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Thermo- and pH-responsive polyelectrolyte complex membranes from chitosan-g-*N*-isopropylacrylamide and pectin

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ABSTRACT

Non-stoichiometric polyelectrolyte complex membranes between chitosan-g-*N*-isopropylacrylamide (PNIPAm) and pectin were prepared and subjected to a thermal treatment by which ionic bonds were converted into amide bonds. Membranes are hydrophilic, with opaque appearance, but vitreous when dry. Swollen membranes undergo a sharp shrinking process with an inflexion point at 33.1 °C. Below LCST, NIPAm chains are hydrated and completely stretched. As temperature increases above LCST, NIPAm chains contract and water is expelled from the polymer matrix, giving rise to the phase transition that is associated to an endothermic peak and is fully thermoreversible. These membranes are not only sensitive to temperature, but also to the pH of the medium whose variation has no influence on LCST. Nevertheless, transition enthalpy decreases when pH increases within studied interval, showing the same trend as equilibrium swelling.

1. Introduction

Nowadays one of the materials research trends is focused on developing smart hydrogels, which are capable of undergoing rapid reversible transitions as a response to local environmental stimuli such pH, temperature, ionic strength or electric field (Dimitrov, Trzebicka, Müller, Dworak & Tsvetanov, 2007; Galaev & Mattiason, 2008; Osada & Ross-Murphy, 1993).

Thermosensitive hydrogels show reversible phase transition associated with a change in hydrophobicity at the Low Critical Solution Temperature (LCST), while pH-responsive polymers normally contain weak ionizable groups and exhibit a reversible swelling–collapse transition when the net charge decreases. Poly(*N*-isopropylacrilamide) and their derivatives are the most widely studied thermosensitive materials (Schild, 1992). They exhibit a sharp hydrophilic–hydrophobic transition when temperature increases above the LCST (~32 °C), given by a reduction of the total number of water molecules structured around hydrophobic groups, which are expelled out from these groups. As a result, hydrophobic interactions are promoted, giving rise to a polymer network or a suddenly shrinking process.

Chitosan is a weak cationic carbohydrate polymer, obtained by the extensive deacetylation of chitin. It is composed of randomly distributed units of β-(1-4)-linked D-glucosamine and *N*-acetyl-D-glucosamine (Muzzarelli, 1977). Because of its cationic character, chitosan can interact with oppositely charged macromolecules, such as carrageenans, carboxymethylcelluloses, pectins, alginates, giving rise to polyelectrolyte complexes (PEC) (Berth, Voigt, Dautzenberg, Donath & Möhwald, 2002; Chavasit, Kienzle-Sterzer & Torres, 1988; Fukuda, 1980; Hugerth, Caram-Lelham & Sundelöf, 1997; Lee, Park & Ha, 1997; Muzzarelli, Stanic, Gobbi, Tosi & Muzzarelli, 2004; Peniche & Argüelles-Monal, 2001).

The swelling behavior and stability of polyelectrolyte complexes depend on pH, temperature, charge density, ionic strength among other environmental conditions. It has been shown that chitosan/pectin polyelectrolyte membranes exhibit pH dependent swelling response which is an attractive property for drug delivery systems (Bernabé, Peniche & Argüelles-Monal, 2005; Yao, Tu, Cheng, Zhang & Liu, 1997).

In a previous work, water soluble chitosan-g-*N*-isopropylacrylamide copolymers were synthesized (Recillas et al., 2009). Their thermo-responsiveness and full reversibility in aqueous solutions was characterized. During heating a phase transition takes

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place, which coincides with a marked increase in G′ and a moderate decrement in G″, due to the formation of a hydrophobic network at the expense of the net amount of sol fraction. It was also found that the connectivity in the gel network is governed by the net number of enthalpic – hydrophobic driven – junctions formed. Both the value of the enthalpy change and the LCST vary with the ionic strength of copolymer solutions, but no dependence was found with the neutralization of the polyelectrolyte chain (Recillas et al., 2009).

The aim of the present work was to obtain non-stoichiometric cross-linked membranes between chitosan-g-NIPAm and pectin and to evaluate their pH- and thermo-responsive behavior as a potential device for smart release.

2. Experimental

2.1. Materials

A commercial low-molecular weight chitosan supplied by Fluka was used. Its viscosity–average molecular weight was 1.3×10^5, estimated at 25 °C in 0.3 M acetic acid/0.2 M sodium acetate (Rinaudo, Milas & Le Dung, 1993) and the degree of *N*-acetylation (DA = 0.26) was determined by ^{1}H NMR, potentiometry and conductimetry.

Citrus pectin was purchased by Faga Lab, its viscosity–average molecular weight was $6.5 \times 10^4 \pm 0.2$, estimated at 25 °C in 1 M NaCl, and degree of esterification of galacturonic units (DE = 88.2 ± 0.8%) and galacturonic acid content (784 ± 6 mg/g) determined by conductimetric titrations and colorimetrically, respectively (Garnier, Axelos & Thibault, 1993).

N-isopropylacrylamide (NIPAm, Sigma–Aldrich) was recrystallized from hexane. Ceric ammonium nitrate (Sigma–Aldrich) was used as initiator without purification. All experiments were carried out with distilled water (conductivity lower than 3 μS cm^{-1}).

2.2. Purification of chitosan

Chitosan was purified as described previously (Recillas et al., 2009), by dissolving in 0.2 M acetic acid solution (5 g L^{-1}) and successively filtered through sintered glass filters (pore diameters: up to 16 μm) and membranes (3, 1.2 and 0.8 μm). Then it was precipitated by drop-wise addition of 1 M ammonium hydroxide until pH ≈ 9, carefully washed with water until no change in conductivity was detected and further washed with ethanol at concentrations 70, 80, 90 and 100% (v/v). The purified polymer was finally dried in vacuum at room temperature.

2.3. Purification of pectin

Pectin was dissolved in distilled water overnight (4 g L^{-1}). The solution was filtered through an ionic exchange resin column Amberlite IR-120 (H$^+$ form), after which the solution was neutralized up to a pH near 7 with 1 M NaOH in order to convert the polyacid into its salt form and successively filtered through membranes (3, 1.2, 0.8, 0.45 and 0.22 μm). NaCl was added to reach a final 2 M concentration. Then the polysaccharide was precipitated by gradual addition of ethanol until a final 70% (v/v) alcohol concentration was attained and washed several times with the same water/ethanol mixture until no change in conductivity was detected. The solid was further washed with ethanol/water mixtures of increasing ethanol concentration: 80, 90 and 100% (v/v). The purified polymer was finally dried in vacuum at room temperature.

Table 1
Identification and composition of chitosan-g-NIPAm copolymers.

Sample	*A*	*D* − *x*	*x*	Grafting (%)
C2	0.263	0.49	0.247	25.7
C3	0.263	0.66	0.071	23.9
C4	0.263	0.69	0.047	8.7

A: acetylated units, *D* − *x*: deacetylated units. *x*: NIPAm-grafted deacetylated units. Grafting percentage: weight of NIPAm per 100 g of copolymer.

2.4. Preparation of chitosan-graft–NIPAm copolymer

Graft polymerization of NIPAm onto chitosan was carried out using different amounts of initiator following the method published elsewhere (Kim, Cho, Lee & Kim, 2000) with some modifications as reported before (Recillas et al., 2009). Briefly, 1% (w/w) chitosan solution in 10% (w/w) aqueous acetic acid was bubbled with nitrogen during 30 min. After addition of NIPAm and initiator the reaction was carried out for 2 h at 25 °C. At the end, the reaction mixture was precipitated in an excess amount of acetone. Remaining homopolymer was removed by Soxhlet extraction with methanol during 48 h. Identification and composition of these samples are shown in Table 1.

2.5. Preparation of chitosan–copolymer/pectin polyelectrolyte complex membrane

The polyelectrolyte complex (PEC) membranes were prepared using chitosan-g-NIPAm and pectin in an equivalent relation of 3:1 (NH_2:COOH). The copolymer was dissolved in 0.1 M hydrochloride acid at 1% (w/w) concentration. At the same time, pectin was dissolved in water at 2% (w/w). Both solutions were left to stand overnight. The solutions were mixed and some drops of hydrochloric acid (32%) were added in order to avoid the formation of the PEC. Subsequently, the pH value was adjusted to about 5.5 by adding 10% (w/w) NaOH solution, to favor the formation of interchain ionic bonds. The disperse hydrogel formed was placed on a sintered glass filter and left to decant and dry for several days. As a result of this the PEC membrane was generated. Then, the membranes were carefully washed with distilled water until no change in conductivity was detected; finally they were dried under vacuum at room temperature.

2.6. Swelling measurements

Dry membranes were weighed and then placed in distilled water under a precise temperature control (±0.1 °C). Water uptake was followed periodically until the equilibrium swelling, W_∞, was attained. The following formula was used to estimate the swelling degree at a given time:

$$W = \frac{M_t - M_0}{M_0} = \frac{M_t}{M_0} - 1$$

where M_t is the weight of swollen membrane at time t and M_0 is the weight of dry membrane. Experiments were run in triplicate; the reported value is the average W.

2.7. Differential scanning calorimetry

Calorimetric measurements were carried out in a micro-DSC-IIIa (Setaram, France). All experiments were performed between 5 and 45 °C with a scanning rate of 0.6 °C/min. Standard Hastelloy vessels were used with approximately 10 mg of equilibrium swollen membrane along with a small excess of the swelling liquid. The latter was also chosen as reference. The same mass of sample and reference were weighted to minimize the differences in heat capacities between them. The samples were equilibrated at 5 °C for 30 min before each scan.

2.8. Sorption and desorption experiments

The dried membranes were cut into fragments of about 20–25 mg. These fragments were weighted accurately and placed in a close chamber saturated with water vapour at 25 °C. The water sorption, *W*, was measured gravimetrically after carefully wiping the surface with a filter paper. The sorption was reported by the same way as the swollen membranes.

Desorption experiments were carried out by isothermal thermogravimetric analysis with a Q500 Thermogravimetric Analyzer (TA Instruments). Thermogravimetric analyses were performed at 25 and 40 °C with 8–10 mg samples of the water-swollen membranes on a platinum pan under nitrogen atmosphere with a nominal gas flow rate of 5 mL/s. Zero time was taken as the moment at which the temperature of the system was stabilised automatically.

The water desorbed at any time, *W*, was calculated from the recorded curve and reported as

$$W = \frac{M_s - M}{M_\infty}$$

where M_s is the weight of the swollen membrane, before desorption, M_∞ is the limiting weight of the membrane after desorption, and *M* is the weight of the membrane at time *t*.

2.9. Scanning electron microscopy

A Phillips XL30 versatile microscope (Einhoven, Netherlands), was employed in both Hi-Vac mode (normal SEM) and Wet mode (Environmental Scanning Electron Microscope, ESEM).

For SEM, the membranes were swollen up to equilibrium with distilled water at 10 °C, frozen in liquid nitrogen and then freeze-dried for 12 h. The freeze dried membranes were fractured and fixed in a sample holder and coated with gold in the surface, using a Fine Coat Jeol-JFC-1100 Equipment (Jeol Ltd., Akishima, Japan). Membrane structure was observed at 20 kV and 50–2000×.

For ESEM, samples were completed swollen in distilled water at 10 °C during 24 h. Excess of water were carefully removed using filter paper, then wetted samples were fixed in aluminum holder and placed in the microscope chamber. To avoid evaporation, temperature inside the chamber was 3 °C. Membrane structure was observed at 15 kV and 500–1000×.

2.10. Solute release measurements

The release experiments were performed using Coomassie Blue as model substance. Previously swollen unloaded membranes were placed in an aqueous solution of Coomassie Blue (4 mg mL^{-1}), keeping a relation of 20 mL of the model substance solution per gram of dry membrane, left to stand for 48 h and then vacuum-dried at room temperature.

Solute release was measured by placing the membranes into the release media at pH 2.95 and 5.92 (1 mL of medium per mg of dry membrane) at 10 and 40 °C and then periodically measuring the absorbance of the supernatants at 587 nm (Cárdenas, Argüelles-Monal, Goycoolea, Higuera-Ciapara & Peniche, 2003).

3. Results and discussion

In order to avoid the pH instability of polyelectrolyte complexes (Tsuchida, Osada & Ohno, 1980), the PEC membranes were subjected to a thermal treatment at 120 °C for 30 and 60 min in an inert atmosphere (Grishina, Rogacheva, Lopatina, Zezin & Kabanov, 1985). This reaction triggers the formation of amide bonds and provides pH stability to the polyelectrolyte complexes. From now on, these membranes will be referred to as PEC2, 3, or 4, according to the constituting copolymer (C2, C3 or C4), and 0, 30, or 60, depending on exposure time to thermal treatment.

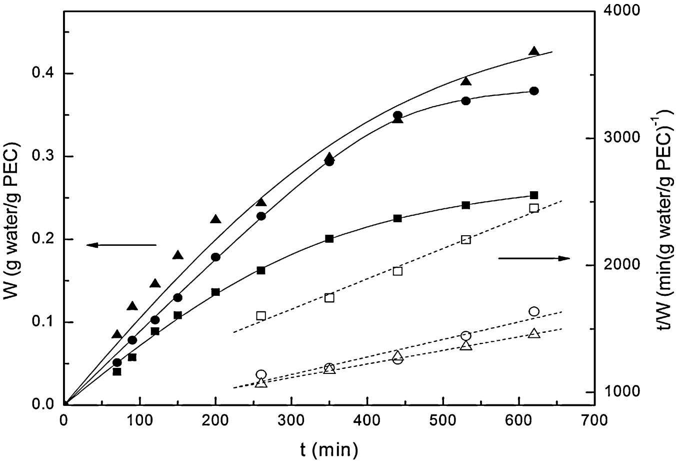

Fig. 1. Plot showing the dependence of the water sorption (*W*) and the reciprocal of the average sorption rate (*t*/*W*) on time at 25 °C. Points correspond to experimental values: (■) PEC2-60; (●) PEC3-60; (▲) PEC4-60. Curves were calculated with equation (5) using the parameters listed in Table 2. Dotted lines represent the linear regression of the corresponding *t*/*W* vs. *t* plots (Eq. (1)).

PEC membranes thus obtained are hydrophilic, with an opaque appearance, but vitreous when dry. However, because of a drop in Tg resulting from the solvent's plasticizing effect, their appearance changes and they become flexible in contact with water.

The morphology of membranes reveals a rough surface structure (micrographs for membrane PEC3 without treatment and with a 60-min thermal treatment is shown in supplementary data). It consists of interconnected heterogeneous spaces within laminated walls leading to highly porous fibrous structures. In general, it was evident that thermal treatment generates no significant change in membrane morphology.

Since chitosan-g-NIPAm copolymer PEC membranes are hydrophilic, their water vapor absorption was evaluated. Experiments were carried out at 25 °C, only for those membranes with 60-min thermal treatment. Fig. 1 depicts the increase in water vapor absorption according to exposure time. It is possible to observe that the amount of adsorbed water is larger for small degree of copolymer grafting. The interaction of $-NH_2$ groups with water molecules is evident in the sorption process.

PEC water vapor sorption process does not display a Fickean behavior. Nevertheless, it is evident in Fig. 1 that membranes present an initial linear dependence of *W* on *t*, which means zero-order sorption kinetics for this initial stage.

In the second stage of the sorption process (times longer than 200 min), data obtained were suitably adjusted to Schott's equation (Schott, 1992):

$$\frac{t}{W} = A + Bt \tag{1}$$

where *W* is the sorption value at time *t*, $B = 1/W_\infty$, the inverse of maximum swelling and $A = 1/(dW/dt)_0$, the reciprocal of the initial swelling rate.

It has been proven that Eq. (1) describes a second-order kinetics regarding the remaining swelling, in this case remaining absorption, expressed as:

$$\frac{dW}{dt} = k(W_\infty - W)^2 \tag{2}$$

Table 2
Values of the parameters k (g PEC × (g H_2O min)$^{-1}$) and K (g PEC/g H_2O)^2, W_∞ (g H_2O/g PEC) and the ratio $kW_\infty^2/(1+KW_\infty^2)$, which is the pseudo-zero-order initial rate constant (ν), obtained by applying Eq. (5) to the water sorption data at 25 °C (Fig. 1). The values of the initial sorption rate estimated from the initial slope are shown in parenthesis.

Sample	W_∞	k	K	$\nu = kW_\infty^2/(1+KW_\infty^2)$
PEC2-60	0.3	0.0517	57.52	7.5×10^{-4} (6.9×10^{-4})
PEC3-60	0.4	0.2713	308.91	8.9×10^{-4} (8.6×10^{-4})
PEC4-60	0.6	0.0282	5.030	12×10^{-4} (11×10^{-4})

where the specific rate constant, k, is related to parameter A in Eq. (1), as follows:

$$k = \frac{1}{AW_\infty^2} \tag{3}$$

The almost linear increase of W with t is a characteristic event of sorption II case that occurs when diffusion rate is much greater than polymer segment mobility and ends when diffusion fronts meet.

In other words, this is a process that initially follows zero-order kinetics ($t < 200$ min) when quantity of water absorbed triggers a shift in polymer matrix structure, and hence, a change in solute mobility (Crank, 1975).

From the moment both diffusion fronts meet, the diffusion phenomenon changes to a second-order kinetics, which is evident for periods longer than 200 min. At this point, membranes have absorbed enough water to display elastic behavior and sorption becomes governed by chain relaxation.

Peniche-Covas, Argüelles-Monal and San Román (1995) have proposed a mathematical model capable of combining zero-order kinetics for the initial part of the adsorption process with second-order kinetics for more prolonged times:

$$\nu = \frac{dW}{dt} = \frac{k(W_\infty - W)^2}{1 + K(W_\infty - W)^2} \tag{4}$$

which, when $W \ll W_\infty$ and $\nu = kW_\infty{}^2/(1+KW_\infty{}^2) = \text{const.}$, gives a zero-order behavior, whereas when $W \approx W_\infty$ and $K(W_\infty - W) \ll 1$ gives $\nu \approx k(W_\infty - W)^2$ and second-order kinetics are described.

Eq. (4) integrates as:

$$\frac{W}{(W_\infty - W)W_\infty} + KW = kt \tag{5}$$

where W_∞, K, and k parameters are equilibrium swelling and two constants, respectively.

It was possible to adjust experimental values for the studied membranes to Eq. (5) using a non-linear least squares method. Estimated values are shown in Table 2. It is evident that equilibrium swelling value shows a PEC2-60 < PEC3-60 < PEC4-60 trend influenced by the composition of membranes. This can be interpreted as a consequence of the different degree of grafting in the copolymer forming the membrane: as the NIPAm graft in chitosan chain becomes smaller, there is a greater quantity of ionic groups available; therefore, the sample absorbs water more easily.

The estimation of diffusion coefficients for a non-Fickean process is very elaborate. However, Hirai and Nakajima calculated reasonable D values for water diffusion in polyelectrolytes, using the following equation (Hirai & Nakajima, 1989):

$$D_{1/2} = 0.049\left(\frac{l^2}{t}\right)_{1/2} \tag{6}$$

where $D_{1/2}$ is the approximate diffusion coefficient, l is sample thickness, t is the time when the amount of water vapor in membrane is half that of the equilibrium. The values of the apparent diffusion coefficients, $D_{1/2}$, during sorption at 25 °C estimated with this equation are 1.03, 0.94 and 1.11×10^{-12} m^2 s^{-1} for PEC2-60, PEC3-60 and PEC4-60, respectively. It is evident that no significant differences are present in $D_{1/2}$, for membranes of the three copolymers.

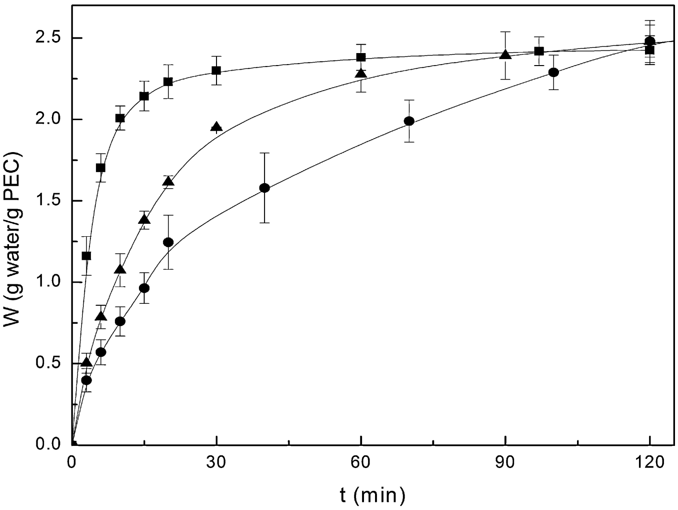

Fig. 2. Swelling behavior of membranes PEC2-30 (■), PEC3-30 (●) and PEC 4-30 (▲) in distilled water at 10 °C.

Thereafter, the behavior of these materials during swelling was studied. Fig. 2 depicts results obtained for PEC membranes at 30-min thermal treatment. Experimental data obtained from swelling kinetics were fit to the model described for a Fickean behavior using the solution of Fick's equation for diffusion through a flat film (Crank, 1975):

$$\frac{W}{W_\infty} = 1 - \sum_{n=1}^{\infty} \frac{8}{(2n+1)^2\pi^2} \exp\left[-\frac{D(2n+1)^2\pi^2 t}{l^2}\right] \tag{7}$$

where l is half the thickness of the membrane, W and W_∞ represent water uptake at time t and equilibrium, respectively, and D is the diffusion coefficient of water in the membrane.

Experimental data were satisfactorily fit to Eq. (7) using a non-linear least squares method (a graphical example of the fitting, as well as a summary of the values of W_∞ and D for all membranes are given in supplementary data). The equilibrium swelling of the complex membranes was low in all three cases and does not vary significantly with composition. There were no major variations in equilibrium swelling for the thermal treatment times used for this system as compared with other PECs (Bernabé et al., 2005), which may be due to the fact that the degree of conversion from ionic bonds into amide bonds resulting from the treatment was low in every case. At this point it should be also considered that if ion pairs are formed before amide bond formation during thermal treatment, the solvation must be very low; so, perhaps the state of the polyelectrolyte complex remains the same as that of uncross-linked membrane and no difference in swelling could be expected. Furthermore, diffusion coefficient values obtained do not show differences either, which agrees with the previous analysis.

The wet surface of swollen membranes at equilibrium was observed using environmental scanning electronic microscopy. Toward this end, samples were swollen in distilled water at 10 °C for 24 h, and placed in the microscope after removing excess water from the surface.

Fig. 3 shows, as an example, micrographs of the PEC4-0 and PEC4-60 membranes dried and swollen at equilibrium. When comparing dried and swollen samples, the rough and porous surface of dried membranes appears smoother in the swollen ones, and enlarged pores are visible, typical of swollen polymer materials.

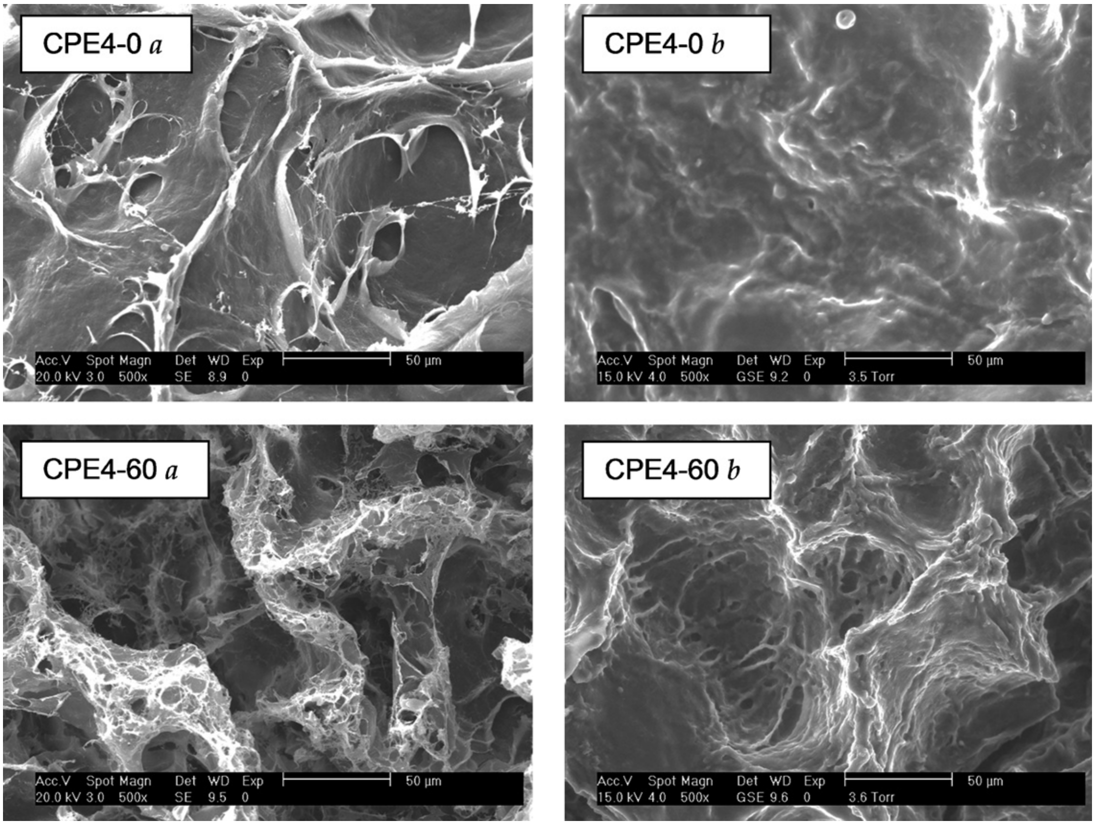

Fig. 3. Micrographs of PEC4-0 and PEC4-60 membranes obtained by (a) SEM and (b) ESEM.

As the swollen membrane is vacuum-dried, the structure becomes rough again (a series of environmental scanning electronic micrographs of the PEC4-60 membrane as it was being vacuum-dried in the microscope is supplied in supplementary data).

Calorimetric studies of PEC swollen membranes show a well-defined endothermic peak between 32.5 and 33.5 °C when temperature rises above LCST (see supplementary data for original calorimetric curves). During cooling, exothermic peaks of the same magnitude were obtained, which proves the process is totally reversible.

Fig. 4 summarizes calorimetric data for all swollen membranes in water. When comparing the thermal behavior during transition, a marked influence of the copolymer composition on the enthalpy values associated to phase transition is evident (PEC2 > PEC3 > PEC4), which is similar to what other authors have previously reported for PNIPAm gels (Grinberg et al., 1999; Lim, Kim & Lee, 1997; Schild, 1992). For PEC membranes of the same composition, no notable changes are evident in the associated enthalpy value. On the other hand, there are no significant differences between different LCST values regardless of the type of copolymer or thermal treatment duration.

Given the similarity in behavior of membranes formed at different compositions, thereafter the work was carried out with only one of them. PEC2-30 membrane was chosen because of its higher NIPAm content and being an intermediate between the non-treated and the thermally modified membranes.

Variation in the degree of equilibrium swelling, W_∞, with temperature is shown in Fig. 5. For this purpose, membrane was initially equilibrated for 12 h at the lowest temperature (15 °C) and its swelling was determined. Then, temperature was raised to desired values, taking as equilibrium value the swelling at 12 h in each case. Each point in the figure represents the average equilibrium swelling value for two measurements.

Phase transition area is located between the two plateaus of the curve, where W_∞ remains virtually constant. When temperature reaches 30 °C, a sharp descent is evident in W_∞ with an inflexion point at 33.1 °C, ending at 35 °C. Within this interval, the gel shrinks discontinuously. This effect is attributed to the “breakdown” of structured water molecules located around NIPAm hydrophobic groups which are expelled from these groups. As a result, hydrophobic interactions between methyl groups from different NIPAm grafted blocks are promoted, giving rise to the

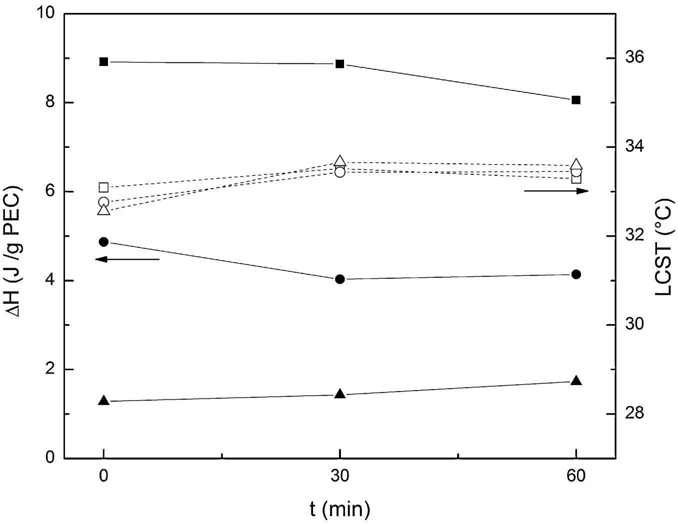

Fig. 4. Influence of the thermal treatment time on the LCST (open symbols, dotted lines) and enthalpy values (filled symbols, solid lines) for PEC2 (■), PEC3 (●) and PEC4 (▲) membranes swelled in water. Data were obtained by micro-DSC at 0.6 °C min^{-1}.

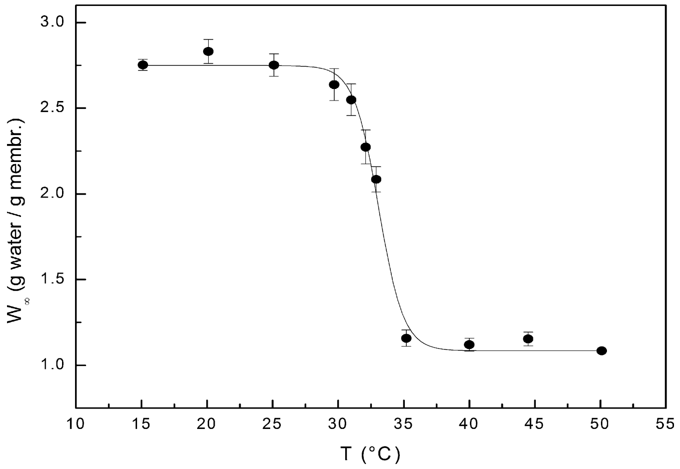

Fig. 5. Swelling equilibrium values, W_∞, as a function of temperature for PEC2-30 membranes. Membranes were swollen in water, pH 5.9, during 12 h in order to achieve equilibrium.

shrinkage of the membrane. It can be observed that the contraction does not eliminate the total amount of water linked to the membrane, which remains solvating the polar groups at pH 5.9.

The PEC2-30 sample was subjected to three continuous swelling–shrinking cycles, induced by the change in temperature of the medium at 10 and 40 °C, for 6-h periods at those two temperatures, below and above LCST, respectively (Fig. 6). The figure depicts the thermoreversible nature of the PEC membranes' swelling process. Below LCST (10 °C) NIPAm chains in the membrane are hydrated and stretched, while as temperature increases sharply to 40 °C, NIPAm chains contract and water is expelled from the polymer matrix, giving rise to phase transition.

Once swelling and thermal behavior of membranes in water was assessed, the effect of the pH of the medium was considered. For this purpose, dried membranes were swollen in solutions where pH was adjusted by adding NaOH or HCl. Value for W_∞ was recorded

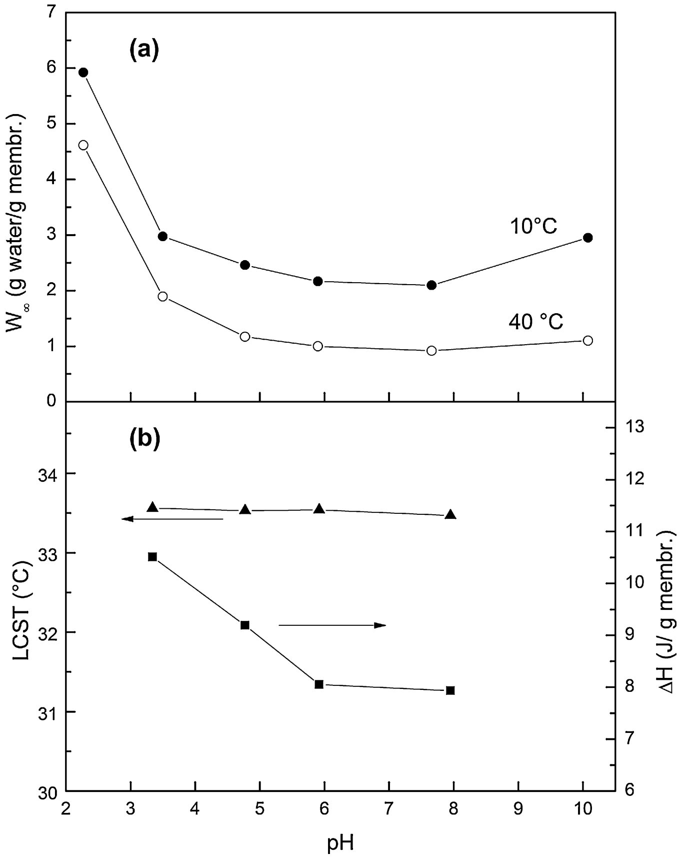

Fig. 7. Influence of pH on the behavior of PEC2-30 membrane. The pH of the solution was adjusted by additions of HCl or NaOH dilute solutions. (a) Equilibrium swelling values at 10 or 40 °C as shown in the graph. (b) LCST (▲) and the enthalpy value associated to the transition (■) of the swollen membrane. The LCST and enthalpy values were obtained by micro-DSC at 0.6 °C min^{-1}.

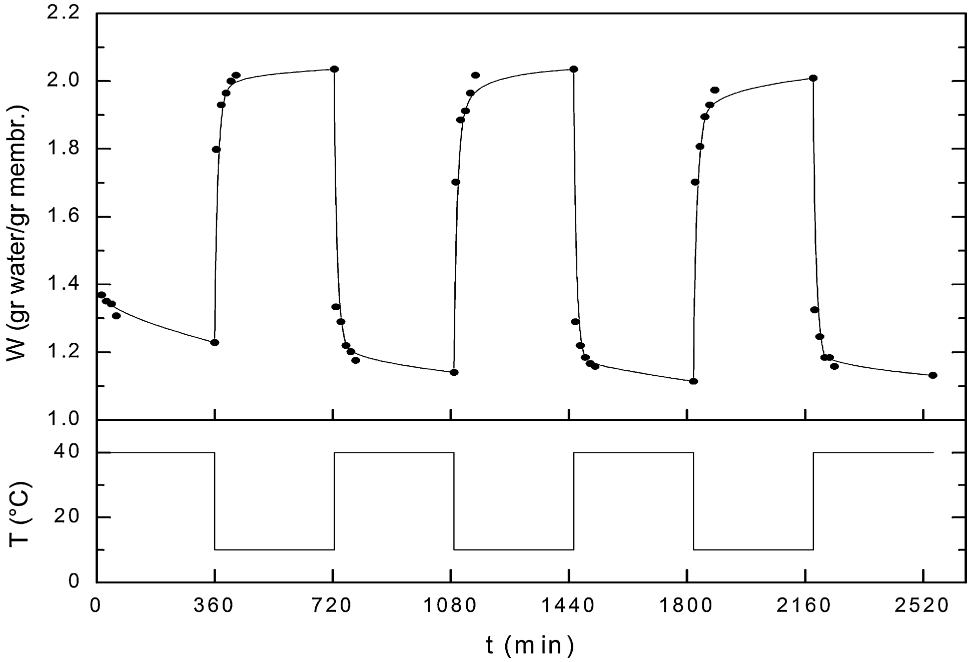

Fig. 6. Variation of swelling in pure water (pH = 5.9) for a PEC2-30 membrane to stepwise periodic changes in temperature between 10 and 40 °C.

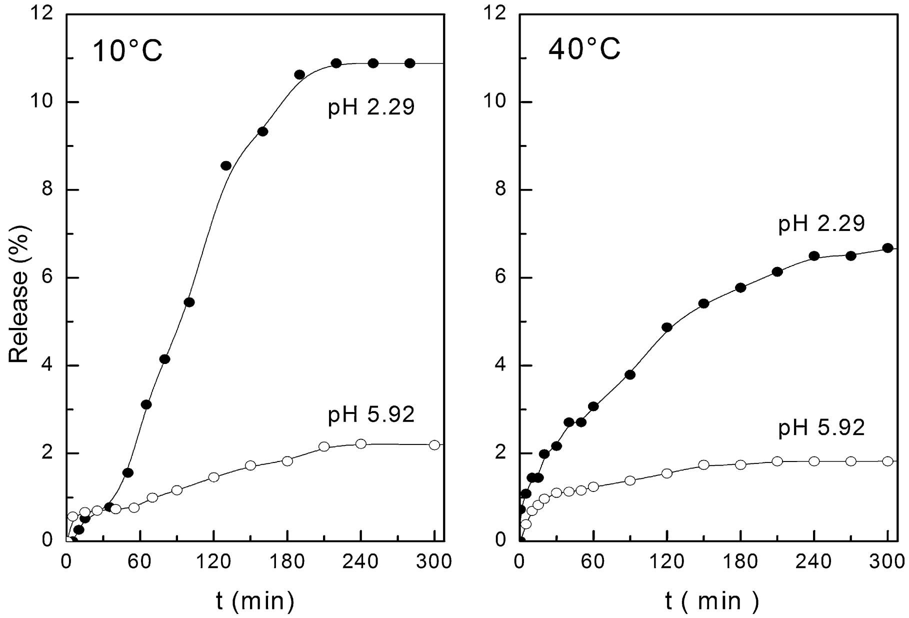

Fig. 8. Coomassie Blue release curves from a PEC2-30 membrane at two different pH values as shown in graph, at 10 °C and 40 °C. Initial content of dye in the membrane was 1.66 ± 0.13 μg mg^{-1}.

after remaining at 10 or 40 °C for 12 h. Results obtained at different pHs are shown in Fig. 7a. It can be noticed that the maximum value for W_∞ recorded at 10 °C was 6 g water/g PEC for a pH close to 2.3. This value gradually decreased to 2.1 at pH 7, but slightly increased at pH 10.

Depending on the pH, more –NH$_2$ or –COOH groups remains able to be ionized at acid or basic conditions. Breakage of interchain salt bonds decreases the cross-linking of the network, while ionization of functional groups causes chain stretching. Both effects determine the expansion of the gel (Flory, 1953; Kokufuta, 2001; Qu, Wirsén & Albertsson, 2000; Yao, Liu, Cheng, Lu & Tu, 1996).

When temperature increases and exceeds LCST (40 °C), the degree of swelling depicts the same tendency with pH as at 10 °C, but with values lower than those recorded at that temperature. These experiments show that swelling of polyelectrolyte complex membranes with chitosan-g-NIPAm is not only sensitive to temperature – given by the NIPAm grafted chains – but also to the pH of the medium – due to the ionization of residual –NH$_2$ and –COOH groups.

The effect of pH on thermal behavior of membranes was also analyzed using DSC. Fig. 7b shows the dependence of LCST and transition enthalpy on the pH of the medium, jointly with equilibrium swelling values at 10 °C. It is evident that variation of pH of the medium has no influence on LCST, similar to findings by varying the degree of neutralization of copolymer chains in solution. Nevertheless, transition enthalpy decreases when pH increases within studied interval, showing the same trend as equilibrium swelling.

Lastly, experiments of the release of a model substance (Coomassie Blue) loaded in the PEC2-30 membrane were carried out. Trials were conducted at two pH values (2.92 and 5.92) and two temperatures, below (10 °C) and above (40 °C) LCST (Fig. 8).

For both temperatures, the greater release of the dye was produced at a pH of 2.92. This result agrees with that shown in Fig. 7a, where swelling of the membrane becomes greater as the acidity of the medium increases, thus favoring a faster diffusion of the model solute.

It can be observed that by increasing the temperature of the acidic medium above LCST, the network's contraction and the possible aggregation of PNIPAm chains in the membrane obstruct the diffusion of the solute, considerably reducing the release. This reduction is less sharp at pH 5.92, where the release was less at both temperatures due to smaller swelling of the matrix in a neutral medium. It is worth noting the low percentage of dye release, even at acidic pH, possibly due to ionic or polar interaction between the membrane and the dye (Coughlan, Quilty & Corrigan, 2004; Shin & Yoo, 1998). Concerning the influence of the pH on this process, possible specific interactions between ionic or hydrophobic groups of the model substance and membrane should not be discarded as well.

Synthesized chitosan-g-NIPAm copolymers, whether in solution or as part of polyelectrolyte complexes, undergo evident changes in their behavior as a response to variation in temperature, ionic strength, and pH of the surrounding medium. It has been established that prepared copolymers may be good candidates to develop innovative materials.

4. Conclusions

Membranes from a non-stoichiometric polyelectrolyte complex between chitosan-g-*N*-isopropylacrylamide and pectin were prepared and covalently cross-linked throughout a thermal treatment at 120 °C. These hydrophilic membranes neither show differences in morphology, nor in swelling with the time exposed to the thermal crosslinking. Swollen membranes undergo a sharp shrinking behavior during heating with an inflexion point at 33.1 °C due to the well-known phase transition of NIPAm chains during which they contract expelling the structural water out from the polymer network. This transition is fully reversible and associated to an endothermic process, the enthalpy of which is proportional to the degree of grafting of NIPAm. These membranes also respond to changes in the pH of the medium. The transition enthalpy decreases when pH increases, showing the same trend as the equilibrium swelling. The LCST does not vary neither with the degree of grafting, nor with the pH of the medium, showing a value around 33 °C in all cases.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2011.06.047.

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