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Time- and pH-dependent self-rearrangement of a swollen polymer network based on polyelectrolytes complexes of chitosan/chondroitin sulfate

André R. Fajardo, Juliana F. Piai, Adley F. Rubira, Edvani C. Muniz *

Grupo de Materiais Poliméricos e Compósitos, GMPC - Departamento de Química Universidade Estadual de Maringá, UEM - Av. Colombo, 5790 – CEP 87020-900, Maringá, Paraná, Brazil

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ABSTRACT

The self-rearrangement of polymer networks based on a polyelectrolyte complex (PN-PEC) resulting from electrostatic interactions between chitosan (CHT) and chondroitin sulfate (CS) was studied in different swelling pH and swelling time conditions. Analysis of the swelling degree and WAXS profiles revealed that the PN-PEC dried sample can self-reorganize depending on both the swelling medium pH and the swelling time. The reorganization is supposed to result from the rearrangement of PN-PEC-forming polymer chains after the release of part of the CS chains, mainly at pH values closer to or higher than the p*K*a of CHT. This reorganization contributes to increase the crystallinity, thermal stability, and average pore size of the polymer network. This phenomenon makes it possible to treat PN-PEC to obtain some interesting application characteristics.

1. Introduction

In recent years, many authors have reported a high interest in the study and application of the so-called biomaterials, especially in biotechnology, due to their several advantages over conventional materials (low toxicity, biocompatibility, etc.) and wide applicability (Narayan & Boehlert, 2008; Sabir, Xu, & Li, 2009), such as controlled release systems (Oh, Drumright, Siegwart, & Matyjaszewski, 2008; Vodn Bubeníková, & Bakoss, 2007), with significant superiority when compared to conventional forms of drug administration (spray, pills, injections) (Huang et al., 2004; Sinha & Kumria, 2001). One of the materials used to produce these systems is chitosan (CHT); a linear polymer derived from chitin, a natural biopolymer (Singla & Chawla, 2001). Many researchers have used CHT, which has excellent physical and biological properties (Aranaz et al., 2009; Gupta & Kumar, 2000; Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). Due to its cationic nature and high density charge in acid solution, CHT can form polymer networks of polyelectrolytes complexes (PN-PEC) with water-soluble polyanionic species (Denuziere, Ferrier, & Domard, 1996; Vasiliu, Popa, & Rinaudo, 2005). PN-PEC is formed by macromolecule networks containing a relatively large number of charged functional groups or, in appropriate conditions; it can be loaded (Berger et al., 2004). Electrostatic attraction, the main interaction in PN-PEC, is stronger than secondary interactions, such as bonds dipole–dipole interactions and H-bonding (Chen, Wang, Chen, & Fan, 2005).

Depending on the environment in which PN-PEC is swollen, it exhibits high charge density and varied swelling behavior (Dai, Li, Zhang, Wang, & Wei, 2008), allowing the diffusion of water and/or molecules of the solutes, such as drugs and proteins (Du, Dai, Liu, & Dankovich, 2006; Ghaffari, Navaee, Oskoui, Bayati, & Tehrani, 2007). Generally, PN-PEC has a higher degree of swelling in a medium with pH between the p*K*a values of the functional groups responsible for its formation. This characteristic makes PN-PEC an interesting material for use as a carrier for controlled drug release in a wide pH range.

In this work, the polyanionic species used to form a polyelectrolyte complex together with CHT was chondroitin sulfate (CS). It is an important structural component of tissues and ligaments and is a key component of cartilage. This biopolymer has been used to treat diseases related to atherosclerosis, thrombosis, and osteoarthritis (Morris & Smith, 2009; Richy et al., 2003) for a long time.

Many reviews have discussed the formation and implementation of these systems (Hartig, Greene, Dikov, Prokop, & Davidson, 2007; Sakiyama, Takata, Kikuchi, & Nakanishi, 1999; Thunemann et al., 2004), but the study of possible structural modifications of these materials under different swelling conditions has not received much attention. This work investigates the influence of swelling medium pH and swelling time as factors in the self-reorganization of chains that form PEC in an attempt to contribute to the post-treatment of PN-PEC formed by CHT/CS to obtain, preserve, or improve interesting features, such as crystallinity or thermal stability.

* Corresponding author. Tel./fax: +55 44 3261 4215.
E-mail address: ecmuniz@uem.br (E.C. Muniz).

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2. Experimental

2.1. Materials

Chitosan (CAS 9012-76-4, Golden-Shell Biochemical, China) 85% deacetylated and M_V of 87×10^3 g mol^{-1} was obtained with the method proposed by Mao et al. (2004). The intrinsic viscosity of the CHT solution in acetic acid (HAc) 2 wt-%/0.2 mol L^{-1} sodium acetate (NaAc) was measured using an Ubbelohde-type capillary viscometer (Model Cannon 100/E534) to 25.0 °C. The solution concentrations were adjusted based on sample viscosity. The Mark–Houwink constants used for chitosan with a deacetylation degree of 85% were $K = 1.38 \times 10^{-5}$ and $a = 0.85$. Chondroitin sulfate, (lot OP1141/08) which was kindly supplied by Solabia Maringá, Brazil, had M_V equal to 22×10^3 g mol^{-1}, according to the method proposed by Wasteson (1971). The viscometry of different solutions consisting of 1, 2, 3, 4, and 5 wt-% of CS, and 0.2 mol L^{-1} sodium chloride (NaCl) was also measured. For this experimental condition, the value of constant K was 5.0×10^{-5} and a was 1.14. All reactants were of analytical grade and used without further purification.

2.2. Formation of polymer network by PN-PEC

To form PN-PEC, the polymer network-forming solution was prepared by combining individual aqueous solutions of CS and CHT. For the first solution, 6.4 g of CHT was dissolved in 400 mL of an aqueous solution of 0.57 mol L^{-1} HCl, which was heated to 65 °C and stirred until complete solubilization, yielding a 1.6 wt/v-% acid aqueous solution. For the second solution, 25.0 g of CS was solubilized in 100 mL of distilled water to produce a 25.0 wt/v-% aqueous solution. After that, both solutions were slowly mixed under magnetic stirring at room temperature. The resulting solution had pH of 1.8. The suspension formed was stored for 24 h for the sedimentation of PN-PEC. After this period, the material formed was separated from the supernatant and purified by immersion in 500 mL of distilled water overnight. Then, the pH of the medium was neutralized with 0.2 mol L^{-1} of aqueous sodium hydroxide (NaOH). Finally, the purified material was cut into small cubes with sides of ca. 1.50 cm and dried at room temperature for 48 h.

2.3. Preparation of buffer solutions

The buffer solutions were prepared according to the National Book of Formulas – United States Pharmacopoeia (USP30-NF2520, 2007). Different buffer solutions were prepared in the range from pH 2 to 10 at a concentration of 50 mmol L^{-1}. The ionic strength was adjusted for a constant concentration of 0.1 mol L^{-1} by adding KCl. The quantities of the starting solutions required to make each buffer solution are described in Table 1.

2.4. Determination of swelling degree (S)

In this work, swelling degree (S) (Yildiz, Isik, & Kis, 2002) was utilized to characterize the swelling capacity of the PN-PEC of CHT/CS in aqueous liquids. S was determined as the ratio of the weight of swollen PN-PEC samples (W_i) and the dry weight of PN-PEC (W_o) using the following equation:

$$S = \frac{W_i - W_o}{W_o} \times 100$$

The dried samples were first weighed and then immersed in 50 mL of buffer. S was determined for samples swollen in buffer with pH 2, 6, 8 and 10. The sample weights taken at set intervals were used to calculate the value of S. The measurements were performed in triplicate.

2.5. Reorganization of PN-PEC in the CHT/CS polymer network

After drying, the small sample cubes were immersed in 30 mL of buffer solutions of pH 2, 6, 8, and 10 with constant ionic strength at 37 °C for 100, 200, 400 and 800 h. The samples were collected, crushed to small pieces, and lyophilized (Christ Gefriertrocknungsanlagen) at −55 °C for 24 h.

2.5.1. Wide-angle X-ray scattering (WAXS) analysis

Powders of lyophilized PN-PEC were characterized by WAXS technique on a Shimadzu diffractometer model XRD-600, equipped with Ni-filtered Cu-K_α radiation. The WAXS data were collected in a scattering range of $2\theta = 5°$–$70°$ with resolution of 0.02° at a scanning speed of 2° min^{-1}. The analyses were made by applying an accelerating voltage of 40.0 kV and a current intensity of 30.0 mA. Next, the pure polysaccharides (CHT and CS), the salts used in the production of the buffers, and the buffers themselves were also characterized by this technique.

2.5.2. Differential scanning calorimetric (DSC) analysis

DSC tests were performed on a calorimeter (Netzsch, model STA 409 PG/4/G Luxx, USA) operating in the following conditions: heating rate of 10 °C min^{-1}, nitrogen flow rate of 20 mL min^{-1}, temperature range from 22 to 400 °C. The analyses were made using tightly closed containers to reduce the effects of loss of humidity.

2.5.3. Thermogravimetric (TGA) analysis

TGA analysis of PN-PEC samples was carried out on a thermogravimetric analyzer (Netzsch, model STA 409 PG/4/G Luxx, USA) at a rate of 10 °C min^{-1} under nitrogen atmosphere with flow rate of 20 mL min^{-1} in a temperature range from 22 to 400 °C.

2.5.4. PN-PEC morphology by SEM

The porous morphology of the dried swollen PN-PEC samples was investigated by scanning electron microscopy (SEM) (Shimadzu, model SS 550). SEM images were obtained after swelling in buffers with different pHs and in swelling time conditions. After swelling, the samples were removed from water and quickly frozen by immersion in liquid nitrogen for 5 min. Thereafter, the frozen samples were fractured and lyophilized by freeze drying at −55 °C for 48 h. The surfaces of the lyophilized samples were sputter-coated with a thin layer of gold for allow the SEM visualization. The images were taken by applying an electron accelerating voltage of 8 kV.

Table 1
Used amount of reactants in the preparation of buffer solutions with constant ionic strength (0.1 mol L^{-1}).

pH	Solute	Solution 0.5 mol L^{-1} (mL)	HCl 0.2 mol L^{-1} (mL)	NaOH 0.2 mol L^{-1} (mL)	Add KCl (g)
2.00	KCl	100	53.0	0	2.94
6.00	KH_2PO_4	100	0	29	3.30
8.00	KH_2PO_4	100	0	234	3.04
10.00	H_3BO_3/KCl	100	0	220	0.45

3. Results and discussion

3.1. Swelling capacity

The swelling capacity of the CHT/CS PN-PEC was evaluated using the swelling degree (*S*). Fig. 1 describes the swelling profiles of PN-PEC samples immersed in different buffer solutions. Each sample was kept in a buffer solution with specific pH for 350 h.

When the PN-PEC samples were swollen in pH 2 buffer, they achieved equilibrium faster than at higher pH values. *S* showed that in this condition, PN-PEC samples can to absorb about 175% of their dry weight. However, this was the lowest *S* value among the tested swelling conditions. In this condition, with pH lower than the p*K*a of CHT ($pKa_{CHT} \approx 6.20$) (Strand, Tommeraas, Varum, & Ostgaard, 2001), the amino groups in the CHT structure are in the ionized form ($—NH_3^+$), and they strongly interact with the sulfonic groups ($—OSO_3^-$) of CS. It must be highlighted that p*K*a of this group is nearly 2.60 (Larsson, Nilsson, & Tjalve, 1981). This strong interaction causes the polymer network to remain stable and entangled in such a way that it does not expand significantly, which reduces the absorption of liquids.

In buffer solutions with pH 6, 8, and 10, the PN-PEC samples swelled to a maximum; after this, the amount of absorbed liquid decreased due to mass loss of the PN-PEC samples. When the samples were swollen in buffers with pH higher than pKa_{CHT}, the amino groups of CHT did not ionize (Strand et al., 2001), while the sulfonic ($pKa \approx 2.60$) (Larsson et al., 1981) and carboxylic groups ($pKa \approx 4.57$) (Larsson et al., 1981) of CS were converted because of their lower p*K*a, when compared to the p*K*a of CHT. With an increase in the density of negative charges due to the ionized groups, the anion–anion electrostatic repulsion forces among the CS chains rose. This repulsion, coupled with the decrease in electrostatic interactions between the two polysaccharides, destabilized the network and afforded mobility to the CS chains. Thus, during the liquid diffusion through the network, the CS chains with greater susceptibility to removal, smaller chains or fragments, were captured and later released during the liquid outflow (Piai, Rubira, & Muniz, 2009). It is worth emphasizing that CS showed good solubility in conditions close to neutrality (Wang, Chen, Wang, & Chen, 2007), which was not observed for CHT (Kurita, 2006), pointing out that sample mass loss was due to the release of part of the CS chains.

The samples swollen in buffers with pH 6 and 8 achieved the highest values of *S*. The increase in the pH of the medium to values greater than pKa_{CHT} minimized the repulsion among the chains and the electrostatic interaction were no longer the main form of physical interaction in the formation of PN-PEC. The protonated amino groups in the CHT structure at high buffer pH react with the basic groups (OH^-) present in the environment ($—NH_3^+ + OH^- \rightarrow NH_2 + H_2O$). Thus, the amino groups formed H-bonds with the sulfonic and carboxylate groups from non-released CS. With a smaller repulsion, the chains thus could get closer and interact. This led the polymer network to regain stability and decrease the distance among the chains. Therefore, for samples swollen in pH 10 buffer, the maximum value of *S* was less than those observed for the samples swollen in buffers with pH 6 and 8.

The determination of parameter *S* for PN-PEC samples swollen in different pH conditions showed that their swelling behavior is pH- and time-dependent, and that this CHT/CS PN-PEC can be classified as a "smart biomaterial" (Furth, Atala, & Van Dyke, 2007).

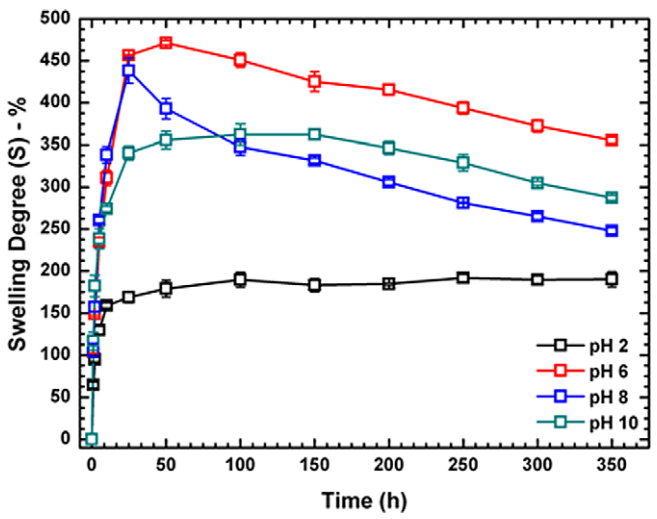

Fig. 1. Determination of swelling degree (*S*) of PN-PEC samples swollen in buffer solutions with different pH values.

3.2. Wide-angle X-ray scattering (WAXS) of PN-PEC

Fig. 2 shows the WAXS profiles of the pure polysaccharides and of dried PN-PEC samples after swelling in buffers with pH 2, 6, 8, and 10 for 100, 200, 400, and 800 h. The diffraction signals at $2\theta = 10.8°$ and $2\theta = 19.9°$ in the CHT diffraction profile were attributed to the crystalline regions of the polysaccharide structure. These crystalline regions are formed by interactions among the CHT chains (H-bonding, for instance) (Pillai, Paul, & Sharma, 2009). A low-intensity diffraction signal at $2\theta = 21.9°$, corresponding to crystalline regions in the CS structure, was also observed. In the formation of the complex, the crystalline regions observed in the pure polysaccharides were broken (Du et al., 2006). The interactions among the chains of the pure polysaccharides were replaced by interactions among CHT and CS chains, with the predominance of electrostatic interactions.

When PN-PEC samples were swollen in buffer solution with pH 2, no diffraction signals were observed in the WAXS profiles of PN-PEC, even after 800 h swelling (see Fig. 2a). This demonstrates that organized regions were not formed in the PN-PEC structure after swelling in this pH condition, independently of the swelling time. As discussed above, when the samples were swollen in this pH condition, the electrostatic interaction among the polysaccharide chains was strong enough to prevent the detachment and subsequent releasing of CS. The partial release of CS from the PN-PEC to the environment is discussed in the literature as a key factor for the chain self-rearrangement that results in the formation of the PN-PEC (Piai et al., 2009). This self-rearrangement is responsible for the formation of ordered regions within the network. Thus, the absence of diffraction signals in the WAXS profiles of samples swollen in buffer at pH 2 allows us to infer that no CS was released nor were the chains rearranged.

Different from the samples swollen in pH 2 buffer, the diffractogram profiles of samples swollen in buffers with pH 6, 8, and 10 show high-intensity signals at $2\theta = 43.8°$ and $2\theta = 64.2°$. The appearance of these signals suggests the formation of ordered regions within the structures of dried PN-PEC samples swollen in the pH range 6–10. The signals also became more intense when the swelling time was increased, indicating that the formation of these regions is pH- and time-dependent. When the samples were pre-swollen in buffer with pH near to or higher than the pKa_{CHT}, as observed in the study of swelling degree, the amount of liquid that flowed through the network was much greater due to the repulsive forces, which caused substantial expansion and network destabilization. As a result of the diffusion of liquid through the network, CS, which no longer interacts strongly with CHT, is partially released. CS was not completely released because in pH range 6–7, the amount of basic groups (OH^-) in the medium was small, and the CHT amino group remained partially protonated, causing the

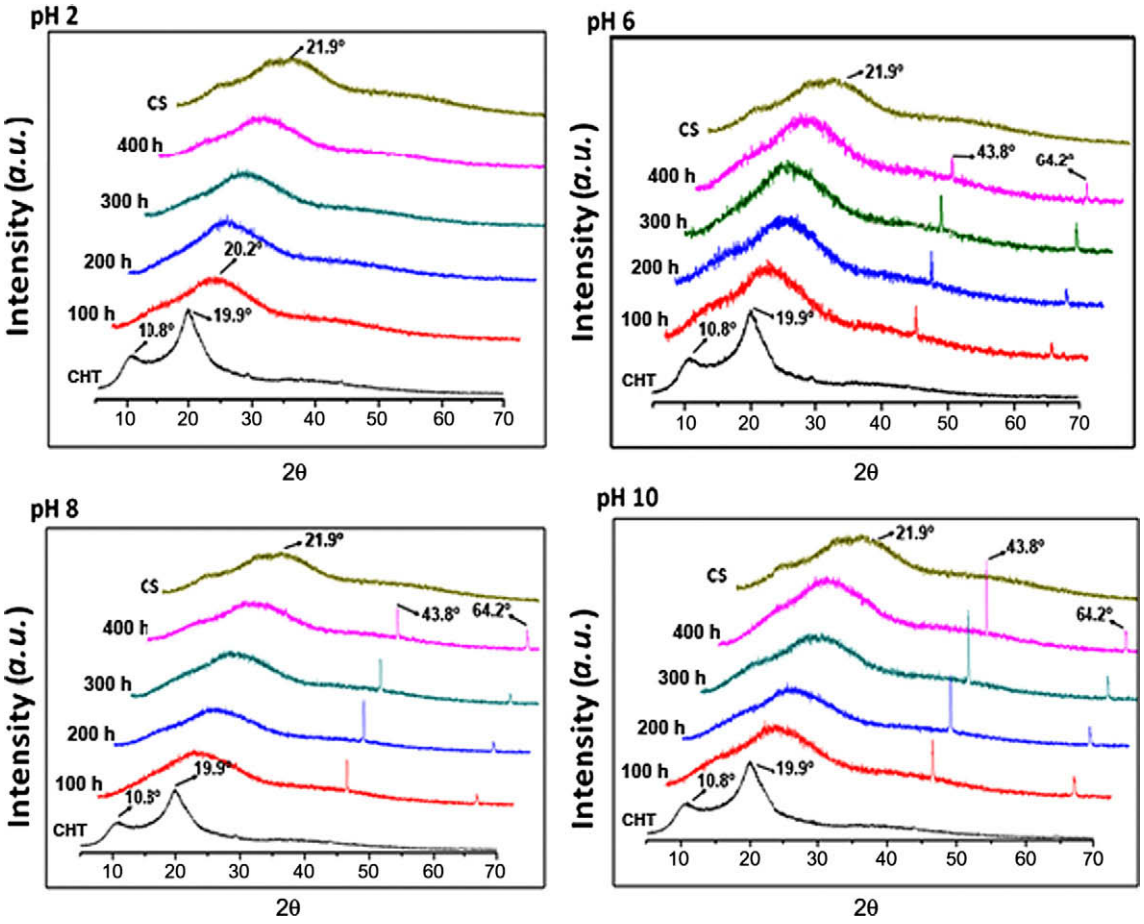

Fig. 2. WAXS profiles of pure polysaccharides and powders of PN-PEC samples after swelling in buffers with pH 2, 6, 8, and 10 for different swelling times.

polymer network also to remain partially entangled. This increase led the CHT $—NH_3^+$ groups to be converted into $—NH_2$ groups. The $—OSO_3^-$ and $—COO^-$ groups of CS were non-released to interact with the CHT $—NH_2$ groups by H-bonding. A change was observed in the interaction forces that form PN-PEC. Initially, electrostatic interactions predominated, and after the material treatment in different pHs conditions, H-bonding occurred and supported the polymer network.

In the formation of PN-PEC and when the same samples were swollen in pH 2 buffer for different time intervals, the electrostatic interaction among the groups of both polysaccharides predominated. In a long-range interaction, like in electrostatic interaction, the polymers chains do not necessarily need to be close for the interaction among groups of opposite charge. In this case, only interactions among the same polysaccharide chains occurred, and the structure formed and/or maintained tended to an entangled state. Therefore, the presence of organized regions was not observed in the WAXS profiles of the dried samples after swelling in pH 2 buffer, and their capacity to absorb liquid was low (Fig. 1). The increase in the swelling buffer pH caused a transient behavior in the PN-PEC structure. The material network expanded due to anion–anion repulsion between CS chains, which gave place to H-bonding electrostatic interactions. These interactions were replaced by inter-chains features and interactions among the chains of a same polysaccharide. H-bonding is less intense than electrostatic interactions, from the point of view of energy, thus requiring that the polysaccharide chains be closer. This chain self-rearrangement resulted in a greater organization and the formation of an organized region, as observed in the WAXS profiles of the samples previously swollen in buffers with pH $\geqslant$6. Samples swollen in pH 10 buffer had ordered chains in the network and improved stability, as shown by the increase in the intensity of the WAXS profile diffraction signals. This increase also depended on the swelling time of the PN-PEC samples. These inferences are consistent with the behavior observed for the *S* parameter. For samples swollen at pH 10 buffer, *S* fell to a value lower than those of samples swollen in buffers at pH 6 and 8, because the network was stabilized by H-bonding.

The WAXS profiles of the chemical substances used to prepare the buffer solutions, H_3BO_3, KCl, KH_2PO_4 and NaOH, and of buffers solutions used as swelling media for PN-PEC (Fig. 3) were performed. No signals were observed at $2\theta = 43.8°$ and $2\theta = 64.2°$ in the WAXS profiles of any of these substances. This means that the ordered region signals at $2\theta = 43.8°$ and $2\theta = 64.2°$ neither originated nor was affected by the PN-PEC-swelling buffer components.

The periodic distances of the PN-PEC ordered regions were evaluated at pHs 6, 8, and 10 by applying Bragg's Law. The values determined for the period of 2.06 Å for $2\theta = 43.8°$ and 1.45 Å for $2\theta = 64.2°$ shows that PN-PEC samples swollen in different pH conditions formed ordered structures with different periodic distances. Both signals were compared by calculating the ratio between the areas of the signals at 64.2° and 43.2°. The values obtained are shown in Table 2.

The area ratio of the WAXS signals at 64.2° and 43.2° gradually increased with the increase in the pH of the PN-PEC pre-swelling buffer solution. Relative to the swelling time, the signal area ratio increased for longer swelling times, indicating that PN-PEC tends to self-rearrange into a polymer structure with ordered regions and a lesser periodic distance among them. The data in Table 2

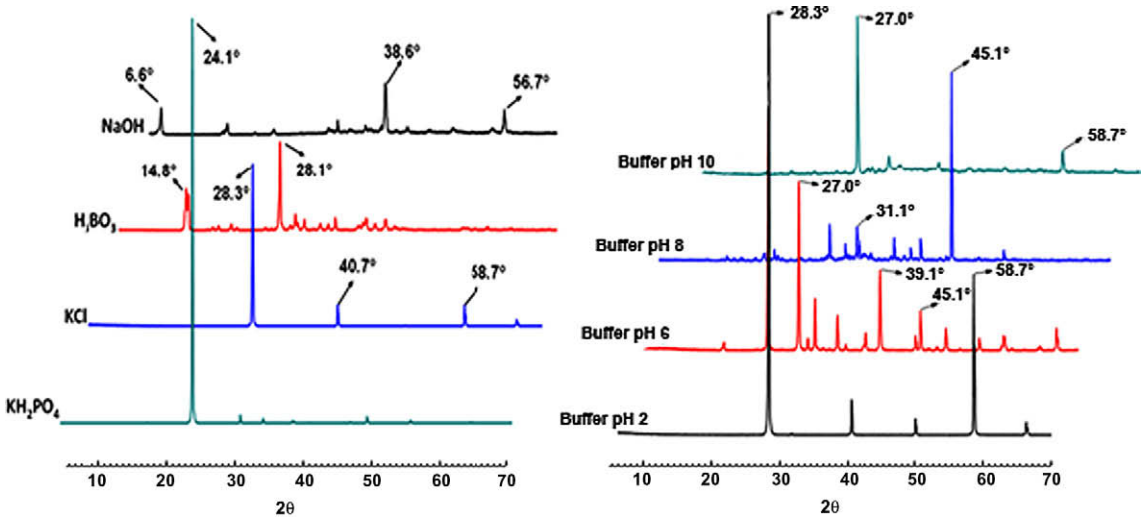

Fig. 3. WAXS profiles of chemical substances used to prepare the buffer solutions and of the swelling buffer solutions of PN-PEC samples.

Table 2
Areas of the signals at 64.2° and at 43.8° observed on X-ray diffractograms of PN-PEC as shown in Fig. 1, and the area ratio between them.

Condition of swelling	Area of the signals (a.u.)		Area ratio (64.2°/43.8°)
	$2\theta = 43.8°$	$2\theta = 64.2°$	
pH 6–100 h	439	131	0.3
pH 6–200 h	554	229	0.4
pH 6–400 h	653	413	0.6
pH 6–800 h	789	676	0.9
pH 8–100 h	333	184	0.5
pH 8–200 h	595	358	0.6
pH 8–400 h	724	525	0.7
pH 8–800 h	990	938	0.9
pH 10–100 h	313	197	0.6
pH 10–200 h	674	515	0.8
pH 10–400 h	778	683	0.9
pH 10–800 h	1054	1023	1.0

show that when PN-PEC was immersed in pH 6 buffer for 200 h, the periodic distance of 2.06 Å of the ordered region predominated. However, when the swelling time for pH 6 was higher than 400 h and/or the buffer pH was higher than 6, the 64.2°/43.2° area ratio increased, showing that the chains remaining inside PN-PEC reorganized and formed structures with smaller periodic distance, 1.45 Å (Fig. 3). Therefore, higher pH conditions and longer swelling times led to the prevalence of finer structure (WAXS signal at 64.2°) as compared to signal at 43.2°, because the CHT chains were still protonated even after 200 h of swelling at pH 6. Probably, the polymer–polymer electrostatic repulsions intensified at pH 6 and swelling times longer than 200 h, or short swelling times when PN-PEC was swollen in buffers with pH higher than 6. In these conditions, the amino groups did not protonate anymore. Thus, negatively-charged moieties should be stabilized by the H-bonds of the amino groups in the CHT structure. As a result, the remaining CS chains reorganized to form more ordered structures, as shown in the scheme in Fig. 4.

Fig. 4 shows a schematic illustration of the entangled structure transition of PN-PEC caused by the variation of the swelling buffer pH. This scheme can be related to the WAXS data of dried PN-PEC samples pre-swollen in different pH conditions and/or in different time intervals. When the samples were swollen in the pH 2 buffer, the strong polymer–polymer interaction prevented the output of the CS chains and the PN-PEC entangled structure was maintained. Increasing the pH of the swelling medium to values near to pKa_{CHT} resulted in the destabilization and expansion of the structure due to the repulsion effect among the negatively-charged groups of CS. The network expansion caused the release of some CS chains from the network due to the significant increase in the diffusion of the liquid within the PN-PEC, as seen in Fig. 1. In an environment with pH between 6 and 8, the CHT amino groups react with basic groups presents in the medium. The amino groups in turn interacted with the sulfonic and carboxylate groups of CS through H-bonding. For this to occur, a self-rearrangement of the CHT and CS chains is required, and that they are close enough to increase H-bonding. The approximation and position of the chains, which are more ordered within the PN-PEC, were responsible for the formation of the ordered regions visualized by the WAXS analysis of PN-PEC samples swollen in buffer with pH greater than 6. When samples were swollen in buffer with pH greater than 8, H-bonding increased and so did the amount of ordered parts. This is associated

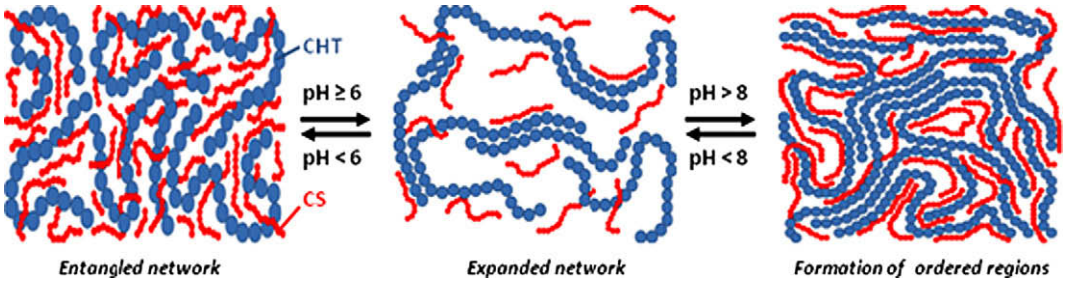

Fig. 4. Schematic illustration of the transition from the entangled structure (at pH <6), the expansion of the network (pH 6–8), and the formation of ordered regions (pH >8) within the PN-PEC.

with the higher intensity of the diffraction signals, which is in accordance with the increase in the pH of the sample swelling buffer or the increase in the swelling time interval.

It is important to infer that the release of a fraction of CS from the polymer network should reduce the polymer chain density inside PN-PEC, thus causing an increase in empty spaces (pores). Additionally, the polymer network formed by CHT and non-released CS chains tended to shrink the chains in order to fill the empty spaces left by the CS movement outward. Therefore, during the release of a fraction of CS, an elastic force developed in the direction opposite to the shrinking due to the tendency of the polymer chains to return to their original swollen state arrangement, thus minimizing the contraction process.

An increase in the ordered regions with a lesser periodic distance was also observed for constant buffer pH and increasing swelling time. Fig. 5 shows the plot of the ratio of the areas of the diffraction signals at 64.2° and 43.2° against the swelling time for three different buffers.

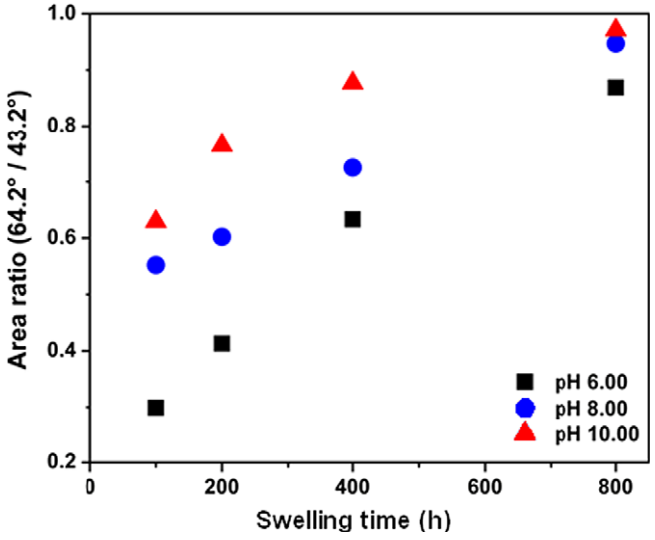

Fig. 5. Ratio between the areas of the diffraction signals at 64.2° and 43.2° against the swelling time for buffers with different pH values.

3.3. DSC and TGA analysis

DSC tests were used to characterize the thermal behavior of the dried PN-PEC due to reorganization of the polymer network chains during swelling for varying pH and/or swelling time. Fig. 6 shows the DSC curves of the pure polysaccharides (Fig. 6a) and the dried PN-PEC samples after swelling in buffers at pH 6 (Fig. 6b), pH 8 (Fig. 6c), and pH 10 (Fig. 6d) for 100–800 h of swelling.

The DSC curves of the pure polysaccharides (Fig. 6a) show endothermic peaks in the temperature range from 50 to 150 °C attributed to the loss of volatile components or a possible chain relaxation. The DSC curve of the CS show a strong exothermic peak at 246 °C, while the DSC curve of CHT present an exothermic peak at 307 °C; both peaks have been attributed to the degradation of the polysaccharides (Fig. 7). The strong electrostatic interactions between CHT and CS charged groups caused the loss of H-bonding. As a consequence, the polymer crystalline structures, which were formed by H-bonding among a same polysaccharide chains (Yen,

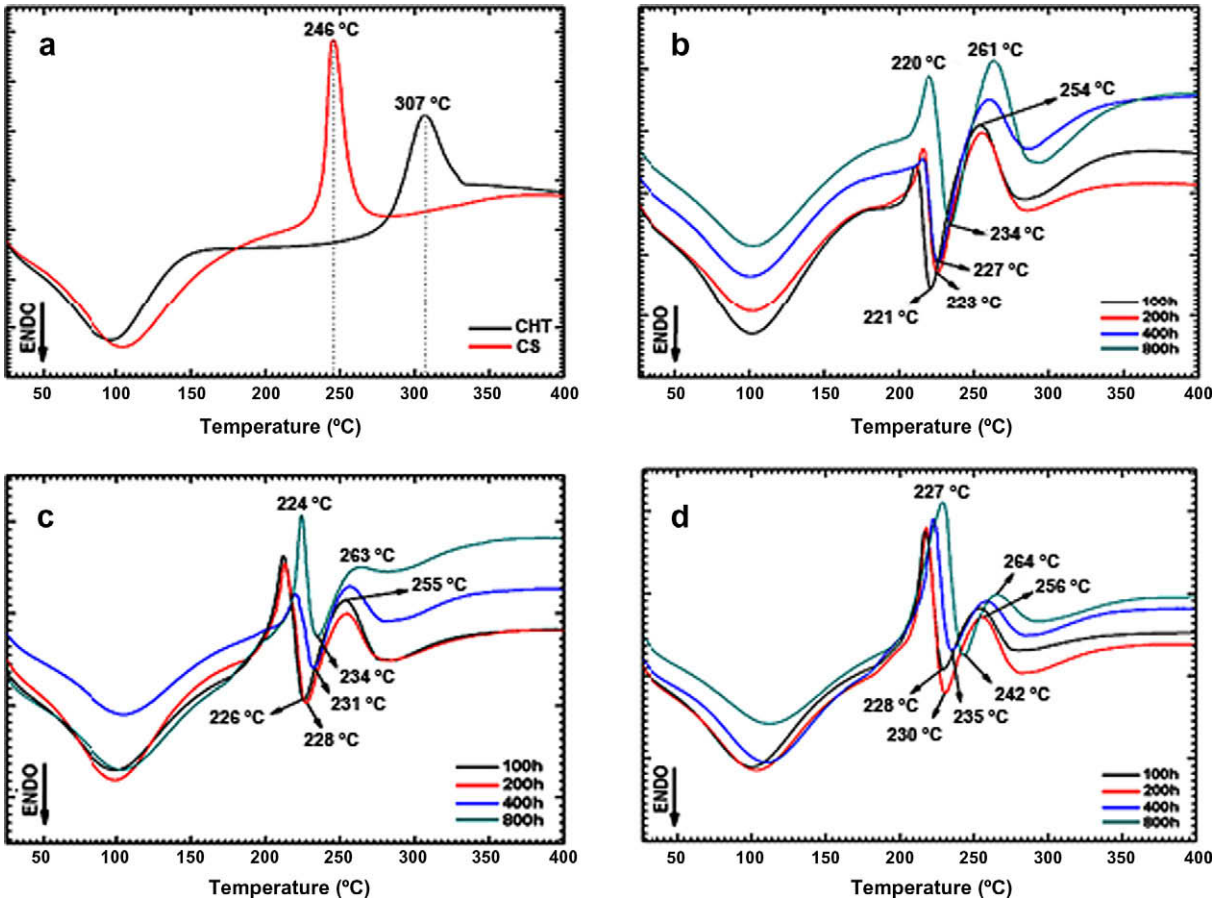

Fig. 6. DSC curves of (a) pure polysaccharides and PN-PEC samples swollen in buffers with pH (b) 6, (c) 8, and (d) 10 for different time intervals.

Yang, & Mau, 2009), ruptured. This indicates that the formation of PN-PEC may change or even end the crystalline structure present in the pure polysaccharide structures, which will consequently change its thermal behavior.

Fig. 6b shows the DSC curves of PN-PEC samples after swelling in pH 6 buffer for 100 h. The exothermic peak at 250 °C was upshifted with the increase in the swelling time, reaching 261 °C for 800 h of swelling. This same behavior was observed when PN-PEC was swollen either in buffer solutions at pH 8 or 10. When PN-PEC was immersed in buffers with pH closer to or greater than pKa_{CHT}, the amino groups (—NH_2) had a neutral form (Strand et al., 2001). This significantly reduced the electrostatic attraction among the amino groups of CHT and the CS sulfonic (—OSO_3^-) and carboxylate (—COO^-) groups, weakening the interaction points that formed the network. In this condition, the CS chains acquire enough mobility to be released out of PN-PEC. After part of CS was released, the CHT chains in the PN-PEC network were as free as the pure CHT, since a similar exothermic peak was observed for pure CHT near to 307 °C. The endothermic peak that appears in the range from 220 °C to 240 °C in Fig. 6b–d is related to electrostatic interactions between the polysaccharide chains of PN-PEC. At pH 10 (Fig. 6d), this peak appeared at 228 °C for 100 h of swelling, and at 242 °C for 800 h, which is probably related to the increased stability of the electrostatic interactions. With the increase in the pH of the PN-PEC swelling buffer to values close or above pKa_{CHT}, the protonated amino groups of CHT stabilized, which increased the stabilization of the density of negative charges because the sulfonic and carboxylate groups did not protonate in this condition. The excess negative charges must increase the repulsion between the CS-forming chains and lead the system to expand. The inward movement of the liquid in the polymer network induced the partial release of the CS chains, causing an increase in the erosion of the material. The DSC curves in Fig. 6b–d show an exothermic peak near to 220 °C. When the pH and the swelling time were increased, the peak intensity increased and the temperature shifted to higher values.

Fig. 7 shows the TGA curves of the dried PN-PEC samples after swelling in buffer solutions with pH 6 (Fig. 7b), 8 (Fig. 7c), and 10 (Fig. 7d) at different time intervals. In all curves, the material mass decreased around 15% between 50 and 150 °C due to the evolution of water and volatile components in the samples. As shown by the DSC tests, increasing the buffer pH caused an increase in the sample degradation temperature. This conclusion is supported by the self-rearrangement of the CHT and CS chains observed in the WAXS profiles. A buffer pH near to or higher than 6 and longer swelling times will contribute to produce a material with higher thermal stability. Thus, it is noteworthy that if the material was pre-treated in the above conditions, the improved thermal stability will enable its application at higher temperatures.

3.4. Morphological analysis

The SEM images of PN-PEC samples swollen in pH 10 buffer and 100, 200, 400, and 800 h are shown in Fig. 8. The SEM images of each sample showed pores of varying sizes heterogeneously distributed on the sample surface. The average pore size was calculated by means of the software Size Meter®, version 1.1 with differentiation threshold set according to the image scale of

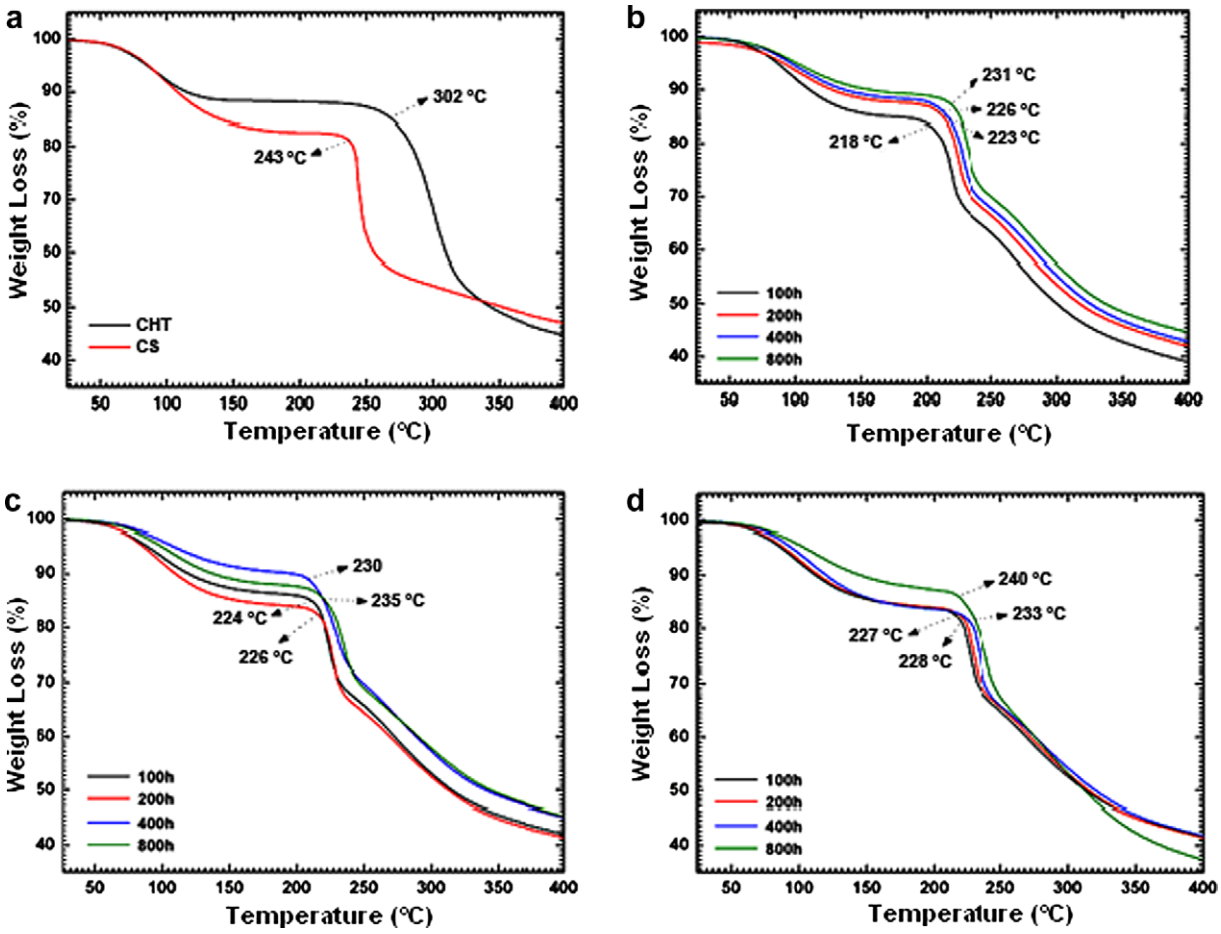

Fig. 7. TGA curves of (a) pure polysaccharides and PN-PEC samples swollen in buffers with pH (b) 6, (c) 8, and (d) 10 in different swelling times.

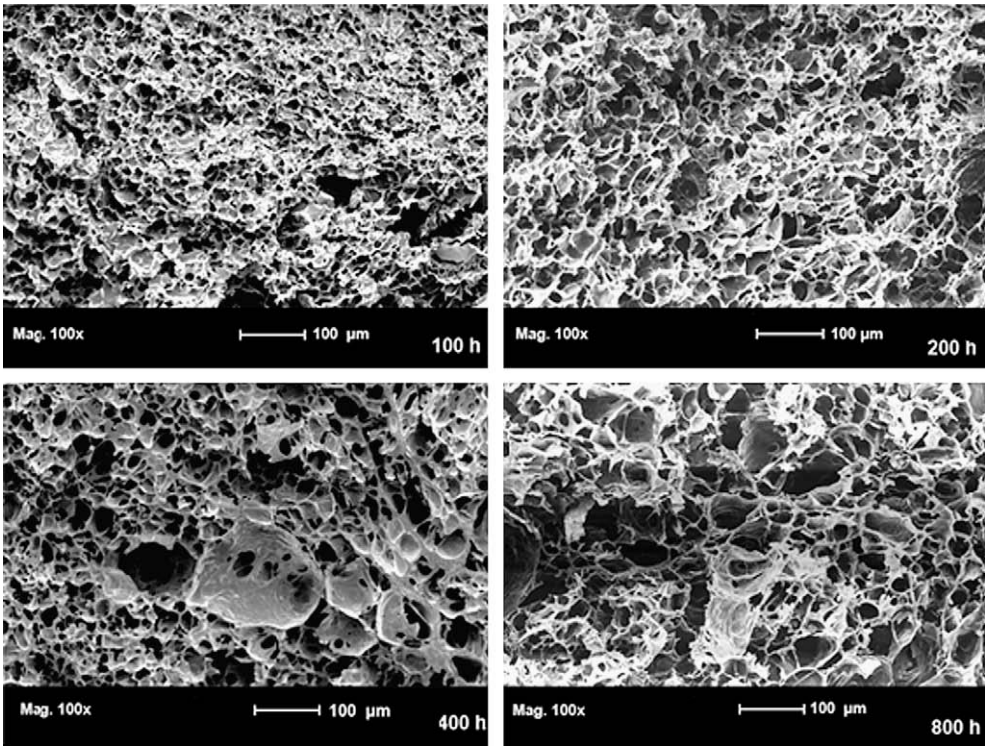

Fig. 8. SEM images obtained from PN-PEC samples dried after swelling in pH 6, 8, and 10 buffer solutions for 100, 200, 400, and 800 h.

100 µm. Since the pore shape was undefined, the measurements were taken between the extreme points of the pores. The average was calculated from the measurement of 50 randomly selected pores. This procedure was performed for each swollen and dried PN-PEC sample. The results are given in Table 3.

According to Table 3, the increase in the average pore size is more pronounced for samples swollen in buffer solutions with pH 6 and 8. It is noteworthy that when the samples were swollen in these pH conditions, they absorbed more liquid, as shown by *S* parameter (Fig. 1). Thus, it is possible to characterize the erosive process due to the increase in the liquid flow within the network. The release of part of solute CS chains increased the number of pores within the material structure. The loss of material also increased the pore size.

Fig. 9 shows SEM images of PN-PEC samples after swelling in buffers with pH 6, 8, and 10 for 800 h. Unlike the samples pre-swollen in buffer solutions with pH 6 or 8, the samples swollen at pH 10 showed a reduction in the average pore size directly related to the increase in the swelling time, as shown by the data in Table 3. This behavior was observed for the samples pre-treated in pH 10 buffer and was confirmed by the measurement of the *S* parameter and the WAXS analysis of the samples. As emphasized before, at a higher pH, the interaction between the CHT amino groups and CS carboxylic groups decreased significantly due o the presence of basic groups like hydroxyl groups (OH^-), which neutralize the protonated CHT amino groups. The self-rearrangement causes the chains to be arranged in a more orderly way, thus reducing the number of pores and their average size. Furthermore, H-bonding stabilizes the network and prevents the release of the rest of the CS chains. This static behavior causes a reduction in the liquid influx and stops erosion. The SEM images of the PN-PEC sample demonstrate this pH-sensitivity property.

Table 3
Average pores size of PN-PEC varying the time of immersion and the pH of the medium.

Immersion time (h)	Average pores size (µm)		
	pH 6	pH 8	pH 10
100	38 ± 5	45 ± 6	48 ± 8
200	42 ± 3	48 ± 5	49 ± 7
400	50 ± 7	50 ± 4	47 ± 6
800	56 ± 9	52 ± 7	42 ± 4

4. Conclusions

A polymer network formed by polyelectrolytes complexes of CHT/CS with capacity of self-reorganizing its structure when swollen in buffers with pH greater than the p*K*a of CHT ($pKa_{CHT} \approx 6.2$) was synthesized. Such reorganization was also dependent on the PN-PEC swelling time. The ratio of the areas of the WAXS profile signals at 64.2° and 43.2° of the dried PN-PEC samples increased as the pre-swelling pH and/or time increased. It is proposed that the self-rearrangement of the chains that form the polymer network after the release of part of the CS chains during swelling occurred mainly at pHs near to or higher than the pKa_{CHT}. In these conditions, the electrostatic interactions stopped forming the polymer network, which continued to be stabilized by H-bonding. The proximity of the CHT and CS chains allowed the formation of these bonds and ordered regions were formed within PN-PEC. These ordered regions increased the crystallinity of the polymer network and led to an increase in its thermal stability. The average pore size became higher in buffer solution with pH between 6 and 8 and lesser when the PN-PEC was swollen in buffers with pH $\geqslant$8.

This in-depth study of the reorganization of the polymer network formed by PEC-cross-linked CHT/CS confirmed that this material is pH sensitive. It can be previously treated to show different structures (entangled or with some crystallinity), higher thermal stability, varying quantities of pores and/or average pore

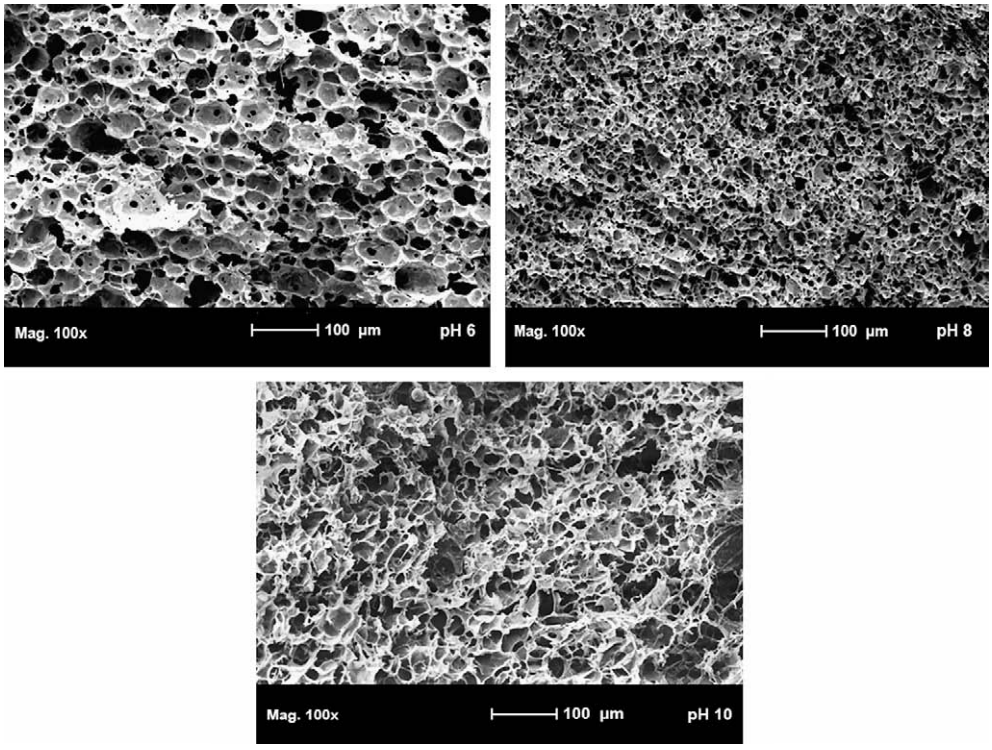

Fig. 9. SEM images obtained from PN-PEC samples dried after swelling in buffers with pH 6, 8, and 10 for 800 h.

size, and swelling behaviors. This biomaterial can be tailored to specific applications after further investigation.

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