, 1420 kDa)	0.17	–	Khalid et al. (2002)
Ch (88%, 70 kDa)	0.24	289	Qu et al. (2000)
Ch (60%, 900 kDa)[a]	0.40	–	Guan et al. (1996)
NaAlg	0.63	308	This work
Ch-HMW/NaAlg	0.62	319	This work
Ch-MMW/NaAlg	0.55	282	This work
Ch/pectin	0.44	202	Guan et al. (1996)
Ch/gelatin	0.36	306	Guan et al. (1996)

[a] Chitosan crosslinked with glutaraldehyde.

metrical curves can be observed what seems to indicate that bound and free water exist simultaneously in polysaccharide–water system. In the case of NaAlg–water systems the observed DSC endotherms for any water content are broad and asymmetric and no distinct peak splitting is observed (Fig. 7B). All these results indicate that water present in hydrogels exists in a continuum of states between the extreme non-freezing and freezing forms.

Differences in the endothermic peak shape for NaAlg samples and uncrosslinked and crosslinked chitosan samples indicate that these polymers differ in their strength of water–polymer interaction. These polysaccharides differ in type of water binding sites (hydrophilic groups) and thus also the strength of water molecules binding. Considering NaAlg, Ch and Ch/NaAlg complex, it can be seen that water molecule can be bound by the following hydrophilic groups: –COONa and –OH in the case of sodium alginate, –OH, $–NH_2$ and –NHAc in the case of chitosan, –OH, $–NH_2$, –NHAc and $–COO^{-}\cdots{}^{+}NH_3–$ in the case of sodium alginate/chitosan complex membrane containing both complexed as well uncomplexed chitosan molecules. The strength of the interaction of water with these groups is different. It is known that interaction of water molecules with the amine group is weaker than with the hydroxyl one (Rueda, Secall, & Bayer, 1999).

The area under the DSC peak represents the change in enthalpy associated with the melting of freezable water (free water and freezable bound water). Fig. 8 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 (Higuchi et al., 1984; Quinn, Kampff, Smyth, & McBrierty, 1988). This procedure gives $\Delta H_m = 319$ J/g and $W_{nf,max} = 0.62$ g/g for Ch-HMW/NaAlg–water system. Values of ΔH_m and $W_{nf,max}$ for all analysed samples are listed in Table 1. In this table there are also presented values of ΔH_m and $W_{nf,max}$ for chitosan samples of different molecular characteristics (different molecular weight and different degree of deacetylation) and for chitosan/anionic polyelectrolyte complexes. The heat of melting of freezable water in all the hydrogels analysed by us is lower than the heat of melting of bulk water, i.e. than 334 J/g. Similar phenomenon has been observed earlier for different non-ionic polymers, ionic polymers and ionic complexes. Fig. 9 shows the dependencies of W_f and W_{nf} values on total water content, W_c, for the samples of Ch, NaAlg and Ch/NaAlg. For all analysed polymers 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.

Our results and literature data (Hatakeyama & Hatakeyama, 1998; Khalid, Agnely, Yagoubi, Grossiord, & Couarraze, 2002; Ohno, Shibayama, & Tsuchida, 1983; Ping et al., 2001; Qu, Wirsén, & Albertsson, 2000) show that there are variations in the amount of non-freezing bound water for different hydrogels. The mechanism of formation of unfrozen water in polymers is not explained (Cha, Hyon, & Ikada, 1993; Müller-Plathe, 1998; Patil, Mark, Apostolov, Vassileva, & Fakirov, 2000). It is generally accepted that unfrozen bound water is formed by the hydrogen bonds between water molecules and polar groups in the polymer. Liu and Yao (2001) analysed the origin of unfrozen bound water in gelatin/water samples by DSC, positron annihilation lifetime spectroscopy and NMR spectroscopy and proposed that nanocavity in polymers is an important reason for the formation of unfrozen water.

Values of $W_{nf,max}$ for chitosan markedly increases when it forms PEC with NaAlg. We suppose that it results from differences in molecular and supermolecular structure of uncrosslinked and ionically crosslinked polymers. When chitosan forms a polyelectrolyte complex with sodium alginate then the number of hydrophilic groups of Ch and NaAlg that interact with water molecules decreases and simultaneously new hydrophilic $–COO^{-}\cdots{}^{+}NH_3–$ groupings appear. Moreover, as results from X-ray spectra, changes in the crystallinity of chitosan samples after complex formation can be observed (Fig. 10). It is well known that crystallinity influences the amount of the non-freezable water. In partly crystalline chitosan–water penetrates only the amorphous regions of the polymer and the crystalline regions remain unaffected. After PEC formation the crystallinity of chitosan decreases and the portion of amorphous regions increases. Thus, the structure is more accessible for water molecules and in result more water molecules penetrate inside polysaccharide matrix and bound to chitosan chains.

It can be also supposed that chitosan and sodium alginate can form PECs of different ionic crosslinking density and/or different supermolecular structure, depending on molecular ratio of reacting polymers. When closely packed structures of high crosslinking density are formed, then molecular chains align regularly, water molecules are excluded from the well-arranged portion and the value of $W_{nf,max}$ is low. In contrary, when bulky three-dimensional networks of low crosslinking density are formed then they can retain a large amount of water and the value of $W_{nf,max}$ is high. In our case Ch:NaAlg molar ratio is equal to about 3.5:1, the crosslinking density is not too high and thus rather "bulky" PECs are formed. Such complexes have tendency to retain a large amount of non-freezing water.

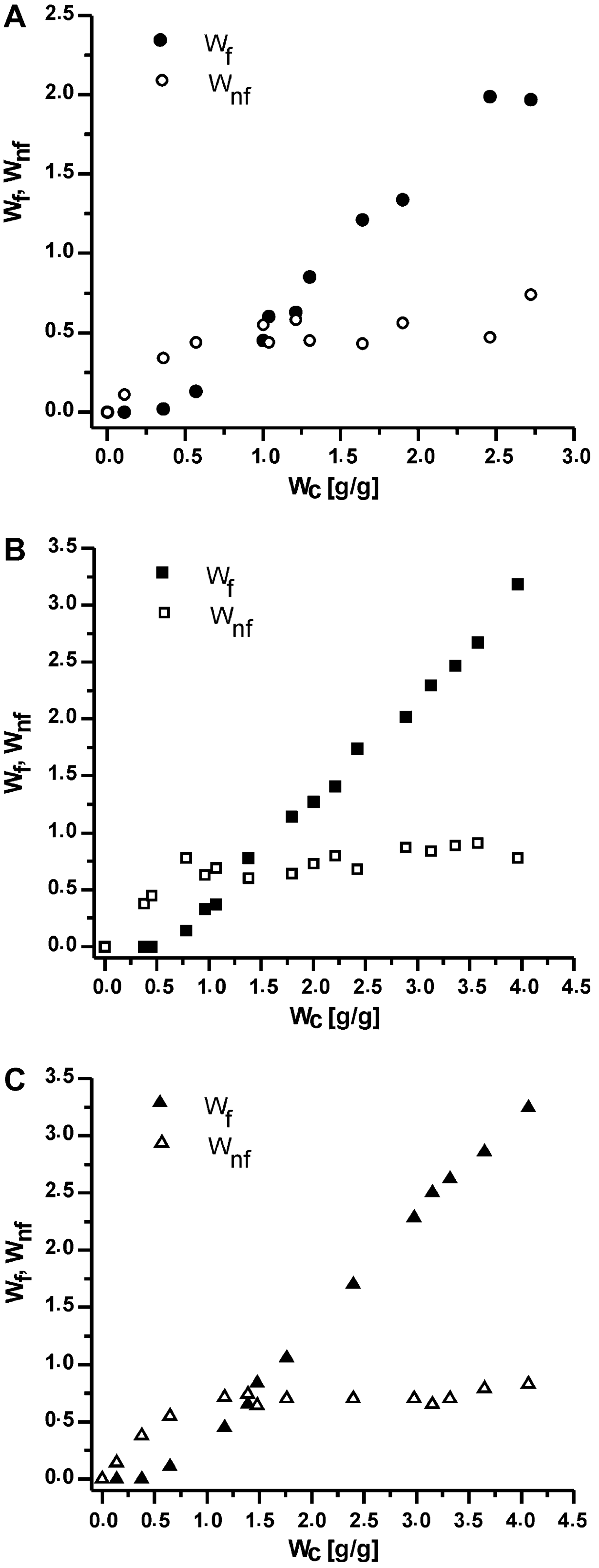

Fig. 9. The amounts of freezing water (W_f) and non-freezing water (W_{nf}) in Ch-HMW (A) NaAlg (B) and Ch-HMW/NaAlg (C) samples with different water content.

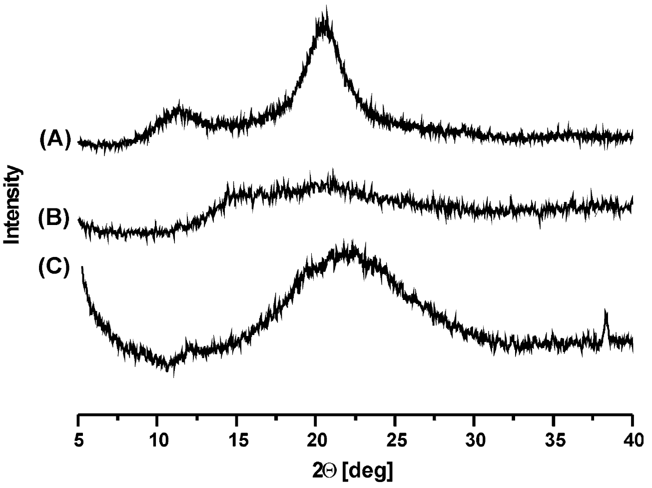

Fig. 10. Wide angle X-ray diffractograms of Ch-HMW (A) NaAlg (B) and Ch-HMW/NaAlg complex (C).

4. Conclusions

The results reported above can be summarized as follows:

1. FTIR, DSC and DTG methods confirmed the formation of chitosan/sodium alginate polyion complexes with ionic bonds between protonated amine groups of chitosan and carboxylate groups of alginate.
2. Addition of sodium alginate to chitosan and formation of ionic crosslinks influenced the state of water in chitosan hydrogel membrane. Both in uncrosslinked as well as in ionically crosslinked chitosan membranes there were observed non-freezing water and freezing water, but their amounts were different.
3. In Ch–water, NaAlg–water and Ch/NaAlg complex–water systems the frozen water began to melt when the water amount was above a characteristic threshold that was dependent on the chemical and physical structure of the polymer.
4. The amount of non-freezable water in the hydrogel polyion complex membrane with Ch:NaAlg molar ratio equal to about 3.5:1 and thus with rather low crosslinking density was relatively large. This result seems to indicate that if the ionic crosslinking density was not too high rather "bulky" PECs were formed that had tendency to retain a large amount of non-freezing water.
5. Molecular weight of chitosan only slightly influenced the state of water, both in chitosan as well as in chitosan/sodium alginate membranes.

References

Avella, M., Di Pace, E., Immirzi, B., Impallomeni, G., Malinconico, M., & Santagata, G. (2007). Addition of glycerol plasticizer to seaweeds derived alginates: Influence of microstructure on chemical–physical properties. *Carbohydrate Polymers, 69*, 503–511.

Berger, J., Reist, M., Mayer, J. M., Felt, O., & Gurny, R. (2004). Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications. *European Journal of Pharmaceutics and Biopharmaceutics, 57*, 35–52.

Berger, J., Reist, M., Mayer, J. M., Felt, O., Peppas, N. A., & Gurny, R. (2004). Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. *European Journal of Pharmaceutics and Biopharmaceutics, 57*, 19–34.

Bhaskar, G., Ford, J. L., & Hollingsbee, D. A. (1998). Thermal analysis of the water uptake by hydrocolloids. *Thermochimica Acta, 322*, 153–165.

Blanco, M. D., Olmo, R., & Teijon, J. M. (2002). Hydrogels. In J. Swarbrick & J. C. Boylan (Eds.), *Encyclopedia of pharmaceutical technology* (pp. 239–260). New York: Marcel Dekker.

Cha, W.-I., Hyon, S.-H., & Ikada, Y. (1993). Microstructure of poly(vinyl alcohol) hydrogels investigated with differential scanning calorimetry. *Makromolekulare Chemie, 194*, 2433–2441.

Chaplin, M. (2008). Polysaccharide hydration. In London South Bank University. Available from: www.lsbu.ac.uk/water/hydrat.html.

Corkhill, P. H., Jolly, A. M., Ng, Ch. O., & Tighe, B. J. (1987). Synthetic hydrogels: 1. Hydroxyalkyl acrylate and methacrylate copolymers – water binding studies. *Polymer, 28*, 1758–1766.

Despond, S., Espuche, E., Cartier, N., & Domard, A. (2005). Hydration mechanism of polysaccharides. A comparative study. *Journal of Polymer Science Part B: Polymer Physics, 43*, 48–58.

Draget, K. I., Smidsrød, O., & Skjåk-Bræk, G. (2002). Alginates from algae. In E. J. Vandamme & S. De Baets (Eds.). *Biopolymers. Polysaccharides II. Polysaccharides from eukaryotes* (Vol. 6, pp. 216–244). Weinheim: Wiley.

Eisenberg, D., & Kauzmann, W. (2005). *The structure and properties of water*. Oxford: Clarendon Press.

Ghaffari, A., Navaee, K., Oskoui, M., Bayati, K., & Rafiee-Tehrani, M. (2007). Preparation and characterization of free mixed-film of pectin/chitosan/Eudragit® RS intended for sigmoidal drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics, 67*, 175–186.

Gierszewska-Drużyńska, M., & Ostrowska-Czubenko, J. (2007). Synteza i charakterystyka membran hydrożelowych na bazie chitozanu i alginianu sodu. *Polimery (Warsaw), 52*, 517–523.

Guan, Y. L., Shao, L., & Yao, K. D. (1996). A study on correlation between water state and swelling kinetics of chitosan-based hydrogels. *Journal of Applied Polymer Science, 61*, 2325–2335.

Hatakeyama, H., & Hatakeyama, T. (1998). Interaction between water and hydrophilic polymers. *Thermochimica Acta, 308*, 3–22.

Higuchi, A., Komiyama, J., & Iijima, T. (1984). The states of water in gel cellophane membranes. *Polymer Bulletin, 11*, 203–208.

Hodge, R. M., Edward, G. H., & Simon, G. P. (1996). Water absorption and states of water in semicrystalline poly(vinyl alcohol) films. *Polymer, 37*, 1371–1376.

Iĺina, A. V., & Varlamov, V. P. (2004). Hydrolysis of chitosan in lactic acid. *Applied Biochemistry and Microbiology, 40*, 300–303.

Khalid, M. N., Agnely, F., Yagoubi, N., Grossiord, J. L., & Couarraze, G. (2002). Water state characterization, swelling behavior, thermal and mechanical properties of chitosan based networks. *European Journal of Pharmaceutical Sciences, 15*, 425–432.

Kim, S.-G., Lim, G.-T., Jegal, J., & Lee, K.-H. (2000). Pervaporation separation of MTBE (methyl *tert*-butyl ether) and methanol mixtures through polyion complex composite membranes consisting of sodium alginate/chitosan. *Journal of Membrane Science, 174*, 1–15.

Kim, S.-G., Kim, Y.-I., Jegal, J., Lim, G.-T., & Lee, K.-H. (2002). Characterization and preparation of polyion complex composite membranes for the separation of methyl *tert*-butyl ether/methanol mixtures. *Journal of Applied Polymer Science, 85*, 714–725.

Kim, S.-G., Lee, K.-S., & Lee, K.-H. (2007). Pervaporation separation of sodium alginate/chitosan polyelectrolyte complex composite membranes for the separation of water/alcohol mixtures: Characterization of the permeation behaviour with molecular modeling techniques. *Journal of Applied Polymer Science, 103*, 2634–2641.

Kishida, A., & Ikada, Y. (2001). Hydrogels for biomedical and pharmaceutical applications. In S. Dumitriu (Ed.), *Polymeric biomaterials* (pp. 135–145). New York: CRC Press.

Kittur, F. S., Harish Prashanth, K. V., Udaya Sankar, K., & Tharanathan, R. N. (2002). Characterization of chitin, chitosan, and their carboxymethyl derivatives by differential scanning calorimetry. *Carbohydrate Polymers, 49*, 185–193.

Knaul, J. Z., Hudson, S. M., & Creber, K. A. M. (1999). Improved mechanical properties of chitosan fibers. *Journal of Applied Polymer Science, 72*, 1721–1732.

Liu, W. G., & Yao, K. D. (2001). What causes the unfrozen water in polymers: Hydrogen bonds between water and polymer chains? *Polymer, 42*, 3943–3947.

Martinsen, A., Skjåk-Bræk, G., Smidsrød, O., Zanetti, F., & Paoletti, S. (1991). Comparison of different methods for determination of molecular weight and molecular weight distribution of alginates. *Carbohydrate Polymers, 15*, 171–193.

McBrierty, V. J., Martin, S. J., & Karasz, F. E. (1999). Understanding hydrated polymers: The perspective of NMR. *Journal of Molecular Liquids, 80*, 179–205.

Muzzarelli, R. A. A., Rocchetti, R., Stanic, V., & Weckx, M. (1997). *In R. A. A. Muzzarelli, & M. G. Peter (Eds.). Chitin handbook (pp. 109–119)*. Grottammare: Atec Edizioni.

Müller-Plathe, F. (1998). Different state of water in hydrogels? *Macromolecules, 31*, 6721–6723.

Neto, C. G. T., Giacometti, J. A., Job, A. E., Ferreira, F. C., Fonseca, J. L. C., & Pereira, M. R. (2005). Thermal analysis of chitosan based networks. *Carbohydrate Polymers, 62*, 97–103.

Ohno, H., Shibayama, M., & Tsuchida, E. (1983). DSC analyses of bound water in the microdomains of interpolymer complexes. *Makromolekulare Chemie, 184*, 1017–1024.

Patil, R. D., Mark, J. E., Apostolov, A., Vassileva, E., & Fakirov, S. (2000). Crystallization of water in some crosslinked gelatins. *European Polymer Journal, 36*, 1055–1061.

Pawlak, A., & Mucha, M. (2003). Thermogravimetric and FTIR studies of chitosan blends. *Thermochimica Acta, 396*, 153–166.

Pearson, F. G., Marchessault, R. H., & Liang, C. Y. (1960). Infrared spectra of crystalline polysaccharides V. Chitin. *Journal of Polymer Science, 43*, 101–116.

Peter, M. G. (2002). Chitin and chitosan from animal sources. In E. J. Vandamme & S. De Baets (Eds.). *Biopolymers. Polysaccharides II. Polysaccharides from eukaryotes* (Vol. 6, pp. 485–574). Weinheim: Wiley.

Ping, Z. H., Nguyen, Q. T., Chen, S. M., Zhou, J. Q., & Ding, Y. D. (2001). States of water in different hydrophilic polymers – DSC and FTIR studies. *Polymer, 42*, 8461–8467.

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

Quinn, F. X., Kampff, E., Smyth, G., & McBrierty, V. J. (1988). Water in hydrogels. I. A study of water in poly(*n*-vinyl-2-pyrrolidone/methyl methacrylate) copolymer. *Macromolecules, 21*, 3191–3198.

Rao, C. N. R. (1963). *Chemical application of infrared spectroscopy*. New York, London: Academic Press.

Ribeiro, A. J., Silva, C., Ferreira, D., & Veiga, F. (2005). Chitosan-reinforced alginate microspheres obtained through the emulsification/internal gelation technique. *European Journal of Pharmaceutical Sciences, 25*, 31–40.

Rosca, C., Popa, M. I., Lisa, G., & Chitanu, G. Ch. (2005). Interaction of chitosan with natural or synthetic anionic polyelectrolytes. 1. The chitosan–carboxymethylcellulose complex. *Carbohydrate Polymers, 62*, 35–41.

Rueda, D. R., Secall, T., & Bayer, R. K. (1999). Differences in the interaction of water with starch and chitosan films as revealed by infrared spectroscopy and differential scanning calorimetry. *Carbohydrate Polymers, 40*, 49–56.

Russo, R., Giuliani, A., Immirzi, B., Malinconico, M., & Romano, G. (2004). Alginate/polyvinylalcohol blends for agricultural applications: Structure–properties correlation, mechanical properties and greenhouse effect evaluation. In M. Precella, G. Galli, & P. Giusti (Eds.). *Current topics in polymer science and technology, macromolecular symposia* (Vol. 218, pp. 241–250). Weinheim: Wiley.

Sankalia, M. G., Mashru, R. C., Sankalia, J. M., & Sutariya, V. B. (2007). Reversed chitosan–alginate polyelectrolyte complex for stability improvement of alpha-amylase: Optimization and physicochemical characterization. *European Journal of Pharmaceutics and Biopharmaceutics, 65*, 215–232.

Sarmento, B., Ferreira, D., Veiga, F., & Ribeiro, A. (2006). Characterization of insulin-loaded alginate nanoparticles produced by ionotropic pre-gelation through DSC and FTIR studies. *Carbohydrate Polymers, 66*, 1–7.

Sartori, C., Finch, D. S., Ralph, B., & Gilding, K. (1997). Determination of the cation content of alginate thin films by FTi.r. spectroscopy. *Polymer, 38*, 43–51.

Smitha, B., Sridhar, S., & Khan, A. A. (2005). Chitosan–sodium alginate polyion complexes as fuel cell membranes. *European Polymer Journal, 41*, 1859–1866.

Soares, J. P., Santos, J. E., Chierice, G. O., & Cavalheiro, E. T. G. (2004). Thermal behaviour of alginic acid and its sodium salt. *Ecletica Quimica, 29*, 57–64.

Tamai, Y., Tanaka, H., & Nakanishi, Y. (1996). Molecular dynamics study of polymer–water interaction in hydrogels. 1. Hydrogen-bond structure. *Macromolecules, 29*, 6750–6760.

Wang, H., Li, W., Lu, Y., & Wang, Z. (1997). Studies on chitosan and poly(acrylic acid) interpolymer complex I Preparation, structure, pH-sensitivity, and salt sensitivity of complex-forming poly(acrylic acid): Chitosan semi-interpenetrating polymer network. *Journal of Applied Polymer Science, 65*, 1445–1450.

Yao, K. D., Peng, T., Goosen, M. F. A., Min, J. M., & He, Y. Y. (1993). PH-sensitivity of hydrogels based on complex forming chitosan: Polyether interpenetrating polymer network. *Journal of Applied Polymer Science, 48*, 343–354.

Yao, K. D., Tu, H., Cheng, F., Zhang, J. W., & Liu, J. (1997). PH-sensitivity of the swelling of a chitosan–pectin polyelectrolyte complex. *Angewandte Makromolekulare Chemie, 245*, 63–72.